UDC 579.6:579.864.1

A COMPARATIVE STUDY OF PROBIOTIC POTENTIAL OF TWO LACTIC ACID BACTERIA ISOLATES OBTAINED FROM CEREAL-BASED PRODUCTS

***Denys Holubchyk*[1,2]**, *2nd Year Master's Student, Specialist of the Department of Biotechnology*
https://orcid.org/0000-0002-5129-6150
***Olexii Dugan*[1]**, *D-r of Sc., Biological, Professor of Department of Industrial Biotechnology and Biopharmacy*
https://orcid.org/0000-0002-5646-917X
***Svitlana Danylenko*[2]**, *D-r of Sc., Engineering, Head of the Department of Biotechnology*
https://orcid.org/0000-0003-4470-4643
***Olena Yalovenko*[1]**, *PhD, Biological, Associate Professor of Department of Industrial Biotechnology and Biopharmacy*
https://orcid.org/0000-0002-5022-143X
***Oksana Potemska*[2]**, *PhD, Technical, Senior Researcher*
https://orcid.org/0000-0003-4725-5847
***Mykola Mykhailenko*[2]**, *Postgraduent, Department of Biotechnology*
https://orcid.org/0009-0008-4678-5858
[1]National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", Kyiv, Ukraine
[2]Institute of Food Resources of the NAAS, Kyiv, Ukraine

https://doi.org/10.31073/foodresources2025-24-12

Subject. *Over the past few decades, interest in probiotics and functional foods has significantly increased across many countries worldwide, accompanied by growing attention to healthy lifestyles. This trend indicates a need for new probiotic strains, especially those derived from underexplored plant sources, as they may prove to be ideal candidates for use in non-dairy functional products.* ***Purpose.*** *To assess and compare the probiotic potential of two lactic acid bacteria isolates obtained from barley and oat processing products.* ***Methods.*** *The purity of the cultures was determined using standard microbiological techniques. Species identification of the isolates was performed based on a combination of morphological, physiological-biochemical, and cultural characteristics. Stress tolerance was assessed by evaluating the survival of the studied microorganisms after exposure to artificial saliva (0.5 h), physiological saline with pH 2.5 (1 h), and a mixture of bile and artificial duodenal juice (2 h). Antibiotic susceptibility was determined using the disk diffusion method.* ***Results****. It was established that the isolate obtained from the barley-based product belongs to the species Lacticaseibacillus casei, while the one from the oat-based product belongs to the species Companilactobacillus nantensis. Their survival rates under simulated conditions of saliva, stomach, and small intestine was 97.12 ± 1.81%, 96.73 ± 0.35%, and 85.46 ± 2.45% for L. casei, and 98.30 ± 0.42%, 97.91 ± 1.39%, and 84.14 ± 1.58% for C. nantensis, respectively – all of which are considered high. Both cultures were sensitive to a range of antibiotics but resistant to kanamycin and streptomycin, indicating the need for further genetic investigation.* ***Scope of results.*** *The findings indicate a high probiotic potential of lactic acid bacteria isolated from lesser-studied plant sources – specifically, barley and oat processing products – and suggest the possibility of further research and application of L. casei and C. nantensis isolates in the food or medical industries.*

Key words: *lactic acid bacteria, Lacticaseibacillus casei, Companilactobacillus nantensis, stress resistance, antibiotic susceptibility*

ПОРІВНЯЛЬНЕ ДОСЛІДЖЕННЯ ПРОБІОТИЧНОГО ПОТЕНЦІАЛУ ДВОХ ІЗОЛЯТІВ МОЛОЧНОКИСЛИХ БАКТЕРІЙ, ОТРИМАНИХ ІЗ ПРОДУКТІВ ПЕРЕРОБКИ ЗЛАКІВ

***Голубчик Д. С.*[1,2]**, *здобувач 2 курсу магістратури, фахівець відділу біотехнології*
https://orcid.org/0000-0002-5129-6150
***Дуган О. М.*[1]**, *д.б.н., проф. кафедри промислової біотехнології та біофармації*
https://orcid.org/0000-0002-5646-917X
***Даниленко С. Г.*[2]**, *д.т.н., завідувачка відділу біотехнології,*
https://orcid.org/0000-0003-4470-4643
***Яловенко О. І.*[1]**, *к.б.н., доц. кафедри промислової біотехнології та біофармації*
https://orcid.org/0000-0002-5022-143X
***Потемська О. І.*[2]**, *к.т.н., старша дослідниця*
https://orcid.org/0000-0003-4725-5847
***Михайленко М. М.*[2]**, *аспірант*
https://orcid.org/0009-0008-4678-5858

[1]Національний технічний університет України
«Київський політехнічний інститут імені Ігоря Сікорського», Київ, Україна
[2]Інститут продовольчих ресурсів НААН, Київ, Україна

Предмет. *Протягом кількох останніх десятиліть серед багатьох країн світу суттєво зросла зацікавленість людей у пробіотиках і функціональних продуктах, а також спостерігається посилення уваги до здорового способу життя. Це означає потребу в нових пробіотичних штамах, особливо отриманих із малодосліджених рослинних джерел, адже вони можуть виявитися ідеальними кандидатами для використання у немолочних функціональних продуктах.* ***Мета.*** *Оцінити та порівняти пробіотичний потенціал двох ізолятів молочнокислих бактерій, отриманих із продуктів переробки ячменю та вівса.* ***Методи.*** *Встановлення чистоти культур здійснювалося стандартними мікробіологічними методами. Видова приналежність ізолятів визначалася за сукупністю морфологічних, фізіолого-біохімічних та культуральних характеристик. Стресостійкість визначалася шляхом оцінки виживаності досліджуваних мікроорганізмів після проходження через штучну слину (0,5 год), фізіологічний розчин з pH 2,5 (1 год) та суміш жовчі і штучного соку дванадцятипалої кишки (2 год). Чутливість до антибіотиків визначалася диско-дифузійним методом.* ***Результати.*** *Було встановлено, що ізолят, отриманий із продукту переробки ячменю, належить до виду Lacticaseibacillus casei, а із продукту переробки вівса – до виду Companilactobacillus nantensis. Їх виживаність у змодельованих умовах слини, шлунка та тонкого кишечника складає 97,12 ± 1,81%, 96,73 ± 0,35% і 85,46 ± 2,45% для L. casei та 98,30 ± 0,42%, 97,91 ± 1,39% і 84,14 ± 1,58% для C. nantensis відповідно, що є високими показниками. Обидві культури чутливі до ряду антибіотиків, але стійкі до канаміцину та стрептоміцину, що означає необхідність їх генетичного дослідження.* ***Сфера застосування результатів.*** *Результати свідчать про високий пробіотичний потенціал молочнокислих бактерій із менш досліджених рослинних джерел – а саме з продуктів переробки ячменю та вівса – та про можливість подальшого дослідження і застосування ізолятів L. casei і C. nantensis у харчовій або медичній промисловості.*

Ключові слова: *молочнокислі бактерії, Lacticaseibacillus casei, Companilactobacillus nantensis, стресостійкість, чутливість до антибіотиків*

Problem Statement. In recent decades, there has been a growing popularity of probiotics in many countries around the world, along with increased public awareness of the beneficial effects that probiotic microorganisms can exert on the body [1]. This trend is driven both by the development of the functional food industry [2], and the spread of a conscious approach to health, especially among younger generations [3]. For example, between January 1, 2007, and December 31, 2022, search queries for the keywords "gut probiotic," "gut microbiome," and "dysbiosis" increased by 829%, 1400%, and 267%, respectively, with Ukraine also playing a certain role in this process. In particular, compared to the other aforementioned terms, the concept of "dysbiosis" stands out in Ukraine due to its high level of search activity [4], which may indicate a significant demand for probiotics and functional foods.

The World Health Organization provides the following definition of probiotics: "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [5]. A comprehensive list of the beneficial properties of probiotics has not yet been fully established; however, it is known that they exert a complex influence on the body's physiological systems. The formation of an optimal gut microbiota contributes to the reduction of blood plasma glucose levels, normalization of body weight, alleviation of nervous disorders, increased bone strength, and accelerated wound healing [6], as well as the modulation of inflammatory responses and immune reactions – resulting in enhanced effectiveness in combating pathogens of not only bacterial but also viral origin [7].

The concept of "probiotics" is not limited to a single genus of microorganisms; this category includes bacteria from various genera, as well as yeasts and even molds. However, the most common group is lactic acid bacteria (LAB) [8]. New bacterial strains with potential probiotic properties can be isolated from various sources: the human intestine, saliva, breast milk, animals, food (with dairy and fermented products being particularly promising), and the environment [9]. Plant-based raw materials and their derivatives (such as flour, fermented grains, and sourdoughs) are also of significant interest [10–12], as LAB isolated from these sources are more easily adapted to non-dairy functional products, which is important for individuals with

lactose intolerance [13]. The majority of potential probiotics are isolated from processed wheat, rye, and spelt products, whereas other cereals, including barley and especially oats, have been studied significantly less [12]. This highlights the relevance of working with these sources and the necessity of investigating the probiotic potential of the isolated LAB, focusing particularly on key selection criteria for probiotic strains – namely, their ability to survive simulated gastrointestinal tract (GIT) conditions [14] and their sensitivity to critically important antimicrobials [15].

The purpose of the study is to assess and compare the probiotic potential of two lactic acid bacteria isolates obtained from barley and oat processing products.

Materials and methods. The isolation of LAB from processed barley and oat products was carried out according to [16]. Specifically, barley and oat flours were mixed with bottled water in equal proportions (100 g : 100 g), covered, and left to sit at room temperature for 24 hours. This was followed by standard microbiological procedures to obtain pure cultures.

The isolates were confirmed as lactic acid bacteria using Gram staining, catalase testing, and gelatin liquefaction ability. To determine the species of the studied microorganisms, a series of tests were conducted to assess their physiological and biochemical characteristics, including growth at different temperatures, the ability to ferment various carbohydrates, arginine hydrolysis, and gas production from glucose (using a Durham tube inserted into the medium). The obtained results were entered into the ABIS online laboratory service [17], designed for the identification of various bacteria, and the final species identification of the studied cultures was determined based on its conclusions.

The determination of LAB cultures' stress resistance was carried out according to the method described by Kim H. et al. [14] with modifications. The studied microorganisms were pre-cultivated in tubes containing 10 mL of MRS broth at 37ºC for 24 hours and then suspended in 100 mL of artificial saliva (0,0561 g KH_2PO_4, 0,0563 g Na_2HPO_4, 0,2478 g $KHCO_3$, 0,0964 g NaCl, 0,0051 g $MgCl_2$, 0,0275 g $CaCl_2$, 0,0048 g citric acid, 100 mL of distilled water) [18].

The bacterial suspensions in artificial saliva were incubated at 37ºC for 30 minutes, after which 10 mL of each LAB suspension was aseptically transferred into 100 mL of physiological saline with a pH of 2.5 (0,85 g NaCl, 1 mL HCl (14%), 100 mL of distilled water) [14], simulating the acidic environment of the stomach. Incubation was carried out at 37ºC for 1 hour.

At the next stage, 10 mL of each microorganism suspension was transferred into a model solution of bile and synthetic duodenal juice, which was prepared by mixing 4 mL of bile solution (10 g of dried purified bile, 100 mL of distilled water) with 17 mL of synthetic duodenal juice (dissolve in 1 L of distilled water: 6,4 g $NaHCO_3$, 0,239 g KCl, 1,28 g NaCl – and adjust the pH to 6.0) [14]. The test samples were incubated at 37ºC for 2 hours. At each stage, samples were collected for plating on Petri dishes and subsequent cell counting.

Survival rate was calculated after each simulated stage of passage through the GIT according to the following formula:

$$SR = \frac{LgN_t}{LgN_{t0}} \cdot 100\%,$$

where N_{t0} – CFU/mL at the beginning of the passage through the simulated human GIT, N_t – CFU/mL at the end of a specific stage.

The sensitivity of the microorganisms to antibiotics was determined using the disk diffusion method according to [15], and the results were interpreted according to [19]. The study considered a selection of key antimicrobial agents to which probiotics are expected to be sensitive, following the recommendations of the European Food Safety Authority (EFSA) [20].

All experiments were conducted in triplicate. Statistical analysis was performed using Student's t-test at a significance level of $p < 0.05$. Letter indices (a, b, c) indicate statistically significant differences between groups.

Results and discussion. Both cultures formed round colonies with smooth edges on agar, with diameters up to 2 mm. The colony surfaces were smooth and glossy, with a convex shape and a soft consistency. The color was translucent white for the isolate from barley processing products and opaque creamy for the isolate from oat processing products.

Thermo-fixed smears were prepared from the agar colonies and stained with crystal violet. The cells of each strain appeared as straight or slightly curved rods of varying sizes, occurring singly, in pairs, or as short chains (Fig. 1). They were Gram-positive, non-motile, non-spore-forming, and lacked flagella and capsules. The cells were small in both length and width, which is characteristic of a heterofermentative type of metabolism (either obligate or facultative) [21].

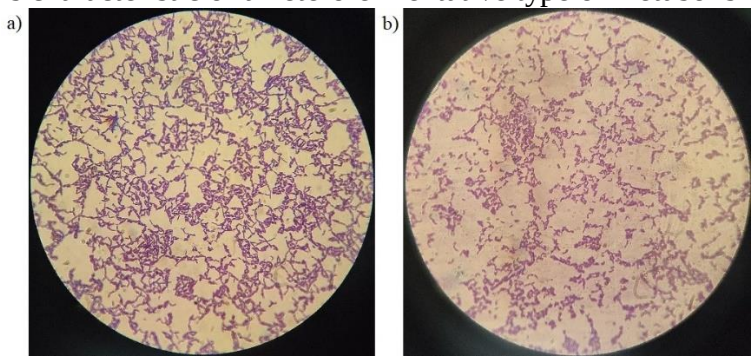

Fig. 1. Microscopic images of the isolates (×1000):
a) from barley processing products;
b) from oat processing products

The phenotypic characteristics of the studied microorganisms (Table 1) indicate that they belong to the group of facultatively heterofermentative LAB, confirming previous observations. A notable feature of their metabolism is the ability to ferment various hexoses as well as ribose. Additionally, unlike obligately heterofermentative LAB, they are unable to produce carbon dioxide in MRS medium with glucose [22].

Table 1

Physiological and biochemical characteristics of the studied LAB

Characteristic		Isolate from barley processing products	Isolate from oat processing products
Growth at different temperatures	15ºC	+	+
	45ºC	-	-
Carbohydrate	Glucose	+	+
	Gluconate	+	-
	Lactose	+	+
	Sucrose	+	+
	Fructose	+	+
	Maltose	+	+
	Mannose	+	+
	Mannitol	+	+
	Melibiose	-	+
	Glycerin	-	-
	Sorbitol	+	+
	Inositol	-	-
	Raffinose	-	w
	Rhamnose	-	w
	Galactose	+	+
	Ribose	+	+
	Trehalose	+	+
	Xylose	-	-
Gelatin hydrolysis		-	-
Arginine hydrolysis		-	-
Gas production		-	-

+ – positive result; w – weakly positive result; - – negative result

The phenotypic characteristics entered into the ABIS online service indicate that *Lacticaseibacillus casei* was obtained from barley processing products (similarity: 91.5%, probability: 92.5%), and *Companilactobacillus nantensis* from oat processing products (similarity: 94.5%, probability: 78.1%).

When passing through simulated GIT conditions (Fig. 2), the survival rate of *L. casei* was 97.12 ± 1.81% after exposure to artificial saliva, 96.73 ± 0.35% after low pH, and 85.46 ± 2.45% after exposure to bile and synthetic duodenal juice, while the survival rate of *C. nantensis* was 98.30 ± 0.42%, 97.91 ± 1.39%, and 84.14 ± 1.58%, respectively. No statistically significant differences were observed between the results for the two cultures at any stage, which may be attributed to the similar characteristics of the environments from which they were isolated – each characterized by low pH and high microbial competition.

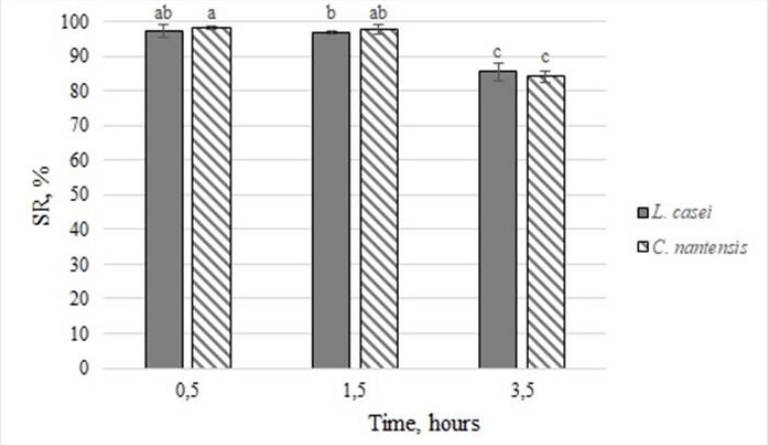

Fig. 2. Survival rate of the isolates under simulated GIT conditions (values with different letter indices indicate statistically significant differences ($p < 0.05$, Student's t-test) between the corresponding groups

The survival rates of both cultures are relatively high, although not the highest reported. For example, data from S. Jung et al. [23] indicate that during passage through simulated models of the oral cavity, stomach, and small intestine, the survival rates of *L. casei* HY2782 were 99.7%, 76.1%, and 48.3%, respectively, and those of *L. casei* ATCC393 were 86.6%, 71.8%, and 29.4%, which are significantly lower than those observed in the cultures studied here. In contrast, the survival rate of *L. casei* DN-114 001 after 2 hours of incubation in simulated gastric fluid (pH 2.0) and 3 hours in simulated intestinal fluid was 92.47% [24], which is a significantly higher value.

Studies investigating the survival of *C. nantensis* under simulated GIT conditions could not be found; however, it is known that probiotic microorganisms used in commercially available preparations retain viability ranging from over 45% to over 91% of cells per capsule (2 min under simulated conditions of saliva (pH 6.9), 80 min – conditions of stomach (pH was gradually decreased from 5.5 to 2.0 by adding a pepsin solution in 0.1 M HCl), 20 min – conditions of duodenum (pH was increased to 5.0 by using a solution of pancreatin and bovine bile salts in 0.1 M $NaHCO_3$), 90 min – conditions of ileum (pH was increased to 6.5 by using a 0.1 M $NaHCO_3$ solution)) [25]. Based on this information, it can be concluded that the obtained cultures meet the survival requirements for probiotics passing through the human GIT very well.

The results of antibiotic susceptibility testing, interpreted according to [19], are presented in Table 2. They demonstrate the variability in the response of the studied cultures to different antimicrobial agents.

Despite differences in numerical values – sometimes considerable – *L. casei* and *C. nantensis* were sensitive to chloramphenicol, erythromycin, and tetracycline, that is, to exactly half of the tested antibiotics. In addition, *C. nantensis* was sensitive and *L. casei* moderately sensitive to ampicillin, which is an entirely acceptable result. On the other hand, both microorganisms were resistant to streptomycin and kanamycin, which, according to EFSA recommendations [20], indicates that they should not be used as probiotics. The study by B. de Souza et al. [26] shows that resistance to kanamycin is characteristic of the majority of *L. casei* strains, and this, among other factors, is related to the widespread aminoglycoside resistance among LAB. However, phenotypic resistance to kanamycin and streptomycin in both studied cultures does not necessarily indicate the presence of resistance genes in the genomes of the microorganisms [27] or their localization on mobile genetic elements [28] – it merely highlights

the need for further genetic research on these characteristics in *L. casei* and *C. nantensis*, which would allow a definitive conclusion to be drawn regarding their compliance or non-compliance with established probiotic requirements.

Table 2

Antibiotic susceptibility determination

Antibiotic (disk content)	Inhibition zone diameter, mm		Susceptibility	
	L. casei	*C. nantensis*	*L. casei*	*C. nantensis*
Ampicillin (10 µg)	13,67 ± 1,53	26,67 ± 1,15	I	S
Chloramphenicol (30 µg)	26,67 ± 0,58	32,33 ± 1,53	S	S
Erythromycin (15 µg)	33,00 ± 1,00	29,33 ± 1,15	S	S
Kanamycin (30 µg)	0,00 ± 0,00	0,00 ± 0,00	R	R
Streptomycin (10 µg)	8,33 ± 0,58	9,67 ± 0,58	R	R
Tetracycline (30 µg)	27,00 ± 1,00	19,00 ± 1,00	S	S

Resistant – R, intermediate – I, susceptible – S

Conclusions. It was established that *L. casei* and *C. nantensis* were isolated from barley and oat processing products, respectively. The survival rate of *L. casei* was 97.12 ± 1.81% after simulated saliva, 96.73 ± 0.35% after low pH, and 85.46 ± 2.45% after bile and synthetic duodenal juice (representing the overall survival rate following all simulated gastrointestinal conditions), while the survival rate of *C. nantensis* was 98.30 ± 0.42%, 97.91 ± 1.39%, and 84.14 ± 1.58% after the corresponding stages. Both microorganisms were sensitive to chloramphenicol, erythromycin, and tetracycline; *C. nantensis* was sensitive and *L. casei* moderately sensitive to ampicillin. The studied cultures also exhibited resistance to kanamycin and streptomycin; therefore, genetic investigation is required before a final conclusion can be made regarding their suitability for human consumption. However, the current results indicate a high probiotic potential of LAB isolated from lesser-studied plant sources – specifically, barley and oat processing products – and suggest the possibility of further research and application of *L. casei* and *C. nantensis* isolates in the food or medical industries.

References

1. Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., Fakiri, E. M. (2013). Health Benefits of Probiotics: A Review. ISRN Nutrition, 2013, 1–7. https://doi.org/10.5402/2013/481651.

2. Rashidinejad, A. (2024). The road ahead for functional foods: Promising opportunities amidst industry challenges. Future Postharvest and Food. https://doi.org/10.1002/fpf2.12022.

3. Firya Fadhila Fathin, Adila Sosianika, Fatya Alty Amalia & Rafiati Kania. (2023). The The Role of Health Consciousness and Trust on Gen Y and Gen Z Intention to Purchase Functional Beverages. Journal of Consumer Sciences,8 (3), 360–378. https://doi.org/10.29244/jcs.8.3.360-378.

4. Pezzino, S., Sofia, M., Mazzone, C., Litrico, G., Agosta, M., La Greca, G., Latteri, S. (2024). Exploring public interest in gut microbiome dysbiosis, NAFLD, and probiotics using Google Trends. Scientific Reports, 14 (1). https://doi.org/10.1038/s41598-023-50190-5.

5. Probiotics in Food: Health And Nutritional Properties And Guidelines for Evaluation. (2006). FAO and WHO.

6. Gul, S., Durante-Mangoni, E. (2024). Unraveling the Puzzle: Health Benefits of Probiotics – A Comprehensive Review. Journal of Clinical Medicine, 13 (5), 1436. https://doi.org/10.3390/jcm13051436.

7. Das, T. K., Pradhan, S., Chakrabarti, S., Mondal, K. C., Ghosh, K. (2022). Current Status of Probiotic and related Health Benefits. Applied Food Research, 100185. https://doi.org/10.1016/j.afres.2022.100185.

8. Ranjha, M. M. A. N., Shafique, B., Batool, M., Kowalczewski, P. Ł., Shehzad, Q., Usman, M., Manzoor, M. F., Zahra, S. M., Yaqub, S., & Aadil, R. M. (2021). Nutritional and Health Potential of Probiotics: A Review. Applied Sciences, 11 (23), 11204. https://doi.org/10.3390/app112311204.

9. Phumkhachorn, P., & Rattanachaikunsopon, P. (2023). Probiotics: Sources, selection and health benefits. Research Journal of Biotechnology, 18(5), 102–113. https://doi.org/10.25303/1805rjbt1020113.

10. Sharma, K., Attri, S., & Goel, G. (2018). Selection and Evaluation of Probiotic and Functional Characteristics of Autochthonous Lactic Acid Bacteria Isolated from Fermented Wheat Flour Dough Babroo. Probiotics and Antimicrobial Proteins, 11 (3), 774–784. https://doi.org/10.1007/s12602-018-9466-z.

11. Priscilla, A., Sellamuthu, P. S., Kulkarni, S. A. (2018). Isolation and characterization of potential probiotics from fermented ragi (Eleusine coracana). International Journal of Pharmacy and Pharmaceutical Sciences, 10 (6), 145. https://doi.org/10.22159/ijpps.2018v10i6.25565.

12. Holubchyk, D., Khablenko, A., Dugan, O., Danylenko, S., & Korzhenivska, A. (2024). Probiotic microorganisms in bread sourdoughs. Food Science and Technology, 18 (1). https://doi.org/10.15673/fst.v18i1.2848.

13. Sornplang, P., Piyadeatsoontorn, S. (2016). Probiotic isolates from unconventional sources: a review. Journal of Animal Science and Technology, 58(1). https://doi.org/10.1186/s40781-016-0108-2.

14. Kim, H., Rwubuzizi, R., Fugaban, J. I. I., Holzapfel, W. H., Todorov, S. D. (2023). Beneficial Properties and Evaluation of Survival in Model Systems of LAB Isolated from Oral Cavity. Acta Microbiologica Bulgarica, 39(1), 36–50. https://doi.org/10.59393/amb23390106.

15. Tan, Q., Xu, H., Aguilar, Z. P., Peng, S., Dong, S., Wang, B., Li, P., Chen, T., Xu, F., Wei, H. (2013). Safety Assessment and Probiotic Evaluation of Enterococcus Faecium YF5 Isolated from Sourdough. Journal of Food Science, 78(4), M587–M593. https://doi.org/10.1111/1750-3841.12079.

16. McKenney, E. A., Nichols, L. M., Alvarado, S., Hardy, S., Kemp, K., Polmanteer, R., Shoemaker, A., & Dunn, R. R. (2023). Sourdough starters exhibit similar succession patterns but develop flour-specific climax communities. PeerJ, 11, Article e16163. https://doi.org/10.7717/peerj.16163.

17. ABIS online – Lactobacillus input. Regnum Prokaryotae. https://www.tgw1916.net/bacteria_Lactobacillus_input.php.

18. Pytko-Polonczyk, J. J., Jakubik, A., Przeklasa-Bierowiec, A., & Muszynska, B. (2017). Artificial saliva and its use in biological experiments. J Physiol Pharmacol, 68(6), 807–813.

19. Charteris, W. P., Kelly, P. M., Morelli, L., Collins, J. K. (1998). Antibiotic Susceptibility of Potentially Probiotic Lactobacillus Species. Journal of Food Protection, 61(12), 1636–1643. https://doi.org/10.4315/0362-028x-61.12.1636.

20. Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. d. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., Villa, R. E., Wallace, R. J., Wester, P., Glandorf, B., Herman, L., Kärenlampi, S., Aguilera, J., Anguita, M., Brozzi, R., & Galobart, J. (2018). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal, 16 (3). https://doi.org/10.2903/j.efsa.2018.5206.

21. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., & Stackebrandt, E. (Eds.). (2006). The Prokaryotes: Vol. 4. Bacteria: Firmicutes, Cyanobacteria (3rd ed.). Springer New York, NY. https://doi.org/10.1007/0-387-30744-3.

22. Wood, B. J. B., Holzapfel, W. H. (Eds.). (2012). The Lactic Acid Bacteria: Vol. 2. The Genera of Lactic Acid Bacteria. Springer.

23. Jung, S. H., Hong, D. K., Bang, S.-J., Heo, K., Sim, J.-J., & Lee, J.-L. (2021). The Functional Properties of Lactobacillus casei HY2782 Are Affected by the Fermentation Time. Applied Sciences, 11 (6), 2481. https://doi.org/10.3390/app11062481.

24. Millette, M., Nguyen, A., Amine, K. M., & Lacroix, M. (2013). Gastrointestinal survival of bacteria in commercial probiotic products. International Journal of Probiotics and Prebiotics, 8(4), 149–156.

25. Naissinger da Silva, M., Tagliapietra, B. L., Flores, V. d. A., Pereira dos Santos Richards, N. S. (2021). In vitro test to evaluate survival in the gastrointestinal tract of commercial probiotics. Current Research in Food Science, 4, 320–325. https://doi.org/10.1016/j.crfs.2021.04.006.

26. De Souza, B. M. S., Borgonovi, T. F., Casarotti, S. N., Todorov, S. D., Penna, A. L. B. (2018). Lactobacillus casei and Lactobacillus fermentum Strains Isolated from Mozzarella Cheese: Probiotic Potential, Safety, Acidifying Kinetic Parameters and Viability under Gastrointestinal Tract Conditions. Probiotics and Antimicrobial Proteins, 11 (2), 382–396. https://doi.org/10.1007/s12602-018-9406-y.

27. Rozman, V., Mohar Lorbeg, P., Accetto, T., Bogovič Matijašić, B. (2020). Characterization of antimicrobial resistance in lactobacilli and bifidobacteria used as probiotics or starter cultures based on integration of phenotypic and in silico data. International Journal of Food Microbiology, 314, 108388. https://doi.org/10.1016/j.ijfoodmicro.2019.108388.

28. Feng, C., Zhang, F., Wang, B., Gao, J., Wang, Y., & Shao, Y. (2019). Evaluation of kanamycin and neomycin resistance in Lactobacillus plantarum using experimental evolution and whole-genome sequencing. Food Control, 98, 262–267. https://doi.org/10.1016/j.foodcont.2018.11.030.UDC 637.334; 637.518; 664.38