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A Comprehensive Analysis of Cytotoxicity of Genetically Modified *Lactobacillus plantarum* 8PA3 (B-11007) Strain Including Identification and Evaluation of Virulence Factors

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Abstract

Introduction. Active development of biotechnologies and genetic engineering techniques broadens the horizons for engineering the probiotic bacterial strains with tailored functional properties, including the strains based on bacteria of the genus *Lactobacillus* that are traditionally considered safe microorganisms. Genetic modification enhances their antagonist activity, resistance to adverse environmental factors, and boosts metabolic efficiency. However, bacterial genome editing may entail risk of introducing the undesirable traits, including cytotoxicity and expression of virulence factors. Safety concerns are particularly relevant in the frame of veterinary practice, where probiotics are widely used to correct the gastrointestinal microbiocenosis in agricultural animals and improve their productivity. The aim of the study is to conduct a comprehensive analysis of cytotoxicity of the genetically modified *Lactobacillus plantarum* 8PA3 (B-11007) strain including identification and evaluation of the virulence factors responsible for inducing cell death and disruption of cellular functions.

Materials and Methods. A genetically modified *Lactobacillus plantarum* 8PA3 (B-11007) strain was the object of the study conducted in the period from January to February 2026. A comprehensive *in vitro* analysis of its cytotoxicity included: assessment of the cytopathic effect in intestinal cell culture, the effect on the viability and metabolic activity of enterocytes, and the state of intestinal epithelial barrier function. Additionally, the analysis of potential virulence factors was performed, along with the study of the mechanisms of cell death, including signs of apoptosis and necrosis.

Results. The recombinant *Lactobacillus plantarum* 8PA3 (B-11007) strain has demonstrated a high biological safety and pronounced biological activity, which enhanced the intestinal epithelial barrier function by 15.2%, the metabolic activity of cells by 8.7% and simultaneously reduced early apoptosis of enterocytes by 31.1% ($p<0.05$), showing no signs of cytotoxic action or activation of caspase-dependent cell death.

Discussion and Conclusion. The conducted comprehensive analysis allows making the unequivocal conclusion about genetically modified *L. plantarum* 8PA3 (B-11007) strain exhibiting no cytotoxic activity *in vitro.* Moreover, by certain properties (cellular metabolic activity, barrier function), it exceeds the original native strain, which makes it a promising candidate for further preclinical and clinical trials as a potential probiotic agent.

Keywords: comprehensive analysis, *Lactobacillus*, *L. plantarum 8PA3 (B-11007)*, probiotics, genetic engineering, cytotoxicity, intestinal cell culture, enterocytes, virulence factors

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Оригинальное эмпирическое исследование

Комплексный анализ цитотоксичности генетически модифицированного штамма *Lactobacillus plantarum* 8РАЗ (В-11007) с выявлением и оценкой факторов вирулентности

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Аннотация

Введение. Активное развитие биотехнологий и методов генной инженерии открывает широкие перспективы для создания пробиотических штаммов с заданными функциональными свойствами, в том числе на основе бактерий рода *Lactobacillus*, традиционно считающихся безопасными микроорганизмами. Генетическая модификация позволяет усиливать их антагонистическую активность, устойчивость к неблагоприятным факторам среды и метаболическую эффективность. Однако вмешательство в бактериальный геном может сопровождаться риском появления нежелательных свойств, включая цитотоксичность и экспрессию факторов вирулентности. Проблема безопасности особенно актуальна в ветеринарной практике, где пробиотики широко применяются для коррекции микробиоценоза желудочно-кишечного тракта сельскохозяйственных животных и повышения их продуктивности. Цель исследования — провести комплексный анализ цитотоксичности генетически модифицированного штамма *Lactobacillus plantarum* 8РАЗ (В-11007) с выявлением и оценкой факторов вирулентности, отвечающих за индуцирование клеточной гибели и нарушение клеточных функций.

Материалы и методы. Объектом исследования, проведенного в период с января по февраль 2026 г., являлся генетически модифицированный штамм *Lactobacillus plantarum* 8РАЗ (В-11007). Комплексный анализ его цитотоксичности с использованием методов *in vitro* включал: оценку цитопатического эффекта на органной культуре кишечника, влияние на жизнеспособность и метаболическую активность энтероцитов, а также состояние барьерной функции кишечного эпителия. Дополнительно выполнялся анализ потенциальных факторов вирулентности и изучались механизмы клеточной гибели, включая признаки апоптоза и некроза.

Результаты исследования. Рекомбинантный штамм *Lactobacillus plantarum* 8РАЗ (В-11007) продемонстрировал высокий профиль биологической безопасности и выраженную биологическую активность, увеличивая барьерную функцию кишечного эпителия на 15,2 %, метаболическую активность клеток на 8,7 % и одновременно снижая ранний апоптоз энтероцитов на 31,1% ($p<0,05$), без признаков цитотоксического действия и активации каспаз-зависимой гибели клеток.

Обсуждение и заключение. Проведённый комплексный анализ позволяет сделать однозначный вывод о том, что генетически модифицированный штамм *L. plantarum* 8РАЗ (В-11007) не обладает цитотоксическим действием в условиях *in vitro*. Более того, по ряду параметров (метаболическая активность клеток, барьерная функция) он превосходит исходный нативный штамм, что делает его перспективным кандидатом для дальнейших доклинических и клинических исследований в качестве потенциального пробиотического средства.

Ключевые слова: комплексный анализ, *Lactobacillus, L. plantarum* 8РАЗ (В-11007), пробиотики, генная инженерия, цитотоксичность, органная культура кишечника, энтероциты, факторы вирулентности

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Introduction. Active development of biotechnologies and genetic engineering techniques broadens the horizons for engineering the probiotic strains with tailored functional properties. In this context, identification and description of the potential factors of virulence are performed using the specialized databases and taxonomies [1]. Modern understanding of the role of probiotics in maintaining human health and regulating metabolic processes fosters the relevance of targeted modification of bacteria designated to enhance their beneficial properties [2]. At the same time, the results of the phenotypic and genomic analysis of *Lactobacillus reuteri* strains demonstrate the need for a comprehensive safety assessment even for commonly used probiotics [3]. Similar data on the importance of comparing the genomic markers and phenotypic characteristics in the context of assessing safety of a given strain are presented in publication [4]. General reviews emphasize that expansion of the range of probiotic products requires strict control of their biological safety and pathogenic potential absence [5].

A comprehensive approach to assessment of the safety of probiotic cultures, including genotypic and phenotypic

analysis, was implemented in a study of *Lacticaseibacillus paracasei* NTU 101, demonstrating the relevance of multi-level testing of new strains [6]. Similar methodological principles were applied in the analysis of *Lactobacillus bulgaricus* IDCC 3601, where potential risks to humans were assessed [7]. A detailed description of the phenotypic and genotypic characteristics of *Levilactobacillus brevis* KU15006 has also proved the need to exclude the factors of virulence and antibiotic resistance [8]. It is known that molecular signaling pathways regulating cell proliferation and survival in condition of inflammatory stress can be modified by microbial factors, which emphasises the importance of studying the cellular mechanisms of the host response [9].

The problem of safety of genetically modified microorganisms is particularly relevant for veterinary practice, where probiotics are used to normalize the microbiocenosis and increase animal resistance to infections, as confirmed by the studies of *Lactococcus lactis* IDCC 2301 [10]. The assessment of four lactobacilli strains with probiotic properties showed that even closely related microorganisms require individual analysis of genetic stability and the absence of undesirable determinants [11]. The activity of enzymes, such as bile salt hydrolases, is based on the interaction of microbiota and the host organism, and has significant importance [12]. Studies of the *Lactobacillus delbrueckii subsp. lactis* strain CKDB001 confirm the need to evaluate not only safety but also functional metabolic effects [13].

In vitro experimental models are widely used to evaluate the cytotoxicity and safety of encapsulated probiotic cultures, as demonstrated for *Lactiplantibacillus plantarum* CRD7 and *Lacticaseibacillus rhamnosus* CRD11 [14]. Changes in intestinal microbiota and metabolic parameters after administration of *Lacticaseibacillus paracasei* AO356 confirm the importance of studying the systemic effect of probiotics [15]. Analysis of the phenotypic and genotypic characteristics of *Weissellacibaria* JW15 also indicates the need for a comprehensive assessment of safety of the new cultures [16]. An additional stage of preclinical testing is *in silico* analysis of potential genes of virulence and resistance, as shown for representatives of the genus *Bacillus* isolated from the bee products [17].

Particular attention is paid to the ability of strains to produce biogenic amines, which can have a toxic effect when accumulated in food products, as demonstrated for lactic acid bacteria isolated from fish products [18]. Genomic sequencing and evaluation of the probiotic potential of *Lacticaseibacillus paracasei* strain LC86 and *Lacticaseibacillus casei* strain confirm the importance of a comprehensive analysis of their safety [19]. The reviews investigating the biogenic amines in the fermented products emphasise the potential risks of uncontrolled microbial activity for the health [20]. Current data on the oral cavity microbiome and its impact on systemic health also indicate the need for rigorous evaluation of probiotic interventions [21].

Experimental studies using a *Clostridioides difficile* infection model demonstrate that probiotic strains can reduce expression of inflammation and restore barrier function of the intestines, thus, confirm their therapeutic potential subject to proven safety [22]. However, even opportunistic lactobacilli strains can, under certain conditions, exhibit cytopathic effect on intestinal epithelium. Therefore, comprehensive analysis of cytotoxicity is a necessary step in preclinical studies of new probiotic strains.

The aim of the study is to conduct a comprehensive analysis of the cytotoxic properties of the genetically modified strain *Lactobacillus plantarum* 8PA3 (B-11007), aiming to identify and evaluate the virulence factors responsible for inducing the cell death and dysfunction of cellular mechanisms.

Materials and Methods. The experimental part of the study was performed at the Genome Center of the North-Caucasus Federal University (Stavropol) and at the bacteriological laboratory of the Department of Epizootology and Microbiology of Stavropol State Agrarian University in the period from January 23 to February 17, 2026.

In this study, the *Lactobacillus plantarum* strain 8PA3 (B-11007) was genetically modified by introducing a recombinant plasmid containing functionally independent expression cassettes encoding heterologous proteins under the control of constitutive promoters.[1] The cytotoxicity of the genetically modified *L. plantarum* strain 8PA3 (B-11007) was assessed in compliance with the author's method of A.V. Frolov "Method for determining the cytotoxicity of bacteria".[2]

The study was conducted using the genetically modified (GM) *L. plantarum* strain 8PA3 (B-11007) and its native analogue as a control. Such an approach makes it possible not only to identify differences in the cytopathic effect between the GM form and the control one but also to assess the preservation or enhancement of strain's beneficial properties, which is extremely important for the objective assessment of the modified strain's safety and functional activity.

Bacterial strains were cultured in the standard MRS nutrient medium (Difco™) in under anaerobic conditions at 37°C using AnaeroGen gas bags (Oxoid, UK). Bacterial suspensions were prepared in sterile phosphate-buffered saline (PBS), adjusted to optical density of 0.5 McFarland standard, corresponding to a concentration of approximately 1.5×10^8 CFU/ml, and then diluted to a working concentration of 1×10^9 CFU/ml.

[1] At the time of writing this article, the patent is undergoing patent registration (incoming no. W 25039202 Russian Federation. Registration No. 2025116719).

[2] USSR patent SU No. 1747483 A1.

Cytotoxicity was assessed using two parallel approaches. In the first step, jejunal organ culture from clinically healthy 10-day-old calves (n=10) were placed in sterile containers over a cover slip with liquid nutrient medium (RPMI-1640) containing a suspension of the studied *Lactobacillus* strain at a final concentration of 1×10^9 CFU/ml. Incubation with the bacterial suspension was carried out for 30 min at 37°C in an atmosphere of 5% CO_2 and 95% humidity using a CO_2 incubator (Binder GmbH, Germany). After exposure, the containers were centrifuged at the rate of 10 000 rpm for 10 min to sediment the cells detached as a result of the cytopathic effect. Each experiment was performed in 5 biological replicates.

In parallel, cytotoxicity was assessed in primary culture of enterocytes that were isolated from the jejunum of 10-day-old calves. Cells were seeded in 96-well plates coated with type I collagen at density of 3×10^4 cells per well and cultured in RPMI-1640 medium supplemented with 10% fetal calf serum until a monolayer formed. Cytotoxicity was assessed using the MTT assay with 24-hour incubation and subsequent measurement of optical density (OD) at 570 nm. Intestinal barrier integrity was assessed by measuring transepithelial electrical resistance (TEER) using an EVOM2 Volt/Ohm meter, recording readings every 4 hours for 24 hours.

To determine the mechanisms of cytotoxicity, fluorescence microscopy was performed using annexin V-FITC and propidium iodide staining to differentiate apoptosis from necrosis. Caspase 3/7 activity was also assessed using the Caspase-Glo 3/7 Assay kit.

Quantitative cytotoxicity (C) was assessed using the formula:

$$C= N/t\ (1- g1/g0), \quad (1)$$

where N — the number of cells detached during centrifugation, counted per 1.96 mm^2 along the diagonals of the cover glass;

g0 — centrifugal acceleration of cell detachment, not treated with a cytotoxic bacterial suspension;

g1 — centrifugal acceleration of cell detachment, treated with a cytotoxic bacterial suspension;

t — exposure time to cytotoxic bacterial suspension, h.

To more thoroughly assess the impact on the cellular functions, an MTT assay was used on a monolayer of primary enterocytes for 24 hours. The assay is based on the Tetrazolium dye (MTT) reduction by mitochondrial dehydrogenases of viable cells. Cell viability percentage (VC) was calculated using the formula:

$$VC\ (\%) = (OD570\ experiment / OD570control) \times 100\ \%, \quad (2)$$

where OD570 experiment — optical density of formazan solution in wells with cells treated with bacteria;

OD570 control — optical density in wells with cells cultured in a pure nutrient medium.

All numerical data obtained were subjected to statistical processing. Results are presented as the arithmetic mean (M) ± standard deviation (SD) or standard error of the mean (SEM). The Shapiro-Wilk test was used to assess the normality of data distribution. Comparison of means between the experimental and control groups was performed using the Student's t-test for independent samples using the Primer of Biostatistics 4.03 software (McGraw-Hill, USA). Differences were considered statistically significant at $p \leq 0.05$.

Research Results. The primary assessment of the potential cytotoxic effect of the genetically modified *L. plantarum* strain 8PA3 (B-11007) was performed in compliance with A.V. Frolov's method on jejunal organ culture and revealed no statistically significant increase in cytotoxicity between the GM strain and its native analogue (Table 1).

As shown in Table 1, the cytotoxicity index of the GM strain did not differ statistically significantly ($p>0.05$) from that of the native control strain. The number of detached cells in both groups (corresponding to a cytotoxicity index in the range of 0.06–0.12 a.u.) was within the reference values established for this organ culture in the settings free of pathogenic influence (0.05–0.15 a.u.). This indicates the absence of cytopathic effect and disruption of tissue adhesive properties under the influence of the genetically modified strain, even under extreme stress testing conditions. It is important to emphasize that the method is based on a short-term (30 min) exposure to an extremely high bacterial concentration (1×10^9 CFU/ml) followed by the application of centrifugal force, which simulates the tissue resistance limit. These conditions are stressful and extreme, simulating not a standard interaction, but rather the tissue's "resistance limit". In the context of safety assessment, identifying an effect under such conditions is a valuable indicator for further, more in-depth studies, but cannot directly indicate pathogenicity *in vivo*, where bacterial concentrations and interaction conditions are significantly milder.

Table 1

Cytotoxicity index (CI) of native and GM forms of *L. plantarum* strain 8PA3 (B-11007) on jejunal organ culture (M ± SD, n=5)

Studied group	Cytotoxicity index (CI), arbitrary units (a.u.)	Significance level (p)
Native (control) strain	0.08 ± 0.02	–
Genetically modified strain	0.09 ± 0.03	>0.05

Visual assessment of the organ cultures after the experiment confirmed the quantitative data obtained. The jejunal mucosa in both studied groups (both those exposed to the native and GM strains) retained characteristic morphological features consistent with the normal values. The surface of the samples had a uniform pale pink color, indicating preserved microcirculation and the absence of signs of ischemia or hemorrhagic lesions. Macroscopic examination revealed a smooth, moist mucosal surface, characteristic of healthy tissue. The absence of areas of turbidity, focal hyperemia, edema, or massive epithelial desquamation further demonstrates the absence of destructive effects from the GM strain.

The results of the MTT test on a monolayer of primary enterocytes showed that after 24 hours of incubation, the metabolic activity of cells treated with the GM strain was statistically significantly higher than that in the control group (Table 2).

The data obtained in Table 2 indicate that the GM strain has a moderate stimulating effect on enterocyte metabolic activity, significantly ($p < 0.05$) increasing it by 8.7% compared to the control strain. This effect may be associated with targeted changes in bacterial metabolism as a result of genetic modification, such as increased production of specific metabolites (short-chain fatty acids, B vitamins) that have a trophic effect on intestinal epithelial cells.

To assess safety at the level of cell death regulation, the analysis of apoptosis and the activity of executioner caspases 3/7 was performed (Table 3).

As shown in Table 3, the GM strain significantly ($p<0.05$) reduced the proportion of cells undergoing early apoptosis by 31% compared to the control, without affecting caspase 3/7 activity ($p > 0.05$). This indicates an anti-apoptotic effect unrelated to the classical caspase-dependent pathway and confirms the strain's safety.

All quantitative data obtained, demonstrating the balance between the safety and beneficial properties of the GM strain, are summarized in Table 4.

Table 2

Effect of native and GM forms of *L. plantarum* strain 8PA3 (B-11007) on the metabolic activity of primary enterocytes (M ± SD, n=5)

Studied group	Metabolic activity, % of control	Significance level (p)
Native (control) strain	100.0 ± 3.5	–
Genetically modified strain	108.7 ± 4.1	<0.05

Table 3

Effect of native and GM forms of *L. plantarum* strain 8PA3 (B-11007) on apoptosis and caspase 3/7 activity in primary enterocytes (M ± SD, n=5)

Studied group	Cells in early apoptosis, %	Activity of caspases 3/7, a.u.	Significance level (p)
Native (control) strain	4.5 ± 0.6	1.00 ± 0.12	-
Genetically modified strain	3.1 ± 0.4	0.95 ± 0.10	<0.05 (for apoptosis)

Table 4

Summary of the effect of GM form of *L. plantarum* strain 8PA3 (B-11007) on organ culture and primary enterocytes

Parameter	Native (control) strain	GM strain	Difference, %	Statistical Significance (p)
Cytotoxicity index (CI)	0.08 ± 0.02 a.u.	0.09 ± 0.03 a.u.	+12.5	>0.05
Metabolic activity	100.0 ± 3.5 %	108.7 ± 4.1 %	+8.7	<0.05
Transepithelial electrical resistance (TEER)	100 %	115.2 % (by 24th hour)	+15.2	<0.05
Early apoptosis	4.5 ± 0.6 %	3.1 ± 0.4 %	-31.1	<0.05

Discussion and Conclusion. Based on the conducted comprehansive studies, it was established that the genetically modified *L. plantarum* strain 8PA3 (B-11007) does not exhibit a cytotoxic effect on the calf jejunal organ culture. The cytotoxicity index of the GM strain (0.09±0.03 a.u.) did not statistically significantly differ ($p>0.05$) from the index of the native precursor strain (0.08±0.02 a.u.) and was within the physiological norm, indicating the preservation of cellular adhesion and the integrity of the histological structure of the tissue. Macroscopic assessment of the mucous membrane showed the preservation of normal

characteristics — pale pink color and typical surface topography with physiological roughness, which further confirms the absence of pathological changes in the tissue.

The GM strain proved to have a positive effect on the functional activity of enterocytes. A statistically significant ($p<0.05$) increase in cellular metabolic activity by 8.7% compared to the that in the control was detected, as well as a reliable enhancement of barrier function, expressed as an increase in transepithelial electrical resistance by 15.2% by 24th hour of coincubation. It was confirmed that the mechanism of interaction between the GM strain and host cells is not associated with the induction of apoptosis or necrosis. In the experimental group, a statistically significant decrease in the proportion of cells in early apoptosis was recorded (3.1% versus 4.5% in the control; $p<0.05$) with maintained background level of caspase 3/7 activity, indicating the safety of the strain in the context of cell death regulation.

The conducted studies allow us to conclude that the genetic modification of the *L. plantarum* strain 8PA3 (B-11007) did not lead to the acquisition of cytopathic properties or virulence factors. The preservation of normal macroscopic characteristics of the intestinal mucosa, combined with a positive effect on cellular functions, indicates that the GM strain has positive effects, expressed in stimulating enterocyte metabolism and strengthening the intestinal epithelial barrier. The identified properties make this strain a promising candidate for the development of new probiotic medications based on it. To fully understand the mechanisms of the observed positive effects and identify specific bacterial metabolites responsible for the identified effects, it is advisable to conduct further proteomic and metabolomic studies. The results obtained are the basis for moving to the next stage of preclinical testing in laboratory animals.

References

1. Liu B, Zheng D, Zhou S, Chen L, Yang J. VFDB 2022: A General Classification Scheme for Bacterial Virulence Factors. *Nucleic Acids Research*. 2022;50(D1):D912–D917. http://doi.org/10.1093/nar/gkab1107

2. Barrea L, Verde L, Auriemma RS, Vetrani C, Cataldi M, Frias-Toral E, et al. Probiotics and Prebiotics: Any Role in Menopause-Related Diseases? *Current Nutrition Reports.* 2023;12:83–97. https://doi.org/10.1007/s13668-023-00462-3

3. Lee BS, Ban OH, Bang WY, Chae SA, Oh S, Park C, et al. Safety Assessment of *Lactobacillus Reuteri* IDCC 3701 Based on Phenotypic and Genomic Analysis. *Annals of Microbiology.* 2021;71:10. https://doi.org/10.1186/s13213-021-01622-y

4. Sarita B, Samadhan D, Hassan MZ, Kovaleva EG. A Comprehensive Review Of Probiotics and Human Health — Current Perspective and Applications. *Frontiers in Microbiology*. 2025;15:1487641. https://doi.org/10.3389/fmicb.2024.1487641

5. Chen CT, Chao WY, Lin CH, Shih TW, Pan TM. Comprehensive Safety Assessment of *Lacticaseibacillus Paracasei Subsp. Paracasei* NTU 101 through Integrated Genotypic and Phenotypic Analysis. *Current Issues in Molecular Biology*. 2024;46(11):12354–12374. https://doi.org/10.3390/cimb46110734

6. Lee M, Bang WY, Lee HB, Yang SY, Lee KS, Kang HJ, et al. Safety Assessment and Evaluation of Probiotic Potential of *Lactobacillus Bulgaricus* IDCC 3601 for Human Use. *Microorganisms*. 2024;12(10):2063. https://doi.org/10.3390/microorganisms12102063

7. Lee MG, Kang MJ, Kim S, Jeong H, Kang DK, Paik HD, et al. Safety Assessment of *Levilactobacillus Brevis* KU15006: A Comprehensive Analysis of Its Phenotypic and Genotypic Properties. *Probiotics and Antimicrobial Proteins*. 2025;17(3):1117–1131. https://doi.org/10.1007/s12602-024-10237-z

8. Tail M, Zhang H, Zheng G, Hatami M, Skutella T, Unterberg A, et al. The Sonic Hedgehog Pathway Modulates Survival, Proliferation, and Differentiation of Neural Progenitor Cells under Inflammatory Stress in Vitro. *Cells*. 2022;11(4):736. https://doi.org/10.3390/cells11040736

9. Kim T, Mondal SC, Jeong CR, Kim SR, Ban OH, Jung YH, et al. Safety Evaluation of *Lactococcus lactis* IDCC 2301 Isolated from Homemade Cheese. *Food Science and Nutrition*. 2022;10(1):67–74. https://doi.org/10.1002/fsn3.2648

10. Lee YR, Bang WY, Baek KR, Kim GH, Kang MJ, Yang J, et al. Safety Evaluation by Phenotypic and Genomic Characterization of Four *Lactobacilli* Strains with Probiotic Properties. *Microorganisms*. 2022;10(11):2218. https://doi.org/10.3390/microorganisms10112218

11. Bourgin M, Kriaa A, Mkaouar H, Mariaule V, Jablaoui A, Maguin E, et al. Bile Salt Hydrolases: at the Crossroads of Microbiota and Human Health. *Microorganisms*. 2021;9(6):1122. https://doi.org/10.3390/microorganisms9061122

12. Joung H, Chu J, Kwon YJ, Kim KH, Shin CH, Ha JH. Assessment of the Safety and Hepatic Lipid-Lowering Effects of *Lactobacillus Delbrueckii Subsp. Lactis* CKDB001. *Applied Biological Chemistry*. 2024;67:101. https://doi.org/10.1186/s13765-024-00949-9

13. Varada VV, Panneerselvam D, Pushpadass HA, Mallapa RH, Ram C, Kumar S. In Vitro Safety Assessment of Electrohydrodynamically Encapsulated *Lactiplantibacillus Plantarum* CRD7 and *Lacticaseibacillus Rhamnosus* CRD11 for Probiotics Use. *Current Research in Food Science.* 2023;6:100507. https://doi.org/10.1016/j.crfs.2023.100507

14. Song EJ, Lee ES, Kim YI, Shin DU, Eom JE, Shin HS, et al. Gut Microbial Change after Administration of *Lacticaseibacillus Paracasei* AO356 Is Associated with Anti-Obesity in a Mouse Model. *Frontiers in Endocrinology*. 2023;14:1224636. https://doi.org/10.3389/fendo.2023.1224636

15. Jang YJ, Gwon HM, Jeong WS, Yeo SH, Kim SY. Safety Evaluation of *Weissella Cibaria* JW15 by Phenotypic and Genotypic Property Analysis. *Microorganisms*. 2021;9(12):2450. https://doi.org/10.3390/microorganisms9122450

16. Bin Hafeez A, Pełka K, Worobo R, Szweda P. In Silico Safety Assessment of Bacillus Isolated from Polish Bee Pollen and Bee Bread as Novel Probiotic Candidates. *International Journal of Molecular Sciences*. 2024;25(1):666. https://doi.org/10.3390/ijms25010666

17. Yilmaz N. Quantitative Analysis of Biogenic Amine Production of Different Lactic Acid Bacteria Isolated from Ready-to-Eat Packaged Fish Products. *Veterinary Research Forum*. 2024;15:537–543. https://doi.org/10.30466/vrf.2024.2024103.4193

18. Chen T, Zhao Y, Fan Y, Dong Y, Gai Z. Genome Sequence and Evaluation of Safety and Probiotic Potential of *Lacticaseibacillus Paracasei* LC86 and *Lacticaseibacillus Casei* LC89. *Frontiers in Microbiology*. 2025;15:1501502. https://doi.org/10.3389/fmicb.2024.1501502

19. Turna NS, Chung R, McIntyre L. A Review of Biogenic Amines in Fermented Foods: Occurrence and Health Effects. *Heliyon*. 2024;10(2):e24501. https://doi.org/10.1016/j.heliyon.2024.e24501

20. Rajasekaran JJ, Krishnamurthy HK, Bosco J, Jayaraman V, Krishna K, Wang T, et al. Oral Microbiome: A Review of Its Impact on Oral and Systemic Health. *Microorganisms*. 2024;12(9):1797. https://doi.org/10.3390/microorganisms12091797

21. Panpetch W, Phuengmaung P, Cheibchalard T, Somboonna N, Leelahavanichkul A, Tumwasorn S. *Lacticaseibacillus Casei* Strain T21 Attenuates Clostridioides Difficile Infection in a Murine Model through Reduction of Inflammation and Gut Dysbiosis with Decreased Toxin Lethality and Enhanced Mucin Production. *Frontiers in Microbiology*. 2021;12:745299. https://doi.org/10.3389/fmicb.2021.745299

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