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# Antimutagenic Potential of Four Strains of Bacteria of the Genus *Lactobacillus*

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## ABSTRACT

***BACKGROUND:*** Bacteria of the genus *Lactobacillus* possessing a number of positive properties on the human body are a promising source for the creation of functional nutrition. The study of antimutagenic activity of lactobacilli will substantiate the use of these bacteria to prevent the effects of genotoxic environmental factors.

***AIM:*** To comparative analysis the antimutagenic potential of four *Lactobacillus* strains.

***MATERIALS AND METHODS:*** Four bacterial strains *Lactobacillus casei* 3184, *L. casei* MB, *L. plantarum* AB, *L. plantarum* B578 were used in this work. The antimutagenic activity of cells suspension and supernatant of *Lactobacillus* culture was evaluated using Ames test.

***RESULTS:*** The supernatant of *L. plantarum* B578 in the stationary growth phase most effectively suppressed the mutagenic effect of sodium azide (45.6%) and 2-nitrofluorene (43.5%). Substantial antimutagenic activity was also observed for the cell suspension of the strains *L. casei* 3184 and *L. plantarum* AB in the exponential growth phase against sodium azide (40.8% and 39.9%, respectively), and for the supernatant of these strains in the stationary growth phase against 2-nitrofluorene (39.8% and 37.5%, respectively). *L. casei* strain MB did not significantly reduce the effect of known mutagens: the antimutagenic activity of all tested samples for this strain in different growth phases ranged from 15.9% to 23.4% against sodium azide, and from 15.6% to 28.5% against 2-nitrofluorene.

***CONCLUSION:*** Analysis of the results obtained suggests that the antimutagenic effect of *L. casei* 3184 and *L. plantarum* AB strains against sodium azide is due to direct binding of the mutagen by lactobacilli cells, and that of *L. plantarum* B578 strain — by exometabolites accumulating in the tested culture media during the stationary growth phase. Reduction of 2-NF mutagenicity by *L. casei* 3184, *L. plantarum* AB and *L. plantarum* B578 strains can also be associated with direct binding of the mutagen, with inhibition of biotransformation enzymes of this compound, and with the antioxidant effect of exometabolites of lactobacilli strains. The data obtained emphasize the dependence of the antimutagenic potential of lactobacilli on the growth phase and indicate the promising application of the strains *L. plantarum* B578, *L. casei* 3184 and *L. plantarum* AB to reduce the negative effects of genotoxic agents.

**Keywords:** *Lactobacilli*; antimutagenicity; Ames test; sodium azide; 2-nitrofluorene.

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# Антимутагенный потенциал четырех штаммов бактерий рода *Lactobacillus*

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**АННОТАЦИЯ**

**Обоснование.** Бактерии рода *Lactobacillus*, обладающие рядом положительных свойств на организм человека, являются перспективным источником создания компонентов функционального питания. Оценка антимутагенной активности лактобацилл позволит использовать препараты на их основе для предотвращения последствий генетически активных факторов окружающей среды.

**Цель** — сравнительный анализ антимутагенного потенциала четырех штаммов бактерий рода *Lactobacillus*.

**Материалы и методы.** В работе были использованы четыре штамма бактерий *Lactobacillus casei* 3184, *L. casei* МБ, *L. plantarum* АВ, *L. plantarum* В578. Оценку антимутагенной активности суспензии живых клеток и супернатанта культуральной жидкости лактобацилл проводили с использованием теста Эймса.

**Результаты.** Супернатант штамма *L. plantarum* В578 в стационарной фазе роста наиболее эффективно подавлял мутагенное действие азида натрия (45,6%) и 2-нитрофлуорена (43,5%). У штаммов *L. casei* 3184 и *L. plantarum* АВ антимутагенная активность более выражена для суспензии живых клеток в экспоненциальной фазе роста в отношении азида натрия (40,8 и 39,9% соответственно) и для супернатанта в стационарной фазе роста в отношении 2-нитрофлуорена (39,8 и 37,5% соответственно). Штамм *L. casei* МБ не оказывал существенного влияния на эффект известных мутагенов: антимутагенная активность всех исследованных образцов для данного штамма в разные фазы роста варьировала от 15,9 до 23,4% в отношении азида натрия и от 15,6 до 28,5% в отношении 2-нитрофлуорена.

**Заключение.** Анализ полученных результатов позволяет предположить, что антимутагенное действие штаммов *L. casei* 3184 и *L. plantarum* АВ в отношении азида натрия обусловлено прямым связыванием мутагена клетками лактобацилл, а штамма *L. plantarum* В578 — экзометаболитами, накапливающимися в культуральной жидкости в стационарной фазе роста культуры. Снижение мутагенного эффекта 2-нитрофлуорена штаммами *L. casei* 3184, *L. plantarum* АВ и *L. plantarum* В578 также может быть обусловлено прямым связыванием мутагена, ингибированием ферментов биотрансформации данного соединения и антиоксидантным эффектом экзометаболитов штаммов лактобацилл. Полученные данные подчеркивают зависимость антимутагенного потенциала лактобацилл от фазы роста культуры и природы мутагенного фактора и свидетельствуют о перспективности использования штаммов *L. plantarum* В578, *L. casei* 3184 и *L. plantarum* АВ для снижения негативных эффектов генотоксичных агентов.

**Ключевые слова:** лактобациллы; антимутагенность; тест Эймса; азид натрия; 2-нитрофлуорен.

**Как цитировать**

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## BACKGROUND

Xenobiotic pollution of the environment is still one of the most serious challenges today. Mutagens are a special class of xenobiotics that can cause a change in the genetic material of an organism, which can be passed on to offspring. The majority of these mutations are recessive; however, as they accumulate in a population, they tend to become homozygous. Mutations in germ cells cause a variety of genetic disorders in humans [1]. Somatic mutations also play a key role in the development of many pathological changes in the body, including the initiation of carcinogenesis. [2, 3]. Studies on the mutagenic activity of several carcinogenic compounds showed a significant correlation between mutagenicity and carcinogenicity [4, 5]. In recent decades, there has been an increased focus on the search for and study of antimutagens to maintain genome stability and increase resistance to the genotoxic effects of various factors. The majority of the identified antimutagens are natural substances such as secondary plant metabolites, vitamins, or amino acids [6]. Antigenotoxic properties have been demonstrated for several representatives of the gut microbiota [7, 8]. Therefore, natural antimutagens can be used in the development of prophylactic agents that effectively protect against the detrimental effects of genotoxic exposure.

Studies on the antimutagenic activity of lactic acid bacteria, which are part of the natural human microbiota and are commonly used to produce probiotics, are particularly interesting.

*The work aimed* to assess the antimutagenic potential of four *Lactobacillus* strains.

## METHODS

### Bacterial Strains

The study used four bacterial strains from the genus *Lactobacillus*: *L. casei* 3184, *L. casei* MB, *L. plantarum* AB (from the Collection of microorganisms of the Federal Research Center "Fundamentals of Biotechnology" of the Russian Academy of Sciences, Moscow), and *L. plantarum* B578 (from the All-Russian Collection of Microorganisms, Pushchino).

Antimutagenic activity was assessed using *Salmonella typhimurium* auxotrophic strains TA100 and TA98 (from the collection of microorganisms of the Department of Genetics of the M.V. Lomonosov Moscow State University) that are reverted from auxotrophy to prototrophy by base substitution and frame shift mutagens, respectively.

### Sample Preparation for Antimutagenic Activity Assessment

A lactobacillus culture from an MRS agar slant was added to 5 mL of MRS broth. After incubation at 37 °C for 17–20 h, 1 mL of the culture was transferred into 50 mL of MRS broth and cultured for 30 h under the same conditions. Samples were taken after 6 and 30 h of incubation, which corresponds to the exponential and stationary growth phases of lactobacilli cultures. Part of the sample was centrifuged at 3000 rpm for 15 min in an LMC-4200R centrifuge (rotor R-12/15, BioSan, Latvia). The supernatant was filtered through a sterile 0.20 μm membrane filter (Corning, Germany). The supernatant and the initial bacterial suspension in MRS broth were used in the experiments.

Antimutagenic activity was assessed using the Ames test [9]. 0.1 mL of bacterial suspension of the tester strain, 0.1 mL of mutagen solution, and 0.1 mL of the test supernatant sample or lactobacilli suspension were added to the top 0.6% agar, mixed, and poured onto the surface of glucose minimal agar plates. Sterile MRS medium was used as a negative (solvent) control, and solutions of known mutagens were used as a positive control. These included sodium azide ($NaN_3$) 10.5 μg/mL and 2-nitrofluorene (2-NF) 100 μg/ml (Sigma-Aldrich). The plates were placed in a 37 °C incubator for 48–72 h. *S. typhimurium* His+revertants were counted after incubation. Notably, lactobacilli require rich, complex nutrient media for growth. This prevents the proliferation and growth of lactobacilli in the glucose minimal agar used in the Ames test.

The percentage of mutagenesis inhibition – antimutagenic effect (AE) was calculated using the formula [10]:

$$AE = \left[1 - \frac{T - N}{M - N}\right] \times 100,$$

where $T$ is the number of His+ revertants per plate in the presence of mutagen and the test sample; $M$ is the number of His+ revertants per plate in the positive control; $N$ is the number of spontaneous His+ revertants corresponding to the negative control.

The antimutagenic effect was considered strong when the inhibitory effect was more than 40%, and moderate when 25–40%. Inhibitory effect of less than 25% was considered as weak and was not recognised as a positive result [10].

The data obtained are presented as the mean in each group and the standard deviation (±σ). The Student's $t$ test was used to assess the significance of The Student's t test was used to assess the significance between the means of two groups. Statistical differences between data were considered significant at $p < 0.05$.

## RESULTS AND DISCUSSION

The genus *Lactobacillus* comprises more than 200 species of gram-positive, microaerophilic lactic acid bacteria with considerable phylogenetic and metabolic

diversity and high functional activity [11]. Lactobacilli are widespread in nature and constitute a significant part of the normal human microbiota. Many lactobacilli species are part of microbial communities of the oral cavity, stomach, and intestine, with the majority found in the large intestine. Moreover, lactobacilli play a crucial role in maintaining vaginal health [11–13].

Probiotic microorganisms, including *Lactobacillus*, have numerous beneficial effects, with anticarcinogenic activity being one of the most significant and controversial. Notably, there is no direct evidence that lactobacilli or lactic acid products can suppress cancer. However, a large body of published data from experiments with tumor cell cultures and experimental animals indicates that lactobacilli may have a role in carcinogenesis inhibition [14, 15].

DNA damages and mutations in genes that regulate cell growth and division play a key role in initiating the process of carcinogenesis [16]. Therefore, the search for and practical use of effective antimutagens preventively protecting cells from the effects of genotoxic factors is a relevant area of research in cancer prevention.

In this work we studied the antimutagenic potential of four *Lactobacillus* strains against two known mutagens, $NaN_3$ and 2-NF, inducing base pair substitution or frame shift gene mutations, respectively).

The cell suspension and supernatant of the studied lactobacilli strains inhibited the mutagenic effect of $NaN_3$ in both the exponential and stationary phases of bacterial growth. The overall antimutagenic activity ranged from 17.1% to 40.8% for bacterial cell suspension and 11.5% to 45.6% for supernatant. For the *L. casei* 3184 and *L. plantarum* AB strains, the cell suspensions had higher activity than the supernatants, with a more prominent effect during the exponential growth phase in both cases. The *L. plantarum* B578 strain showed a significant antimutagenic effect against $NaN_3$ during the stationary growth phase; moreover, the activity of the supernatant (45.6%) was higher than that of the cell suspension (37.3%). The *L. casei* MB strain showed low antimutagenic activity against sodium azide: 17.1% for cell suspension and 15.9% for supernatant in the exponential growth phase; 19.2% for cell suspension and 23.4% for supernatant in the stationary growth phase (Table 1).

Table 2 shows the results of assessment of the antimutagenic effect of the studied lactobacilli strains against 2-NF. The mean antimutagenic activity for the *L. casei* 3184 and *L. plantarum* AB strains was 30.8%–39.8% and 29.3%–37.5%, respectively. The cell suspension and supernatant of the *L. plantarum* B578 strain showed a significant antimutagenic potential (>40%) against 2-NF in the stationary growth phase. However, the *L. casei* MB strain slightly inhibiteded the mutagenicity of 2-NF, demonstrating relatively weak antimutagenic activity ranging from 15.6% to 28.5% (Table 2).

Notably, supernatants of all four studied strains inhibited the mutagenic effect of 2-NF more effectively than cell suspensions.

Other *Lactobacillus* species have previously been found to be capable of inhibiting the mutagenic effect of $NaN_3$. In the study [17], the supernatant of cells from various growth phases was used to assess the antimutagenic potential of extracellular metabolites of five lactobacilli strains. The supernatants of *L. plantarum* ATCC8014, *L. casei* ATCC11578, and *L. delbrueckii* subsp. *lactis* ATCC4797 exhibited greater antimutagenic activity against $NaN_3$ in the stationary phase than in the exponential phase. The supernatants of two strains, *L. plantarum* WCFS1 and *L. sakei* 23K had a greater effect in the exponential growth phase. Four lactobacillus strains (*L. casei* T2,

**Table 1.** Antimutagenic effect (AE) of four lactobacilli strains against sodium azide ($NaN_3$) in the Ames test (*Salmonella typhimurium* TA100 strain)

| Variants | Cell suspension | | Supernatant | |
|---|---|---|---|---|
| | Number of $His^+$ revertants/plate | AE, % | Number of $His^+$ revertants/plate | AE, % |
| Negative control | 68.2 ± 5.3 | – | 125.4 ± 17.3 | – |
| Positive control ($NaN_3$) | 626.0 ± 36.3 | – | 978.9 ± 57.1 | – |
| $NaN_3$ + *L. casei* 3184 (1) | 398.3 ± 25.5* | 40.8 | 707.0 ± 21.2* | 31.8 |
| $NaN_3$ + *L. casei* 3184 (2) | 412.3 ± 23.5* | 38.3 | 793.2 ± 42.2* | 21.8 |
| $NaN_3$ + *L. casei* MB (1) | 530.2 ± 11.7 | 17.1 | 848.2 ± 42.3 | 15.9 |
| $NaN_3$ + *L. casei* MB (2) | 516.3 ± 15.7 | 19.2 | 784.0 ± 23.3* | 23.4 |
| $NaN_3$ + *L. plantarum* AB (1) | 403.1 ± 29.3* | 39.9 | 693.0 ± 44.0 | 33.9 |
| $NaN_3$ + *L. plantarum* AB (2) | 414.2 ± 11.2 | 37.9 | 887.4 ± 37.3 | 11.5 |
| $NaN_3$ + *L. plantarum* B578 (1) | 520.3 ± 25.4* | 18.9 | 733.8 ± 22.9 | 29.2 |
| $NaN_3$ + *L. plantarum* B578 (2) | 417.7 ± 18.4* | 37.3 | 593.2 ± 17.1* | 45.6 |

*Note*. *Values are statistically significantly different from the positive control, $p < 0.05$; 1 – exponential growth phase, 2 – stationary growth phase.

**Table 2.** Antimutagenic effect (AE) of four lactobacilli strains against 2-nitrofluorene (2-NF) in the Ames test (*Salmonella typhimurium* TA98 strain)

| Variants | Cell suspension | | Supernatant | |
|---|---|---|---|---|
| | Number of $His^+$ revertants/plate | AE, % | Number of $His^+$ revertants/plate | AE, % |
| Negative control | 35.0 ± 1.2 | – | 75.7 ± 5.2 | – |
| Positive control (2-NF) | 280.8 ± 11.7 | – | 567.3 ± 37.3 | – |
| 2-NF + *L. casei* 3184 (1) | 205.2 ± 15.3* | 30.8 | 382.0 ± 21.7* | 37.6 |
| 2-NF + *L. casei* 3184 (2) | 19.8 ± 10.7* | 35.4 | 371.8 ± 22.2* | 39.8 |
| 2-NF + *L. casei* MB (1) | 240.3 ± 12.5* | 16.3 | 488.6 ± 29.7* | 15.6 |
| 2-NF + *L. casei* MB (2) | 225.2 ± 10.4* | 22.6 | 427.1 ± 23.5* | 28.5 |
| 2-NF + *L. plantarum* AB (1) | 208.8 ± 19.2* | 29.3 | 398.9 ± 19.8* | 34.3 |
| 2-NF + *L. plantarum* AB (2) | 195.2 ± 15.3* | 34.8 | 382.6 ± 27.3* | 37.5 |
| 2-NF + *L plantarum* B578 (1) | 219.3 ± 11.5* | 25.0 | 372.0 ± 12.9 | 39.7 |
| 2-NF + *L. plantarum* B578 (2) | 179.2 ± 10.5* | 41.3 | 353.0 ± 15.2* | 43.5 |

*Note*. *Values are statistically significantly different from the positive control, $p < 0.05$; 1 – exponential growth phase, 2 – stationary growth phase.

*L. casei* T4, *L. plantarum* T5, and *L. brevis* T9) out of 25 isolated from tarhana, a traditional Iranian fermented product, exhibited antimutagenic activity against sodium azide, with a higher effect for the supernatants than for the cell suspensions [18]. Desmutagenic activity was observed in the Ames test with $NaN_3$ for two strains, *L. reuteri* DDL 19 and *L. alimentarius* DDL 48, isolated from feces of healthy goats [19].

Ahmad et al. studied the antimutagenic potential of *L. plantarum* isolate obtained from fermented durian [20]. Notably, only a suspension of living lactobacilli cells significantly reduced the mutagenic effect of both $NaN_3$ and 2-NF. A suspension of *L. paracasei* subsp. *tolerans* JG22 cells isolated from hot pepper leaves showed a desmutagenic effect against 2-NF [21]. Mohabati et al. [22] reported that a cell suspensions of *L. acidophilus* and *L. bulgaricus* isolated from Iranian yogurt was more effective in inhibiting the mutagenic effect of 2-NF than supernatant or inactivated cell suspension.

According to published data, the main mechanisms of antimutagenic activity of lactobacilli include: 1) mutagen binding (desmutagenic effect); 2) transformation of mutagen into a non-genotoxic compound; 3) inhibition of promutagen biotransformation into mutagen; 4) antioxidant effect; 5) DNA repair stimulation [7, 8].

A comparative analysis of our findings and published data suggests the antimutagenic effect of the *L. casei* 3184 and *L. plantarum* AB strains against sodium azide is largely determined by direct binding of mutagen by actively multiplying lactobacilli cells. However, the *L. plantarum* B578 strain's ability to inhibit the mutagenic effect of $NaN_3$ is primarily determined by the strain's exometabolites produced during the stationary growth phase. Mutagenic activity of 2-NF was inhibited by both cell suspensions and supernatants of the strains *L. casei* 3184, *L. plantarum* AB, and *L. plantarum* B578; the effect was higher in the stationary growth phase.

It is known that the nitroreductases of intestinal bacteria play an important role in the reduction of various nitroaromatic compounds to N-nitroso compounds, hydroxylamines, or aromatic amines, most of which are carcinogenic and mutagenic agents [23]. Nitroreductases of *Salmonella* have broad substrate specificity, reducing 2-NF, 1-nitrocyclohexene, aliphatic nitroalkenes, and nitrobenzene, with a substrate conversion efficiency of more than 95% [24]. As a result, during the biotransformation of 2-NF by bacterial nitroreductases, in this case produced by the *S. typhimurium* TA98 strain, the formation of genotoxic metabolites, reactive oxygen species capable of inducing primarily oxidative damage to DNA and gene mutations in the test strain cells, is possible [25]. Inhibiting nitroreductase activity in both intestinal cells and gut microbiota is considered a promising technique for reducing levels of mutagenic and carcinogenic metabolites (for example, in the colon) [23]. Notably, an alkaline environment is optimal for the activity of nitroreductases; therefore, the organic acids produced by lactobacilli can actually inhibit the activity of these enzymes [26].

Using gas chromatography-mass spectrometry, significant amounts of phenyllactic (mandelic) and para-hydroxyphenyllactic acids [27], known to be potent antioxidants [28], were detected among the exometabolites of lactobacilli and bifidobacteria in addition to lactic acid.

Therefore, the antimutagenic effect of the studied lactobacilli strains against 2-NF could be attributed to both direct mutagen binding and inhibition of biotransformation enzymes for this compound, as well as the antioxidant effect of *L. casei* 3184, *L. plantarum* AB, and *L. plantarum* B578 exometabolites.

## CONCLUSION

Lactobacilli are a promising material for developing probiotics and functional nutrition products due to a variety of beneficial effects on the human body. The data obtained in this work indicate that three of the four studied *Lactobacillus strains have antimutagenic properties.* The antimutagenic effect of both the cell suspensions and the supernatants of the *L. plantarum* B578, *L. plantarum* AB, and *L. casei* 3184 strains depends on the culture growth phase. Supernatants of the *L. plantarum* B578, *L. plantarum* AB, and *L. casei* 3184 strains significantly inhibited the mutagenic effect of 2-NF in the stationary growth phase (antimutagenic effect was 43.5%, 37.5%, and 39.8%, respectively). The cell suspensions of the *L. casei* 3184 and *L. plantarum* AB strains in the exponential growth phase, as well as the supernatant of the *L. plantarum* B578 strain in the stationary growth phase, showed the highest antimutagenic activity against sodium azide (40.8%, 39.9%, and 45.6%, respectively). The antimutagenic effect of the studied lactobacilli strains may be attributed to direct mutagen binding, the impact on its biotransformation, as well as the activity of secreted metabolites.

## ADDITIONAL INFO

**Author contribution:** N.S. Karamova: preparation of samples, evaluation of of antimutagenic activity, analysis and discussion of results, literature review, writing the main part of the text; O.N. Ilinskaya: concept and design of the study, discussion of results, literature review, making final edits, funding acquisition. The authors have approved the version for publication and have also agreed to be responsible for all aspects of the work, ensuring that the accuracy and integrity of any part of it is properly considered and addressed.

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**Statement of originality:** The authors did not use previously published information (text, illustrations, data) to create this paper.

**Data availability statement:** All data obtained in the present study are available in the article.

**Generative AI:** Generative AI technologies were not used for this article creation.

**Provenance and peer-review:** This work was submitted to the journal on its own initiative and reviewed according to the standard procedure. Two external reviewers, and a member of the editorial board participated in the review.

## ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

**Вклад авторов.** Н.С. Карамова — подготовка образцов, исследование антимутагенной активности, анализ и обсуждение результатов, обзор литературы, написание текста; О.Н. Ильинская — концепция и дизайн исследования, обсуждение результатов, обзор литературы, редактирование текста, привлечение финансирования. Авторы одобрили версию для публикации, а также согласились нести ответственность за все аспекты работы, гарантируя надлежащее рассмотрение и решение вопросов, связанных с точностью и добросовестностью любой ее части.

**Источники финансирования.** Исследование выполнено при поддержке Российского научного фонда (грант № 24-14-00059).

**Раскрытие интересов.** Авторы заявляют об отсутствии отношений, деятельности и интересов за последние три года, связанных с третьими лицами (коммерческими и некоммерческими), интересы которых могут быть затронуты содержанием статьи.

**Оригинальность.** При создании настоящей работы авторы не использовали ранее опубликованные сведения (текст, иллюстрации, данные).

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**Генеративный искусственный интеллект.** При создании настоящей статьи технологии генеративного искусственного интеллекта не использовали.

**Рассмотрение и рецензирование.** Настоящая работа подана в журнал в инициативном порядке и рассмотрена по обычной процедуре. В рецензировании участвовали два внешних рецензента и член редакционной коллегии.

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