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IMMUNOMODULATORY ACTIVITY OF THE *LACTOBACILLUS DELBRUECKII* SUBSP. *BULGARICUS* LB 86 ENZYME LYSATE

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Abstract. The aim of the study was to immunomodulatory activity of the enzyme lysate of probiotic culture *Lactobacillus delbrueckii* subsp. *bulgaricus* LB86 for the development of a functionally active substance based on it with a deliberate action forapplication in cosmetology and medicine. *In vitro* and *in vivo* experiments, using an experimental model of intact BALB/c mice, investigated the effect of enzyme lysate on the functional activity of animal peritoneal exudate macrophages and the production of immunoregulatory cytokines, using conventional research methods. The enzyme lysate of *L. delbrueckii* subsp. *bulgaricus* LB86 showed immunomodulatory activity aimed at altering the activity of macrophages, as well as the production of pro- and anti-inflammatory cytokines, which are involved in balancing the immune response of the Th1- and Th2- type. Under the action of this enzyme lysate *in vitro*, the indicator of phagocytosis (PhI) of the peritoneal cavity macrophages of intact BALB/c mice was enhanced and after its intragastric administration to these mice, the increase of the parameters of spontaneous and stimulated NBT test of macrophages was observed and increase (on the 6th day after its introduction) in the levels of IL-12 and IFN-γ in the blood serum of mice treated with this enzyme lysate was noted against the background of an increase in the ratio of INF-γ/IL-4, which also indicated its potential ability to activate the cellular immune response. It has been established that the enzyme lysate of *L. delbrueckii* subsp. *bulgaricus* LB86 after intragastric administration to intact mice does not directly participate in modulation of intestinal microbiota and throughout the entire observation period in the intestinal contents the number of lacto- and bifidobacteria in the intestinal contents was maintained at the level of control indicators. The number of aerobic and optional anaerobic bacteria, including staphylococci, streptococci, colimorphic bacteria and microscopic fungi also did not change. Obtained data of immunomodulatory activity of the enzyme lysate of *L. delbrueckii* subsp. *bulgaricus* LB86 may indicate the potential prospects for its practical use in therapeutic and prophylactic drugs.

Keywords*:* *Lactobacillus*, enzyme lysate, cytokines, macrophages, immunomodulatory effect.

Introduction. Formulation of the problem

Current global trends in pharmaceutical and cosmetic markets put forward new requirements for drugs that are developed on the base of microbial cells, especially probiotics with high efficiency [1-5]. The development of drugs with high functional activity, containing lysates of lactic acid bacteria and their components, which have no potential side effects, is relevant. Probiotic lysates are of particular interest and are aimed at regulating the immune system [6,7].

Analysis of recent research and publications

So far the pharmaceutical industry presents preparations of lysates used in the treatment of patients with recurrent respiratory tract infections (Lantigen B, Italy; Broncho-munal, Slovenia), there is a prospect of using lysates for the treatment of eczema and various types of atopy [8]. It was shown that lysates are involved in normal wound healing where they stimulate keratinocyte proliferation and/or migration [9]. Probiotics lysates have become widely used in dermocosmetology. A line of cosmetic products for skin care has emerged on their basis. These products enhance the regenerative activity and restore immunity of skin, which is confirmed by the results of many scientific researches [10-12]. It is believed that the efficacy of probiotics and their lysates is primarily related to their immunomodulatory action, which is realised through stimulation of both cellular and humoral immunity [13-15]

Recently, interest in paraprobiotics – inactivated microbial cells and cell fractions – has increased, that have a positive effect on the host's body: peptidoglycans, surface proteins, lipoteichoic acid, exopolysaccharides and other compounds that are components of cell lysates [16]. Thus, it has been shown that preparations of the *Lactobacillus* cell wall and lysates of *L. plantarum* or *L. casei* increase the host's resistance and stimulate its innate immune system [17-20]. It is possible that activation of the innate immune system by lysates may occur through Toll-like receptors [21]. *L. acidophilus* lysates combined with anti-CTLA-4 antibody blockade have been shown to enhance antitumor immunity in mouse models of CRC by synergistically enhancing T-cell antitumor immunity [22]. The authors found that lysates of *Lactobacillus rhamnosus GG* and its individual components can effectively alleviate inflammation in the intestinal epithelial cells of pigs and strengthen the integrity of the intestinal barrier by altering the gut microbiota [7,23]. Microorganism lysates have been reported to positively modulate protective antibody responses against a number of viruses that cause respiratory disease in mouse models of infection, such as respiratory syncytial virus (RSV) and influenza virus [24]. And understanding the immunostimulating activity of probiotic lysates can provide additional opportunities for their use.

Thus, some experience has been gained in studying the multifactorial effect of bacterial lysates. However, it should be noted that the analysis of data from the references revealed a relationship between the properties of lysates and the features of cell structure on the basis of which they are obtained, as well as the way of their disintegration [25] This makes it expedient to develop new active lysates preparations with high functional activity and targeted action mechanism, and understanding the immunostimulating activity of probiotic lysates can provide additional opportunities for their use.

The purpose of the study was to determine the immunomodulatory activity of the enzyme lysates of probiotic culture *Lactobacillus delbrueckii* subsp. *bulgaricus* LB86 for the development of a functionally active substance with targeted action for application in cosmetology and medicine on its basis.

The following **tasks** are set for the realization of the goal:

- to determine the phagocytic activity and oxygen-dependent bactericidal activity of macrophages of the peritoneal cavity of BALB/c mice upon administration of enzyme lysate in vitro and in vivo;
- to investigate changes in the level of cytokines IL-12, INF-γ, IL-4 (pkg/ml) and the INF-γ / IL-4 ratio in the blood serum of mice injected with enzyme lysate;
- to determine the effect of the lysate on the intestinal microbiome of the studied animals.

Research materials and methods

The subject of the study was a enzyme lysate of the probiotic strain *L. delbrueckii* subsp. *bulgaricus* LB86 (VKPM B-5788). from the Plant of biological enzyme preparations "Enzyme". The destructive agent used suspension of the Cytal-Rk enzyme in distilled water (G-10X, activity – 7000 U/g), a research sample of which was obtained from a culture liquid of *Streptomyces albus* UN44 at SE Enzyme (Ladyzhyn).

The reaction mixture included a suspension of lactic acid bacteria cells (10^9 cells/ml) and Cytal-Rk with a concentration of 100 mg/ml in a 1:1 ratio within 180 min at pH 6.5 [26]. The lytic sensitivity of LAB strains was determined by the turbidimetric method: on PEC-3 (10 mm cuvette, 540 nm wavelength) according to the change of the optical density of the primary and final samples and was calculated in % according to the formula (1):

$$OD,\% = \frac{OD_{init}^{540} - OD_{fin}^{540}}{OD_{init}^{540}} \cdot 100\% \quad (1)$$

where $OD,\%$ – lytic activity of the enzyme in relation to the test culture, %;

OD_{init}^{540} – the optical density of the cell suspension before incubation;

OD_{fin}^{540} – the optical density of the cell suspension after incubation.

Heat treatment of the lysate was performed by autoclaving in the mode: 0.8 atm, 30 min.

Studies on the immunomodulatory activity of this enzyme lysate were performed on male intact BALB/c mice at 8 weeks of age (weighing 18-20 g). Mice were kept under standard vivarium conditions at 22±1°C, were provided with complete compound feeds and had free access to automatic drinkers [27]. All our studies were approved in accordance with Law of Ukraine No 3447-IV "On the Protection of Cruelty Animals", "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" of 18 March 1986 (Strasbourg, 1986) and "General Ethics for Animal Experimentation" (First National Congress on Bioethics, Ukraine, 2001).

Mice were receiving the enzyme lysate intragastrically once a day for 7 days at a concentration of 300 μl per animal, the hydrolyzate of the average daily dose of probiotics of 10^8 CFU/ml, according to the recommendations World Gastroenterology Organisation and our previous studies [28,29]. Two groups of animals (5 mice each) were formed: 1 group miceis, which were treated with enzyme lysate, 2 group miceis, which were similarly introduced with distilled water (control group) [30].

The functional activity of cells of the phagocytic system – peritoneal cavity macrophages was evaluated using conventional methods of examination of

absorptive and oxygen-dependent bactericidal activity on days 1, 3, 6 and 9. The indicator of phagocytosis (PhI) was determined by counting the number of cells containing latex particles (in %) in the field of view of the microscope; and phagocytic number (PhN) – the average number of latex particles that are absorbed by phagocytes (in units). Oxygen-dependent bactericidal activity of phagocytes was studied in a spontaneous and stimulated nitroblue tetrazolium recovery test (NBT test) by spectrophotometric method. The functional activity of cells of the phagocytic system and oxygen-dependent bactericidal activity of phagocytes were evaluated in experiments both *in vitro* and *in vivo* [30].

The concentration of interleukins (IL)-4, IL-12 and interferon (IFN)-γ in the blood serum of mice was determined with enzyme-linked immunosorbent assay on days 1, 3 and 6. Determination was carried out according to the manufacturer's instructions (IBL INTERNATIONAL GMBH, Germany), attached to the set. The concentration of cytokines in serum was expressed in pg/ml.

The effect of enzyme lysate on the intestinal microbiota was determined using conventional microbiological testing methods on days 1, 3, 6, 9 and 18. An aliquot plating from intestinal contents of mice was carried out on eight nutrient media, namely: meat-peptone agar (MPA) – medium for cultivation of aerobic and optional anaerobic bacteria; BAIRD-PARKER-Agar ("Merck", Germany) – for the cultivation and enumeration of staphylococci; KF-Streptococcus agar ("Merck", Germany) – for the cultivation and enumeration of streptococci, MRSA (de Man, Rogosa and Sharpe Agar; "Himedia", India) and Bifidobacterium Agar ("HiMedia", India) – for the cultivation and enumeration of lactobacilli and bifidobacteria respectively; ENDO ("HiMedia", India) – for the cultivation and enumeration of colimorphic bacteria, Sabouraud agar ("Himedia", India) – for the cultivation and enumeration of microscopic fungi. After culturing at 37 °C for 24 h, the number of Colony forming units/mg (CFU/mg) on the Petri dish was calculated, given that one such colony corresponds to one bacterium.

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 ± 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 of the research and their discussion

Obtaining bacterial enzyme lysate is carried out by disintegration of their cell walls by various methods or by a combination of several methods [31]. The most gentle method of disintegration is enzymatic hydrolysis, which allows to obtain enzyme lysate with intact intracellular structures. However, the destruction of lactic acid bacteria cells is a significant problem, which is related to the peculiarity of their cell wall structure. The practical value of the enzyme preparation Cytal-Rk used in our work is determined by the nature of its proteolytic proteins – glycosidases and peptidases, which destroy the peptidoglycan of cell walls. The presence in Cytal-Rk of enzymes with different substrate specificity provides this preparation with significant advantages over enzymes containing only one lytic agent, such as lysozyme.

In our previous studies, it was shown that the maximum sensitivity to the enzyme preparation of cells of the probiotic strain *L. delbrueckii subsp. Bulgaricus LB86* was shown et 14th-16th hour of growth, which corresponded to the transition of the culture from the logarithmic growth stage to stationary [32]. With the load of the microbial mass in the reaction suspension $1 \cdot 10^9$ cells/ml, the concentration of the enzyme – 100 mg/ml, the age of culture 14-16 h, the temperature – 55°C, pH – 6.5 and the duration of the hydrolysis of 180 min, cell destruction reached more than 70% [26].

In order to determine the mechanisms of activity of the enzyme lysate of *L. delbrueckii* subsp. *bulgaricus* LB86 we examined its immunomodulatory activity. It is known that the basis of the immunomodulatory action of certain strains of lactobacilli is the change of many factors of innate and acquired immunity – activation of cells of the phagocytic system and dendritic cells, balancing the production of immunoregulatory cytokines of different opposition groups due to their interaction the cell surface receptors of the immune cells, Toll-like receptors (TLR) above the others [33]. It should be noted that under the influence of lactobacilli, the production of IFN-γ and IL-12 can be altered, which contribute to the differentiation of Th0-helper cells into Th1-cells, and, consequently, the development of the cellular immune system, which is important for the formation of an effective immune response in many infectious, inflammatory and other pathological processes [34].

The study of the immunomodulatory action of the enzyme lysate obtained was performed in vitro and in vivo on an experimental model of BALB/c immunodeficient mice. It was shown that in vitro this enzyme lysate partially increased the functional activity of peritoneal exudate macrophages obtained from intact BALB/c mice.

Thus, the indicator of phagocytosis (PhI) of macrophages when the lysate was introduced increased by 1.6 times compared to the control ($p < 0.05$) against a background of an unchanged PhN phagocytic number (Table 1). However, in vitro, the enzyme lysate had no significant effect on the oxygen-dependent bactericidal

activity of macrophages: spontaneous and stimulated NBT test were at a control level. The functional reserve (FR; the difference between stimulated and spontaneous NBT test) of macrophages treated with this enzyme lysate was also maintained at the control leve p > 0,05).

In experiments conducted *in vivo*, after the treatment of mice with the enzyme lysate, PhI and PhN of peritoneal exudate macrophages were maintained at the control level throughout the observation period (Table 2). At the same time, on the days 1 and 9 after the introduction of this enzyme lysate to mice, the parameters of spontaneous and stimulated NBT test of macrophages increased almost twice compared to the control parameters, which may indicate an increase in effector potential of phagocytes under its influence. At other times of observation, on days 3 and 6, the oxygen-dependent bactericidal activity of the macrophages of mice from the experimental group was maintained at the control level.

When studying the level of cytokinins in the blood serum of mice injected with the lysate, it was established, that

on day 1 and day 3 after its introduction to mice, IL-12, and IFN-γ levels did not change, compared to controls. In contrast, significant activation of IL-12 and IFN-γ production under influence of this enzyme lysate was observed on day 6: the level of these cytokines in blood serum was 4.2 and 2.3 times higher, respectively, than in control (Table 3).

The obtained results confirmed that *in vitro* tests can be only one of the screening criteria for immunomodulatory drugs of microbial origin with a targeted effect on various links of immunity, because *in vivo* they can use other targets. This is evidenced by balancing under the influence of the lysate of the strain L. *delbrueckii subsp. bulgaricus LB 86 in vivo* production of cytokines Th1-(interleukin (IL)-12), interferon (INF)-γ) and Th2-(IL -4) type. The level of IL-12 and INF-γ in the blood serum increased 6 days after administration of this lysate to mice against the background of a decrease in serum IL-4 levels, which indicates the dominant development of Th1-type lymphocytes. Th1 activation is known to stimulate T-lymphocyte and macrophage functions, to the development of an immune response by cellular type, which has a crucial role in protection against intracellular microorganisms and tumor cells. Therefore, increasing the indicators of the functional activity of macrophages of the peritoneal exudate on the 9th day after lysate administration to mice *L. delbrueckii subsp. bulgaricus LB86* may be the result of activation of Th-type cytokine production.

The influence of the lysate on the intestinal microbiota of BALB/c mice was determined. It was shown that the change in the functional activity of macrophages and the level of Th1- and Th2-type cytokinins in the blood serum of intact mice injected with the lysate was not accompanied by a change in the quantitative and qualitative composition of their intestinal microbiota. As can be seen from Table 4, the number of lacto- and bifidobacteria in the intestines of mice of the experimental group remained at the level of control indicators.

Table 1. Phagocytic activity and oxygen-dependent bactericidal activity of macrophages of the peritoneal cavity of mice treated with enzyme lysate *in vitro*

Research option	Indicators				
	PhI ,%	PhN, CU	NBT spont., %	NBT stimul., %	FR, %
Peritoneal exudate, control	26.6 ± 1.0	2.3 ± 0.3	14.5 ± 1.2	18.6 ± 1.8	4.1 ± 1.0
Peritoneal exudate with lysate	43.6± 1.9*	3.0 ± 0.9	12.1 ± 1.0	15.8 ± 2.4	3.7 ± 0.9

* The difference is probable with respect to the performance of intact mice not treated with enzyme lysate (control) (p< 0.05)

Table 2. Phagocytic activity and oxygen-dependent bactericidal activity of macrophages of the peritoneal cavity of mice. treated with enzyme lysate

Group of animals	Obser-vation period	Parameters				
		PhI, %			PhI, %	
Intact mice (control)	-	26.6 ± 1.0	Intact mice (control)	-	26.6 ± 1.0	Intact mice (control)
Fermento-lysate treated mice	Day 1	23.3 ± 2.0	Fermento-lysate treated mice	Day 1	23.3 ± 2.0	Fermento-lysate treated mice
	Day 3	20.0 ± 3.9		Day 3	20.0 ± 3.9	
	Day 6	21.3 ± 2.2		Day 6	21.3 ± 2.2	
	Day 9	26.6 ± 1.5		Day 9	26.6 ± 1.5	

* The difference is probable with respect to the performance of intact mice not treated with enzyme lysate (control) (p< 0.05)

Table 3. The level of cytokines in the blood serum of mice, treated with enzyme lysate

Group of animals	Observation period	Cytokine level, pg/ml			INF-γ/ IL -4 correlation
		IL-12	INF-γ	IL -4	
Intact mice (control)	-	16.4 ± 2.1	25.4 ± 1.0	5.9 ± 1.1	4.3 ± 1.0
Enzyme lysate treated mice	Day 1	14.8 ± 1.9	25.9 ± 2.1	3.2 ± 0.2	8.0 ± 0.8*
	Day 3	14.7 ± 1.8	20.7 ± 1.1	7.9 ± 0.2	2.6 ± 0.9
	Day 6	68.0 ± 5.8*	58.0 ± 5.1*	10.0 ± 1.0*	5.9 ± 0.1*

* The difference is probable with respect to the performance of intact mice not treated with enzyme lysate (control) ($p< 0.05$)

Table 4. Intestinal microbiota of mice treated with enzyme lysate

Group of animals	Obser-vation period, day	The number of microorganisms sown on nutrient media, Lg CFU/mg						
		Aerobic and optional anaerobic bacteria	*Staphylococci*	*Streptococci*	*Lactobacilli*	*Bifidobacteria*	*Colimorphic bacteria*	*Microscopic fungi*
Intact mice (control)	-	4.80 ± 0.11	2.10± 0.09	3.40± 0.15	2.70 ± 0.08	2.40 ± 0.09	4.30 ± 0.17	2.60± 0.08
Fermento-lysate treated mice	3	4.92 ± 0.07	2.32 ± 0.10	3.86 ± 0.09	3.02 ± 0.07	2.73 ± 0.09	4.82 ± 0.09	2.35 ± 0.02
	6	4.33 ± 0.04	2.11 ± 0.06	4.20 ± 0.09	2.70 ± 0.04	2.54 ± 0.06	4.61 ± 0.05	1.43 ± 0.04
	9	4.52 ± 0.06	2.41 ± 0.03	3.52 ± 0.04	2.63 ± 0.08	2.39 ± 0.03	4.52 ± 0.07	2.33 ± 0.06
	18	4.72 ± 0.08	2.37 ± 0.08	3.29 ± 0.07	2.83 ± 0.07	2.52 ± 0.06	4.30 ± 0.08	2.58 ± 0.07

* The difference is probable with respect to the performance of intact mice not treated with enzyme lysate (control) ($p< 0.05$)

The number of aerobic and optional anaerobic bacteria, including staphylococci, streptococci, colimorphic bacteria and microscopic fungi also did not change compared control group.

It is obvious that this enzyme lysate does not take a direct part in the modulation of the intestinal microbiota. But there is an assumption that the components of bacterial cells can affect the intestinal microbiota through mechanisms of immune protection [21]. Probably, the immunomodulatory effect of enzyme lysate *L. delbrueckii subsp. bulgaricus LB86 in vivo* is manifested by its activation under the influence of TLR, which is expressed on the surface of immune and epithelial cells in the gut, but further research is needed to confirm this.

Therefore, further research will be directed at studying the effect of the lysate on the expression of Tol-like receptors on the surface of epitheliolytic and immune system cells, because activation of receptors of this type after recognition of pathogen-bound molecular structures of microorganisms leads to activation of cytokine production and modulation of the immune response.

Conclusion

Thus, the enzyme lysate of *L. delbrueckii* subsp. *bulgaricus* LB86 showed immunomodulatory activity aimed at altering the activity of macrophages, as well as the production of pro- and anti-inflammatory cytokines, which are involved in balancing the immune response of the Th1- and Th2- type.

Under the action of this enzyme lysate *in vitro*, the indicator of phagocytosis (PhI) of the peritoneal cavity macrophages of intact BALB/c mice was enhanced and after its intragastric administration to these mice, the increase of the parameters of spontaneous and stimulated NBT test of macrophages was observed and increase (on the 6th day after its introduction) in the levels of IL-12 and IFN-γ in the blood serum of mice treated with this enzyme lysate was noted against the background of an increase in the ratio of INF-γ/IL-4, which also indicated its potential ability to activate the cellular immune response.

It has been established that the enzyme lysate of *L. delbrueckii* subsp. *bulgaricus* LB86 after intragastric administration to intact mice does not directly participate in modulation of intestinal microbiota and throughout the entire observation period in the intestinal contents the number of lacto- and bifidobacteria in the intestinal contents was maintained at the level of control indicators. The number of aerobic and optional anaerobic bacteria, including staphylococci, streptococci, colimorphic bacteria and microscopic fungi also did not change.

Obtained data of immunomodulatory activity of the enzyme lysate of *L. delbrueckii* subsp. *bulgaricus* LB86 may indicate the potential prospects for its practical use in therapeutic and prophylactic drugs.

References

1. Zerrin Erginkaya, Gözde Konuray-Altun. Potential biotherapeutic properties of lactic acid bacteria in foods Food Bioscience. [Internet]. 2022 April; 46, 101544. Available from: https:// 10.1016/j.fbio.2022.101544
2. Oyetayo VO, Oyetayo FL. Potential of probiotics as biotherapeutic agents targeting the innate immune system. Afr. J. Biotechnol. 2005; 4 (2): 123-127. Available from: http://www.academicjournals.org/AJB
3. Dzuba O.S, Bohdan T.Z., Oriabinska. L.B., Dugan O.M. Biotherapeutic properties of lactobacilli. Problems of environmental and medical genetics and clinical immunology. 2013; 4(118) :19-29 (in Ukrainian).
4. Julio Plaza-Diaz, Francisco Javier Ruiz-Ojeda, Mercedes Gil-Campos, Angel Gil Mechanisms of Action of Probiotics. Advances in Nutrition (Bethesda, Md.). 2019;10 (1):49–66. Available from: https 10.1093/advances/nmy063
5. Markowiak P, Śliżewska K. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. Nutrients. 2017;9(9):1021. https://doi.org/10.3390/nu9091021
6. Pfefferle P I, Prescott S L, Kopp M. Microbial influence on tolerance and opportunities for intervention with prebiotics/probiotics and bacterial lysates. J. AllergyClin.Immunol. [Internet]. 2013;131(6):14533 Aviable frome: https:// 10.1016/j.jaci.2013.03.020
7. Zhuo Q, Yu B, Zhou J. et al. Lysates of *Lactobacillus acidophilus* combined with CTLA-4-blocking antibodies enhance antitumor immunity in a mouse colon cancer model. Sci Rep 9, [Internet]. 2019; 20128. Available from: https:// 10.1038/s41598-019-56661-y
8. Crespo C. Bacterial derivatives of *Lactococcus lactis* and ectoin for atopic dermatitis: dermal compatibility and cosmetic acceptability. Pharm Pharmacol Int J. 2017;5(6):226-231. Available from: https:// 10.15406/ppij.2017.05.00143
9. Mohammedsaeed W, Cruickshank S, McBain AJ, O'Neill CA. *Lactobacillus rhamnosus GG* fermentolysate increases re-epithelialization of keratinocyte scratch assays by promoting migration. Scientific reports. [Internet]. 2015; 5, 16147. Available from: https:// 10.1038/srep16147
10. Lew L, Liong M. Bioactives from probiotics for dermal health: functions and benefits. J Appl Microbiol. 2013; 114:1241–1253. Available from: https:// 10.1111/jam.12137
11. Sharma D, Kober M M, Bowe W P. Anti-aging effects of probiotics. Journal of drugs in dermatology. 2016; 15(1):9–12.
12. Kim H, Kim HR, Kim NR, Jeong BJ, Lee JS, Jang S, Chung DK. Oral administration of *Lactobacillus plantarum* lysates attenuates the development of atopic dermatitis lesions in mouse models. J Microbiol. 2015 Jan;53(1):47-52. Available from: https:// 10.1007/s12275-015-4483-z. Epub 2014 Dec 4. PMID: 25471185
13. Ashraf, Rabia, Shah, Nagendra. Immune System Stimulation by Probiotic Microorganisms. Critical reviews in food science and nutrition. 2014; (54). 938–956. Available from: https:// 10.1080/10408398.2011.619671
14. Ding YH, Qian LY, Pang J, Lin J. Y, Xu Q, Wang L.H, Huang D.S, Zou, H. The regulation of immune cells by Lactobacilli: a potential therapeutic target for anti-atherosclerosis therapy. Oncotarget. 2017; 8(35): 59915–59928. Available from: https:// 10.18632/oncotarget.18346
15. Md. Abul Kalam Azad, Manabendra Sarker, Dan Wan Immunomodulatory effects of probiotics on cytokine profiles. Biomed Res Int. [Internet]. 2018: 8063647. Available from: https://10.1155/20)18/8063647
16. Teame T, Wang A, Xie M, Zhang Z, Yang Y, Ding Q, Gao C, Olsen RE, Ran C, Zhou Z. Paraprobiotics and Postbiotics of Probiotic Lactobacilli. Their Positive Effects on the Host and Action Mechanisms: A Review. Front Nutr. [Internet]. 2020 Oct 22; 7:570344. Available from: https:// 10.3389/fnut.2020.570344. PMID: 33195367; PMCID: PMC7642493.
17. Thorakkattu P, Khanashyam A C, Shah K, Babu K S, Mundanat A. S, Deliephan A et al. Postbiotics: current trends in food and pharmaceutical industry. Foods. 2022; 1: 3094. Available from: https:// 10.3390/foods11193094
18. Sashihara T, Sueki N, Ikegami S. An analysis of the effectiveness of heat-killed lactic acid bacteria in alleviating allergic diseases. J Dairy Sci. 2006 Aug;89(8):2846-55. Available from: https:// 10.3168/jds.S0022-0302(06)72557-7. PMID: 16840600.
19. Khan MW, Zadeh M, Bere P, Gounaris E, Owen J, Klaenhammer T, Mohamadzadeh M. Modulating intestinal immune responses by lipoteichoic acid-deficient *Lactobacillus acidophilus*. Immunotherapy. 2012 Feb;4(2):151-61. Available from: https:// 10.2217/imt.11.163. PMID: 22339459; PMCID: PMC3286340.
20. Izuddin WI, Loh TC, Foo HL et al. Postbiotic *L. plantarum* RG14 improves ruminal epithelium growth, immune status and upregulates the intestinal barrier function in post-weaning lambs. Sci Rep 9. 2019; 9938. Available from: https:// /10.1038/s41598-019-46076-0
21. Zakostelska Z, Kverka M, Klimesova K, Rossmann P, Mrazek J, Kopecny J, Hornova M, Srutkova D, Hudcovic T, Ridl J, Tlaskalova-Hogenova H, Ryffel B. Lysate of probiotic *Lactobacillus casei* DN-114 001 ameliorates colitis by strengthening the gut barrier function and changing the gut microenvironment. PLoS ONE. 2011November; 6:e27961. Available from: https:// 10.1371/journal.pone.0027961
22. Gao K, Wang C, Liu L, Dou X, Liu J, Yuan L, Zhang W, Wang H. Immunomodulation and signaling mechanism of *Lactobacillus rhamnosus GG* and its components on porcine intestinal epithelial cells stimulated by lipopolysaccharide. J Microbiol Immunol Infect. 2017 Oct; 50(5): 700-713. Available from: https:// 10.1016/j.jmii.2015.05.002. Epub 2015 May 14. PMID: 26055689
23. Yoon K, Lee S, Keum GB, Song K, Park J, Song B, Yu SY, Cho JH, Kim ES, Doo H, Kwak J, Kim S, Eun J, Lee JH, Kim HB, Lee J, Kim J. Characteristics of *Lactococcus petauri* GB97 lysate isolated from porcine feces and its in vitro and in vivo effects on inflammation, intestinal barrier function, and gut microbiota composition in mice. Microbiol Spectr. 2024; 12:e01334-23. Available from: https:// 10.1128/spectrum.01334-23
24. Coviello S, Wimmenauer V, Polack FP, Irusta PM. Bacterial lysates improve the protective antibody response against respiratory viruses through Toll-like receptor 4. Hum Vaccin Immunother. 2014;10(10):2896-902. Available from: https:// 10.4161/hv.29784. Epub 2014 Nov 19. PMID: 25483455; PMCID: PMC5443103.
25. Probiotics: Biology, Geneticsand Health (Min–Tze Liong – editor) – Malaysia: "Springer Science&Business Media" 2011.
26. Oriabinska LB, Bohdan TZ, Todosiichuk TS. Lactic acid bacteria fermentolysates and their effect on skin hydration. Food Science and Technology. 2021; 15(4): 32-39. Available from: https://10.15673/fst.v15i4.2255
27. Zapadnyuk IP, edition. Laboratory animals: breeding, maintenance, use in experiment. Kyiv: Higher school; 1983 (in Ukrainian).
28. World Gastroenterology Organisation Global Guidelines. Probiotics and prebiotics. 2023 February. Available from: https://www.worldgastroenterology.org/UserFiles/file/guidelines/probiotics-and-prebiotics-english-2023.pdf
29. Lazarenko LM, Babenko LP, Gichka SG, Sakhno LO, Demchenko OM, Bubnov RV, Sichel LM, Spivak MYa. Assessment of the Safety of *Lactobacillus casei* IMV B-7280 Probiotic Strain on a Mouse Model. Probiotics Antimicrob Proteins. 2021 Dec;13(6):1644-1657. doi: 10.1007/s12602-021-09789-1. Epub 2021 Apr 20. PMID: 33876388; PMCID: PMC8055307.
30. Modzolevsky AF, Dyachenko NS, Spivak N Ya, edition. Modern methods of diagnosis of viral respiratory infections and their therapy using interferon preparations (Methodological recommendations). Kyiv; 1994 (in Ukrainian).
31. Shehadul Islam M, Aryasomayajula A, Selvaganapathy PR. A review on macroscale and microscale cell lysis methods. Micromachines (Basel). 2017 Mar 8;8(3):83. Available from: https:// 10.3390/mi8030083
32. Akulevich OV, Oryabinska L.B, Dugan O.M. Kinetics of growth of lactic acid bacteria of the genus *Lactobacillus* on nutrient media with various sources of carbon and nitrogen nutrition. KPI Science News. 2013; 3: 7-11. (in Ukrainian).

33. 17.34Yoon-Doo Lee, Yi-Fan Hong, Boram Jeon, Bong Jun Jung, Dae Kyun Chung, and Hangeun Kim Differential cytokine regulatory effect of three *Lactobacillus* strains isolated from fermented foods. J. Microbiol. Biotechnol. 2016; 26(9):1517–1526. Available from: https://10.4014/jmb.1601.01044
34. 18.35Gad M, Ravn P, Søborg DA, Lund-Jensen K, Ouwehand AC, Jensen S. Regulation of the IL-10/IL-12 axis in human dendritic cells with probiotic bacteria. FEMS Immunol Med Microbiol. Available from: https://10.1111/j.1574-695X.2011.00835.x

ІМУНОМОДУЛЮВАЛЬНА АКТИВНІСТЬ ФЕРМЕНТОЛІЗАТУ *LACTOBACILLUS DELBRUECKII SUBSP. BULGARICUS* LB 86

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Анотація Метою дослідження було визначення імуномодулюльної активності ферментолізату пробіотичної культури *Lactobacillus delbrueckii* subsp. *bulgaricus* LB86 для розроблення на його основі функціонально активної речовини з цілеспрямованою дією для застосування в косметології та медицині. В експериментах *in vitro* та *in vivo* на експериментальній моделі інтактних мишей лінії BALB/c досліджували вплив ферментолізату на функціональну активність макрофагів перитонеального ексудату тварин і продукцію імунорегуляторних цитокінів за допомогою традиційних методів дослідження. Ферментолізат *L. delbrueckii subsp. bulgaricus* LB86 виявив імуномодулювальну активність, спрямовану на зміну активності макрофагів, а також продукцію про- та протизапальних цитокінів, які беруть участь в імунній відповіді Th1- і Th2-типу. Під дією ферментолізату *in vitro* спостерігалося підвищення показника фагоцитозу (PhI) макрофагів черевної порожнини інтактних мишей лінії BALB/c. Внутрішньошлункове введення лізату приводило до підвищення у сироватці крові мишей показників спонтанного і стимульованого NBT - тесту макрофагів, збільшення (на 6 добу після його введення) рівнів IL-12 і IFN-γ та зростання співвідношення INF-γ/IL-4, що вказувало на його потенційну здатність активувати клітинну імунну відповідь. Встановлено, що ферментолізат *L. delbrueckii subsp. bulgaricus LB86* після внутрішньошлункового введення інтактним мишам не бере безпосередньої участі в модуляції кишкової мікробіоти. Протягом усього періоду спостереження чисельність аеробних та факультативно анаеробних стафілококів, стрептококів, коліморфних бактерій, лакто- та біфідобактерій, а також мікроскопічних грибів залишалась на рівні контрольних значень. Отримано дані щодо імуномодулювальної активності ферментолізату *L. delbrueckii subsp. bulgaricus* LB86 можуть вказувати на потенційні перспективи його практичного використання в лікувально-профілактичних засобах.

Ключові слова: *Lactobacillus*, ферментолізат, цитокіни, макрофаги, імуномодулювальний ефект.

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