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ABSTRACT

of the dissertation presented for the degree of Doctor of Philosophy

EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF PROBIOTICS APPLIED TO THE SURFACE OF FRUIT PLANTS

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Applicant: Shabnam Adalat Mirzayeva

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The dissertation work was performed at the Department of Biology and Ecology of Lankaran State University and the Research Laboratory of Microbiology and Virology of Baku State University.

Scientific superviso

gical sciences, professor Knugavergi Ganbar Ganbarov

Official opponents:



Doctor of biological sciences, professor Mirmusa Mirish Jafarov

Doctor of biological sciences, professor Farida Khosrov Gahramanova

PhD in biology Kamala Kamaleddin Isayeva

FD 1.07 Dissertation Council of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at the Institute of Microbiology, MSE RA

Chairman of the Dissertation Council:

Holee Pro

Corresponding member of ANAS, Doctor of biological sciences, professor **Panah Zulfugar Muradov**

Scientific secretary of the Dissertation Council:

PhD in biology, associate prof. Gunel Ali Gasimova

Chairman of the scientific seminar:

Doctor of biological sciences, professor Konul Farrukh Bakhshaliyeva

INTRODUCTION

Relevance of the topic and degree of development. Lactic acid bacteria have been used in daily life since ancient times. Louis Pasteur's discoveries in the 19th century brought attention to these bacteria, and researchers have since studied them. Ilya Mechnikov, a Russian scientist, worked at the Pasteur Institute in France. He studied the beneficial properties of these bacteria for humans and named them probiotics (which means 'for life' in Greek) and *The World Health Organisation has adopted this term as a synonym for bacteria that promote good health*¹.

*"Lactic acid bacteria can produce antimicrobial proteins"*² in addition to lactic acid as the main metabolite. The food industry commonly uses products such as yogurt, kefir, kumiss, chal, cheese, and sour cream. Lactic acid bacteria are also used in the production of rye bread, canning fruits and vegetables, and preparing silage for animals. *"Live bacteria cells are administered as preparations in medicine to enhance digestion, eliminate toxins from the body, and manage digestive organ dysbiosis*³.

Extensive research work on lactic acid bacteria has been carried out in the conditions of Azerbaijan, and home (spontaneous) yogurt, shor, airan, cheese were usually used to obtain these bacteria. Ganbarov X.Q. and Jafarov M.M. Since 1998, studied "physiological properties of 131 strains of 11 species of genera Lactobacillus and Streptococcus isolated from home yogurt in 84 settlements of 5 agroclimatic regions of Azerbaijan and obtained probiotics with high antibacterial effect"⁴.

¹ Кайбышева, В.О., Никонов, Е.Л. Пробиотики с позиции доказательной медицины // –Москва: Доказательная гастроэнтерология, –2019. Т. 8, №3, с.45-54.

² Kerry, R. Benefaction of probiotics for human health: A review / R. Kerry, J. Patra, S. Gouda [et al.] // Jour. FoodandDrugAnalysis, – 2018. V.26, N3, – P. 927-939

³ Николаева, С.В., Золотарев, Ю.В., Горлова, А.В. Применение пробиотиков в медицинской практике // –Москва: Медицинское обозрение, –2018. N 8 (2), – С.84-87

⁴ Qənbərov, X. Azərbaycan ərazisində evdə hazırlanan (spontan) qatıqlarınmikrobiologiyası / X.Qənbərov, M.Cəfərov – Bakı: Elm, – 2013, – 344 s.

Abdullayeva H.F. isolated Lactobacillus bacteria from samples of homemade cheese in various regions of Azerbaijan⁵. She also isolated strains that synthesize bacteriocin-like inhibitory substances. Mirzayeva F.O. obtained 32 strains of six species from samples of homemade yogurt in the Kur-Araz agroclimatic province of Azerbaijan as pure culture. She studied their antibacterial effect on common pathogenic bacteria. A.F. Akhmedova isolated 10 strains of Enterococcus and Lactobacillus bacteria from homemade yogurt, cheese, cottage cheese, and soft cheese in Azerbaijan. She showed that the bacteriocin substance synthesized by these strains inhibits the growth of Bacillus cereus and Listeria monocytogenes pathogens⁶. Huseynova (Huseynova N.F.) isolated Enterococcus bacterial strains from buttermilk and cheese samples in Azerbaijan. She obtained an enterocin substance from the E. faecium 55 strain, which has antibacterial properties against pathogenic bacteria⁷. Gulahmedov S.G. isolated 66 bacteriocin-synthesising strains from Lactobacillus and Enterococcus from samples of domestic cheese, salt, yoghurt and airan in Azerbaijan and studied the antibacterial properties of the substance⁸. Masumikia R.Y. isolated strains of Lactobacillus and Streptococcus bacteria from samples of cottage cheese and salted cheese from settlements in the Azerbaijani territories of the Republic of Azerbaijan and Iran.

⁵Абдуллаева, Н.Ф. Изучение биохимических и антимикробных свойств бактериоцинподобных ингибирующих веществ, полученных из бактерий рода *Lactobacillus* / Автореферат диск. На соискание ученой степени доктора философии / – Баку, – 2010, –21 с.

⁶ Ахмедова, А.Ф. Изучение протеолитической и антимикробной активности молочнокислых бактерий, выделенных из традиционных молочных продуктов Азербайджана / Автореферат дис. на соискание ученой степени доктора философии / – Баку, 2012, 23 с.

⁷ Гусейнова, Н.Ф. Биохимические и антимикробные свойства энтероцина, полученного из молочнокислых бактерий рода *Eterococcus*/ Автореферат дис. на соискание ученой степени доктора философии / – Баку, 2015, –21 с.

⁸ Гюльахмедов, С.Г. Метаболиты молочнокислых бактерий Азербайджана с антимикробными свойствами и их практическое значение / Автореферат дис. на соискание ученой степени доктора наук / –Баку, 2016, –45с.

The antimicrobial activity against intestinal pathogens, acidity, and bile resistence of these strains were studied 9 .

Microorganisms, including bacteria, naturally thrive on plant surfaces. Despite this, lactic acid bacteria isolation from plant surfaces has been largely overlooked. In China, *the Lactobacillus genus was found in fruits and leaves of white mulberry and acacia leaves*¹⁰. In Bulgaria, *Enterococcus and Streptococcus bacterial strains were found on the surface of leaves, flowers and stems of salvia*¹¹, and *bacterial strains of the genera Lactobacillus and Pedicoccus were isolated from sugar cane and tea leaves*¹².

Currently, there is no literature available on the distribution of lactic acid bacteria on the surface of plants in Azerbaijan. To gain insight into this topic, it is important to study the distribution of probiotics in the phyllosphere of plants and research the properties of their pure cultures. This will lead to the identification of practically important strains.

Object and subject of the research.The study focused on lactic acid bacteria, specifically probiotics, found on the surface of fruit plants in Azerbaijan. The study aimed to quantify the probiotics in the phyllosphere of the plants, assess their antibacterial and antifungal activity, protease activity, resistance to bile, and acidity.

Goals and objectives of the research. The thesis aimed to study probiotic distribution patterns in the phyllosphere of fruit plants in

⁹ Masoumikia, R.Y. Müxtəlif turşsüd məhsullarından ayrılmış

probiotikbakteriyaların bəzi mədə-bağırsaq patogenlərinə qarşı antaqonistliyinin tədqiqi / Biologiy aüzrə fəlsəfə doktoru dis. avtoreferatı / – Bakı, 2015. – 22 s.

¹⁰ Kui-Kui, N. Selection and characterization of Lactic acid bacteria isolated from different origins for ensiling *Robinia pseudoacacia* and *Morus alba L*. leaves / N. Kui-kui, Y. Hui-kino, H. Wei [et al.] // Journal of Integrative Agriculture, – 2016. V.15, N10, – P. 2353- 2362.

¹¹ Teneva-Angelova, T., Beshkova, D. *Genus Saliva* – ecosystem for isolation of lactic acid bacteria // Journal of Microbiology, Biotechnology and food sciences, – 2015. V.5, N2, – P. 103-108.

¹²Tanasupawat, S.Identification of lactic acid bacteria from fermented tea leaves in Thailand and proposals of *Lactobacillus* and *Pedicoccus* / S. Tanasupawat, A. Pakdecto, C. Thawai [et al.] // The Journal of General and Applied Microbiology, – 2007. V.53, – P. 7-15.

Azerbaijan, isolate their pure cultures, and investigate their practical properties.

To achieve this, the following objectives were set:

To study the quantitative characteristics of probiotics in aboveground organs of apple, pear, quince, cherry, blackberry, white and black mulberry;

- To isolate pure cultures of probiotics and study their antibacterial and antifungal properties;

- Select strains with high antimicrobial activity and carry out their identification;

- to study the resistance of selected strains to acidity;

- to study the resistance of selected strains to bile;

- determine proteolytic activity of selected strains.

Research methods. Conventional microbiological methods were used to determine the number of live bacteria. Probiotics were cultured on MRS nutrient medium under microaerophilic conditions. The antibacterial and antifungal activity of probiotics was determined by agar-diffusion of their culture liquid. The genus and species of the bacteria were identified using Bertschi's identifier (Bergey). The test bacteria were cultured on meat-peptone agar, and the test fungi were cultured on malt-nutrient agar medium. We studied the stability of probiotics to acidity by incubating their cell suspension in a buffer solution with pH 2.5 acidity for 3 hours. We determined their resistance to saturation by recording the time of inhibition (in minutes) of cell growth in liquid nutrient medium MRS containing 0.3% bovine bile. We monitored cell proliferation (growth) using a McFarland densitometer. The probiotics' protease activity was measured using the modified Anson method, spectrophotometrically. The experiments were included four replications, and statistical analysis was conducted using Student's test.

The main provisions of the defense:

1. Fruits and flowers from above-ground organs of plants are more favorable for the development of bacteria, including probiotics.

- 2. Spherical probiotics are significantly more abundant in the phyllosphere of fruit plants than rod-shaped probiotics.
- 3. Among probiotic strains, Lactobacillus and Streptococcus exhibit the highest antibacterial activity, while *Peptococcus* and *Pedicoccus* strains exhibit the highest antifungal activity.
- 4. *Streptococcus lactis* strain LDU-155 exhibits high antimicrobial and protease activity, and demonstrates maximum resistance to acid and bile.

Scientific novelty of the research. The distribution patterns of probiotics in the phyllosphere of fruit plants in Azerbaijan were studied for the first time. The study found that probiotics make up 18.5-28.6% of the total number of bacteria, with the highest concentration found in fruits and flowers, and the lowest in green stems.

Spherical probiotics, specifically those belonging to the *Streptococcus, Pedicoccus, Peptococcus*, and genus *Leuconostoc*, are commonly found in the phyllosphere of apple, pear, and quince. On the other hand, both spherical and rod-shaped probiotics are common on the plant surface of cherry, blackberry, white and black mulberry plants. *Lactobacillus,* which are rod-shaped probiotics, were also found to be common. Spherical bacteria accounted for 76% of the total number of probiotics. The study analysed the antimicrobial activity of 81 strains that were isolated as pure cultures. The results showed that *Lactobacillus* and *Streptococcus* strains exhibited the highest antibacterial activity, while *Peptococcus* and *Pedicoccus* strains exhibited the highest antifungal activity.

Gram-negative bacteria were found to be more sensitive to probiotics than Gram-positive bacteria.

The study found that several strains of *Lactobacillus acidophilus* LDU-127, *L. brevis* LDU-183 and *Streptococcus lactis* LDU-155, *S.salivarius* LDU-164, and LDU-65 exhibited high resistance to both acid and bile.

Additionally, *Lactobacillus helveticus* LDU-159, *L. plantarum* LDU-20, and LDU-136, as well as *Streptococcus salivarius* LDU-

164, demonstrated maximum proteolytic activity. Notably, *Streptococcus lactis* strain LDU-155 exhibited high antibacterial, antifungal, and protease activity, as well as resistance to acid and bile.

Theoretical and practical significance of research. The scientific results enhance our understanding of the environmental, physiological, and biochemical properties of probiotics. The distribution patterns of probiotics can aid researchers in obtaining pure probiotic cultures from plants.

Strains with strong antimicrobial properties can be utilized in the production of antibiotics against bacteria and fungi.

Lactobacillus helveticus LDU-159, L. plantarum LDU-20, and LDU-136 strains, along with *Streptococcus lactis* LDU-155, which exhibit maximum proteolytic activity, can be used to produce high-quality fermented milk products and improve the quality of meat products.

Approbation of the work, publications. 14 publications related to the thesis topic were published, including 5 articles and 9 abstracts. The thesis materials were presented at several conferences, including the IX International Scientific Conference on "Innovative Approaches in Modern Biology"(Baku, 2019), the XXIII Republican Scientific Conference for Doctoral Students and Young Researchers (Baku, 2019), and the 2nd International Scientific and Practical online Conference on "Integration of Education, Science and Conditions" (Dnipro, Ukraine, 2020). Business in Modern Additionally, they were presented at an online Republican Scientific Conference for Doctoral Students and Young Researchers on the theme of "Information". Science, technologies, and university perspectives were discussed in various international scientific conferences such as the III International Scientific and Practical Conference 'European Scientific Discussions' (Rome, Italy, 2021), the Online Republican Scientific Conference "Actual Problems of Turkic World" (Lankaran, 2021), the XII International Scientific and Practical Conference "International Scientific Innovations in Human Life" (Manchester, UK, 2022), and the V International Scientific and Practical Conference "International Scientific Innovations in Human

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Life" (Manchester, UK, 2022). Reports were presented at the V International Scientific and Practical Conference "Progressive Research in the Modern World" (Boston, USA, 2023) and the XX International Scientific Conference "Ways of Distance Learning in Modern Conditions" (Munich, Germany, 2023).

Name of the organization where the dissertation work was performed. The thesis was conducted at the Department of Biology and Ecology at Lankaran State University and the Research Laboratory of Microbiology and Virology at Baku State University.

The structure and content of the dissertation The dissertation consists of an Introduction and 5 chapters, Final Analysis, Results, and a list of references. Excluding the bibliography, which includes 12 tables, 20 graphs, 3 figures, and 326 sources. In total, the dissertation consists of 164373 marks. The Introduction is 10744 marks, Chapter I is 48402 marks, Chapter II is 10985 marks, and Chapter III is 22076 marks. Chapter IV has 33613 marks, while Chapter V has 22200 marks, the Final Analysis has 13801 marks, and the Conclusion has 2552 marks).

CHAPTER I

DISTRIBUTION OF PROBIOTICS IN NATURE AND THEIR PRACTICAL PROPERTIES

In paragraph 1.1, the general characterisation and classification of lactic acid bacteria and their main characteristics are given.

Paragraph 1.2 outlines the primary sources of these bacteria, which include homemade fermented milk products (such as yogurt, cheese,and sour cream), biopreserved fruits and vegetables, silage, humanand animal cavities and faeces, and plant and soil surfaces.

Paragraph 1.3 of the chapter discusses the practical properties of probiotics, including their antimicrobial activity against pathogenic and opportunistic bacteria and fungi, as well as their acidity and sputum tolerance and proteolytic activity.

CHAPTER II MATERIAL AND METHODS

The thesis focuses on researching lactic acid bacteria (probiotics) found on the surface (phyllosphere) of fruits and plants. The number

of bacterial cells was determined using methods commonly accepted in microbiology, specifically MRS agar. Identification of bacteria with high antimicrobial activity was based on Berger's determination and their characteristic features. The study analysed the Gram-positive colouration, lactic acid production, catalase, oxidase, gelatinase and nitrate assay, ability to absorb sugars and sugar alcohols, temperature, and pH range¹³. The antimicrobial activity of bacterial strains against opportunistic Gram-positive and Gram-negative bacteria and Candida yeast fungi was determined using the agar $diffusion^{14}$ method. The acid and bile resistance of the strains was studied by sowing them on nutrient medium (MRS)¹⁵. The enzyme solution used was the culture fluid of bacterial strains with high antimicrobial activity. Protease activity was determined using a modified Anson's method and expressed as µmol/min/mg protein $(unit/mg \ protein)^{16}$, based on the amount of enzyme capable of producing 1 μ mol tyrosine (0.181 mg) from casein at 30°C in 1 *minute*. The activity was measured in *µmol/min/mg* of protein. The protein concentration in the culture fluid was measured using a spectrophotometer (UV-vis Specord 250, Germany) at a wavelength of $280 \ nm^{17}$.

¹³ Bergey's manual of Systematic Bacteriology / William B. Whitman director of the editoral office – Springer Dordrecht Heidelberg. London, New York, – 2009. V.3, – 1319p.

 ¹⁴ Balouiri, M., Sadiki, M., Koralchi, S. Methods for in vitro evaluating antimicrobial activity: a review // Journal of Pharmaceutical analysis, – 2016. N6, – P.71-79.
¹⁵ Wyronimus, B., Le, M. Acid and bile tolerance of sporeforming lactic acid bacte-

ria // International Journal of Food Microbiology, -2007, V.61, N2-3, -P. 103-116. ¹⁶ Лабораторный практикум по технологии ферментных препаратов / И.М.

Грачева, Н.Т. Грачев, М.С. Мосичев [и др.], –Москва: книга по требованию, – 2021, – 177с.

 $^{^{17}}$ Withakker, F.R., Granna, P.E. An absolute method for protein determination based of differences in absorbance at 235 and 260 mm // Analitical Biochemistry, - 1980, V.109, - P. 156-159.

CHAPTER III PROBIOTICS IN THE PHYLLOSPHERE OF FRUIT PLANTS

The study analysed the quantity of common bacteria and probiotics (lactic acid bacteria) found in samples taken from various parts of fruit trees (apple, pear, quince, cherry) and blackberry bushes grown in homestead plots in Lankaran. The results are presented in Table 3.1. The highest number of both common bacteria and probiotics were found on flowers and fruits, while the lowest were found on the green branches (stems).

Table 3.1

Fruit crops		Tetal Destarial Count 104 CEU /	Probiotic Count		
		Total Bacterial Count, 10 ⁴ CFU/g	10 ⁴ CFU/g	%	
Apple	Leaf	21.8±1.2	3.9±0.1	17.8	
	Stem	8.6±0.4	1.6 ± 0.04	18.6	
	Flower	26.9±1.2	5.8±0.2	21.5	
	Fruit	28.4±1.3	6.7±0.3	23.6	
D	Leaf	25±1.2	5.6±0.2	22.4	
	Stem	8.7±0.3	2.0±0.1	22.9	
Pear	Flower	28.2±1.3	6.6±0.3	23.4	
	Fruit	29±1.4	7.8±0.3	26.8	
	Leaf	20.8±1.1	4.8±0.2	23.1	
Guince	Stem	6.8±0.3	2.6±0.1	38.2	
Guince	Flower	26.2±1.2	5.6±0.2	21.4	
	Fruit	24.8±1.2	5.0±0.2	20.2	
	Leaf	25.2±1.3	6.4±0.3	25.4	
Chammy	Stem	16.2±0.06	3.4±0.1	21.0	
Cherry	Flower	28.5±1.4	7.0±0.3	24.6	
	Fruit	31.2±1.0	7.8±0.3	25.0	
Blackberry	Leaf	27.9±1.3	7.8±0.3	28.0	
	Stem	25.0±1.2	6.0±0.3	24.0	
	Flower	29.4±1.4	8.6±0.4	29.3	
	Fruit	30.8±1.6	9.9±0.4	32.1	

The distribution of bacteria in the above-ground organs of fruit plants.

Of the total probiotic bacteria, were found on the surface of

flowers, fruits, leaves, and stems, respectively. The percentage was 18.0-31.5%, 15.3-36.7%, 15.8-31.2%, and 12.6-27.3%, respectively. This indicates that the fruits and flowers of above-ground plant organs provide a more favourable environment for bacterial growth than the leaves and stems.

Table 3.2 shows that blackberry plants have the highest number of both common bacteria and probiotics in the phyllosphere of fruit plants, while mulberry trees have the lowest number. It is important to consider these variations when studying the distribution of bacteria in the phyllosphere. The number of probiotics on blackberry plants is 1.8 times that of apple, pear, quince, cherry, white mulberry and black mulberry. It is 1.5 times that of cherry plants, and 1.4 times that of apple, pear, quince, white mulberry and black mulberry. The ratios for white mulberryand black mulberryare 1.8 and 3.4 respectively, while that for cherry is 1.3. The text has been edited for clarity, conciseness, coherence, and grammatical correctness. The language has been made more direct and unambiguous, and the use of standardized language and precise word choice has been ensured. The original content has been maintained without any addition of new aspects. The probiotic content of pear is 2 times higher than that of apple, quince, white mulberry and black mulberry. Additionally, the probiotic content of pear is 1.4 times higher than the initial value. The probiotic content of apple is 1.2 times higher than the initial value. The probiotic content of apple trees is 2.6 and 2.3 times higher than that of white mulberry and black mulberry respectively. The probiotic content of apple trees is 2.1 and 1.9 times higher than the initial value.

Probiotics were distributed in the phyllosphere of apple, pear, quince, cherry, white mulberry, black mulberry, and blackberry plants. The total bacterial counts were 21.0%, 24.2%, 22.9%, 24.1%, 18.5%, 19.8%, and 28.6%, respectively.

Table 3.2.

Emit anong	Total Postarial Count 104 CEU /a	Probiotic Count		
Fruit crops	Total Bacterial Count, 10 ⁴ CFU/g	10 ⁴ CFU/g	%	
Apple	8.57±0.4	1.8 ± 0.05	21.0	
Pear	9.09±0.5	2.2±0.1	24.2	
Guince	7.86±0.3	1.8 ± 0.06	22.9	
Cherry	10.11±0.5	2.46±0.1	24.1	
White mulberry	4.4±0.2	0.85 ± 0.04	18.5	
Black mulberry	5.1±0.2	0.95 ± 0.05	19.8	
Blackberry	11.31±0.6	3.23±0.1	28.6	

Number of bacteria distributed in the phyllosphere of fruit plants

The sexual composition of 412 bacterial strains obtained as pure culture was studied. Out of the 412 strains isolated, 35% belonged to *Streptococcus*, 24.2% to *Pedicoccus*, 17.5% to *Lactobacillus*, 13.3% to *Peptococcus*, and 10% to *Leuconostoc* (Table 3.3).

Table 3.3.

Genus composition of strains isolated from the phyllosphere of fruit plants

	Genera of bacteria							
Crops	Lactobacillus	-		-	Streptococcus			
Apple	0	0	5	2	14			
Pear	0	3	6	3	16			
White mulberry	19	12	23	13	24			
Black mulberry	18	10	22	12	26			
Guince	0	0	6	4	11			
Cherry	13	6	14	7	21			
Blackbarry	22	10	24	14	32			
Total number of strains	72	41	100	55	144			

Only spherical probiotics were found in the phyllosphere of apple, pear, and quince. On the surface of white mulberry, black mulberry, cherry, and blackberry plants, both spherical and bacilliform probiotics were found. The number of spherical bacteria was 3.2 times higher than that of bacilliform bacteria, accounting for 76%.

Out of the 412 strains obtained, 81 relatively active strains were selected for further studies. The strains were classified into different genera, with the majority (25.8%) belonging to *Lactobacillus*, followed by *Streptococcus* (23.5%), *Pedicoccus* (17.3%), *Peptococcus* (19.8%), and *Leuconostoc* (13.6%).

CHAPTER IV ANTIMICROBIAL ACTIVITY OF PROBIOTICS ISOLATED FROM THE PHYLLOSPHERE OF FRUIT PLANTS

The study analysed the antibacterial and antifungal properties of 81 probiotic strains isolated from the surface of fruit plants in Azerbaijan.

Results showed that *Bacillus subtilis*, *B. mesentericus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were sensitive to 60%, 60%, 60%, 77%, 91% and 79% of the probiotic strains, respectively, indicating potential for their use as antimicrobial agents against opportunistic bacteria.

The antibacterial activity of *Lactobacillus* bacteria, which have a relatively high level of antibacterial activity, is shown in Table 4.1. 17 strains of *Lactobacillus* have a high inhibitory effect on both gram-positive and gram-negative bacteria. Only four strains (LDU-10, LDU-127, LDU-131, and LDU-222) were able to inhibit the growth of gram-negative bacteria. The strains LDU-159 and LDU-165 showed the maximum antimicrobial effect on both gram-positive and gram-negative bacteria. The *Streptococcus* bacteria have antimicrobial activity against both gram-positive and gram-negative bacteria in two directions. Among them, 15 strains have activity against both types of bacteria, while 4 strains only affect gram-negative bacteria (see Table 4.2). It has been established that 17 strains of the *Lactobacillus* genus, 15 strains of the *Streptococcus*

genus, 5 strains of the *Pedicoccus* genus, and 2 strains of the *Leuconostoc* genus have a broad-spectrum antibacterial effect.

Lactobacillus strains were found to be effective against 100% of Gramnegative bacteria and 8% of Gram-positive bacteria. *Lactobacillus* strains were found to be effective against 100% of Gram-negative bacteria and 8% of Gram-positive bacteria. *Streptococcus* strains were effective against 100% of both Gram-positive and Gram-negative bacteria. *Lactobacillus* strains were found to be effective against 100% of Gram-negative bacteria and 8% of Gram-positive bacteria.

Table 4.1

	Test cultures and their lysis zone					
Strains	Bacillus subtilis	Bacillus Megatericus	Staphylococcus aureus	Escherichia coli	Klebsiella pneumoniae	Pseudomonas aeruginosa
LDU-9	21±1.0	20±1.0	16±0.5	18±0.7	20±0.9	12±0.5
LDU-10	0.0	0.0	0.0	20±0.8	22±1.0	18±0.7
LDU-20	21±1.0	21±1.0	16±0.8	17±0.6	18±0.8	13±0.4
LDU-60	18±0.9	19±0.8	17±0.8	22±1.0	20±0.8	15±0.7
LDU-68	16±0.6	15±0.7	16±0.7	20±1.0	27±1.2	15±0.6
LDU-87	10±0.4	11±0.5	12±0.6	16±0.6	13±0.7	12±0.5
LDU-105	12±0.6	13±0.6	12±0.4	14±0.7	14±0.7	14±0.5
LDU-127	0.0	0.0	0.0	27±1.3	28±1.3	20±1.0
LDU-131	0.0	0.0	0.0	9.0±0.3	11±0.5	9.0±0.2
LDU-134	11±0.5	10.6±0.6	12±0.4	14±0.6	16±0.7	14±0.6
LDU-136	18±0.9	18.5±0.6	20±1.0	24±1.2	16±0.6	17±0.8
LDU-141	12±0.6	11.6±0.5	13±0.6	15±0.6	16±0.8	10±0.3
LDU-143	12±0.6	12.4±0.6	13±0.5	15±0.7	16±0.7	10±0.4
LDU-159	27±1.3	26±1.2	24±1.2	28±1.2	28±1.3	20±0.1
LDU-165	24±1.2	26±1.3	25±1.3	22±1.1	32±1.5	17±0.7
LDU-170	17±0.8	17±0.8	16±1.0	20±1.0	27±1.3	16±0.8
LDU-177	15.5±0.7	15.5±0.7	16±0.5	18±0.9	21±1.0	14±0.6
LDU-183	16.4±0.6	16.4±0.6	17±0.7	19±0.9	20±0.7	15±0.7
LDU-195	13.2±0.5	13.2±0.5	14±0.7	16±0.7	18±08	12±0.6
LDU-210	15.3±0.7	15.3±0.7	16±0.8	17±0.7	20±1.0	14±0.7
LDU-222	0.0	0.0	0.0	8±0.3	15±0.7	11±0.5

The antibacterial activity of Lactobacillus strains

Table 4.2.

Antibacterial activity of Streptococcus strains						
	Test cultures and their lysis zone, mm					
Strains	Bacillus subtilis	Bacillus mesentericus	Staphylococcus Aureus	Escherichia Coli	Klebsiella Pneumonia	Pseudomonas aeruginosa
LDU-4	12±0.6	12±0.5	13±0.6	16±0.7	18±0.9	22±1.0
LDU-12	17±0.5	16±0.6	16±0.8	18±0.8	19±0.8	18±0.9
LDU-14	19±0.6	20±1.0	18±0.7	14±0.7	14±0.7	20±1.0
LDU-15	16±0.7	17±0.6	15±0.6	18±0.6	20±1.0	20±0.9
LDU-34	15±0.6	16±0.6	15±0.7	16±0.8	19±0.8	23±1.1
LDU-35	14±0.7	14±0.7	13±0.7	16±0.9	18±0.8	20±1.0
LDU-56	16±0.8	15±0.7	14±0.7	23±1.1	27±1.3	28±1.2
LDU-65	18±0.8	19±0.8	22±1.0	18±0.9	20±1.0	26±1.3
LDU-82	16±0.8	18±0.9	20±1.1	19±0.7	20±1.0	24±1.2
LDU-115	12±0.4	12±0.6	13±0.7	15±0.7	20±0.8	15±0.7
LDU-140	20±1.0	21±1.0	26±1.3	18±0.8	23±1.1	20±1.0
LDU-155	12±0.6	13±0.6	12±0.6	18±0.9	21±1.0	20±1.0
LDU-164	20±0.8	21±1.0	26±1.2	18±0.7	20±1.0	24±1.2
LDU-167	18±0.7	19±0.7	24±1.2	17±0.4	18±0.6	28±1.4
LDU-171	22±1.0	23±1.1	26±1.3	18±0.6	21±1.0	30±1.5
LDU-200	0.0	0.0	0.0	12±0.6	14±0.5	11±0.6
LDU-208	0.0	0.0	0.0	14±0.7	16±0.7	12±0.5
LDU-216	0.0	0.0	0.0	15±0.8	17±0.7	12±0.6
LDU-226	0.0	0.0	0.0	18±0.9	18±0.6	14±0.7

Antibacterial activity of Streptococcus strains

Leuconostoc strains were effective against 100% of Gram-positive bacteria and 18% of Gram-negative bacteria. *Pedicoccus* strains were effective against 100% of Gram-positive bacteria and 36% of Gram-negative bacteria. *Peptococcus* strains were effective against 37% of Gram-positive bacteria and 63% of Gram-negative bacteria. Overall, probiotics were found to be more effective against Gram-negative bacteria than Gram-positive bacteria.

The antifungal activity of probiotics was tested against the opportunistic microorganisms *Candida albicans* and *Candida tropicalis*. Out of the 21 strains of the *Lactobacillus* genus, 10 showed antifungal activity against both types of fungi (see graph 4.1).

There was no significant difference in the sensitivity of fungal species to *Lactobacillus* probiotics. *Leuconostoc* probiotics showed similar antifungal activity against *Candida albicans* and *C. tropicalis* yeasts. Six out of the eleven strains of this genus studied had high antifungal activity against both fungal species. Similarly, eleven out of the fourteen *Pedicoccus* strains had comparable antifungal activity against *Candida albicans* and *C. tropicalis* fungi. Strain LDU-106 showed the highest activity against both fungal species. Out of 16 *Peptococcus* strains, 10 exhibited antifungal activity against *Candida albicans* and *C. tropicalis* fungi species was similar, with strains LDU-48 and LDU-33 showing the highest activity.



Graph 4.1 Antifungal activity of *Lactobacillus* strains: 1 – LDU-9; 2 – LDU-20; 3 – LDU-60; 4 – LDU-127; 5 – LDU-136; 6 – LDU-159; 7 – LDU-165; 8 – LDU-170; 9 – LDU-183; 10 – LDU-210; 11 – LDU-10, LDU-68, LDU-87, LDU-105, LDU-131, LDU-134, LDU-141, LDU-143, LDU-177, LDU-195 və LDU-222. A – *Candida albicans*, B – *Candida tropicalis*.

Of the 19 *Streptococcus* strains, 11 exhibited antifungal activity against yeast fungi (graph 4.2). The antifungal effect of probiotics on both types of yeast fungi was similar. The strains LDU-171 and

LDU-65 showed the highest activity against the yeast fungus *Candida albicans*.

The antifungal action of probiotics on *Candida albicans* and *C. tropicis* fungi was similar. The maximum effect on both types of fungi was observed in *Lactobacillus* LDU-127, *Leuconostoc* LDU-116, LDU-160 and LDU-2, and *Pedicoccus* LDU-106. *Peptococcus* was observed in strains LDU-48 and *Streptococcus* LDU-171. Among the probiotics, *Pedicoccus* and *Peptococcus* strains with high antifungal activity have the maximum antifungal activity of *Lactobacillus*, *Leuconostoc*, and *Streptococcus* strains.The antifungal activity was correspondingly 1.2 times and 1.2-1.3; 1.3-1.4 times higher. For example, the antibacterial activity of *Lactobacillus*



Graph 4.2 Antifungal activity of *Streptococcus* strains: 1 – LDU-4, 2 – LDU-14, 3 – LDU-56, 4 – LDU-65, 5 – LDU-155, 6 – LDU-164, 7 – LDU-171, 8 – LDU-200, 9 – LDU-208, 10 – LDU-216, 11 – LDU-226, 12 – LDU-12, LDU-15, LDU-34, LDU-35, LDU-82, LDU-115, LDU-140, LDU-167. A – *Candida albicans*, B – *Candida tropicalis*

strains exceeded the antifungal activity by 1.7 times, *Peptococcus* strains by 1.2 times, and *Streptococcus* strains by 1.8 times. The antibacterial activity of probiotics was higher than the antifungal activity.

CHAPTER V PRACTICAL PROPERTIES OF PROBIOTICS WITH HIGH ANTIMICROBIAL ACTIVITY

The acid and bile resistance and protease activity of probiotics with high antimicrobial activity were studied. Among the *Lactobacillus* species tested, the *L. paracasei* LDU-9 and *L. acidophilus* LDU-127 strains exhibited the highest acid resistance. The *L. brevis* LDU-183, *L. brevis* LDU-60, *L. helveticus* LDU-159, *L. paracasei* LDU-165, *L. plantarum* LDU-20, and *L. plantarum* LDU-136 strains showed lower levels of acid resistance, with acid tolerance percentages 1.2, 1.4, 1.8, 1.2, 1.7, and 1.5 times higher, respectively (graph 5.1). There were differences in acid tolerance between species and strains. The acid tolerance of Lactobacillus paracasei LDU-165 cells was 1.3 times lower than that of LDU-9 strain, and the acid tolerance of L. brevis LDU-60 strain was 1.3 times lower than that of LDU-183 strain.

Leuconostoc probiotics vary in acid tolerance, with the highest tolerance found in *Leu. lactis* strain LDU-71 and the lowest in *Leu. mesenteroides* LDU-100. The acid resistance of *Leu. Lactis* LDU-71 cells was 1.14, 1.24, and 1.4 times higher than that of *Leu. Lactis* LDU-31, *Leu. mesenteroides* LDU-6, and LDU-100, respectively. In general, *Leuconostoc* probiotic cells exhibited weak resistance to acidity.



Graph 5.1. Antiacidity activity of *Lactobasillus* strains: 1 - L. *acidophilus* LDU-127; 2 - L. *brevis* LDU-183; 3 - L. *brevis* LDU-60; 4 - L. *helveticus* LDU-159; 5 - L. *paracasei* LDU-9; 6 - L. *paracasei* LDU-165; 7 - L. *plantarum* LDU-20; 8 - L. *plantarum* LDU-136.

High acid tolerance of probiotics of the genus *Pedicoccus* was observed in strains *P. acidilactici* LDU-142 and *P. pentasaceus* LDU-8. Acid tolerance of *Pedicoccus acidilactici* strain LDU-142, strains *P.cerevisiae* LDU-19, *P. cerevisiae* LDU-158 and *P. halophilus* LDU-59, respectively, are 1.2; 1.3 and 1.4 times greater, and the acid tolerance of *Pedicoccus pentasaceus* strain LDU-8, respectively, is 1.1 1.2 and 1.3 times greater than the acid tolerance of *P. cerevisiae* LDU-158 and *P. halophilus* LDU-59. High acidity resistance of probiotics of the genus *Peptococcus* by *Pep. niger* LDU-209 and Pep. activus was recorded in strains LDU-26. The acid tolerance of *Peptococcus* strains were 1.2 and 1.3 times more resistant to acidity than *Pep.niger* LDU-209, *Pep. activus* LDU-157 and *Pep. aeragenes* LDU-144, respectively.

All the studied *Streptococcus* probiotic strains showed high resistance to acidity and maximum resistance was observed in strains LDU-164 and LDU-65 of *S. salivarus* species. Thus, the percentage of acid tolerance of strains showing maximum resistance is 1.3 1.14;

1.2 and 1.1 times higher respectively from the percentage of resistance of *Streptococcus aureus* strains LDU-171, *S. bovis* LDU-56, *S. cremoris* LDU-14 and *S.lactis* LDU-155; (graph 5.2).



Graph 5.2. Antiacidity activity of *Streptococcus* strains: 1: 1 –*S. aureus* LDU-171; 2 –*S. bovis* LDU-56; 3 –*S. cremoris* LDU-34; 4 –*S. lactis* LDU-155; 5 –*S. salivarius* LDU-164, 6 – *S. salivarius* LDU-65.

The resistance of probiotics to bile was determined by measuring the period of inhibition in liquid nutrient medium MRS containing bile. The probiotic strains tested were *L. acidophilus* LDU-127, *L. brevis* LDU-183, *L. plantarum* LDU-20, LDU-136, *L. helveticus* LDU-159, and *L. paracasei* LDU-170. The strains *L. acidophilus* LDU-127, *L. brevis* LDU-183, *L. plantarum* LDU-20, and LDU-136 showed high resistance to bile, while *L. helveticus* LDU-159 and *L. paracasei* LDU-170 showed low resistance. The highest resistance was observed in *L. acidophilus* strain LDU-127, and the lowest resistance in *L. brevis* LDU-127 strains of Lactobacillus acidophilus was 1.2, 1.4, 1.8, 2.1, 2.7, and 3.0 times greater than that of LDU-183, *L. plantarum* LDU-136, *L. brevis* LDU-159 strains, respectively (see graph 5.3).



Graph 5.3. Bile resistance of *Lactobacillus* strains: 1 - L. *acidophylus* LDU-127; 2 - L. *brevis* LDU-183; 3 - L. *brevis* LDU-60; 4 - L. *helveticus* LDU-159; 5 - L. *paracasei* LDU-9; 6 - L. *paracasei* LDU-170; 7 - L. *plantarum* LDU-20; 8 - L. *plantarum* LDU-136.

Among bacterial strains of the Leuconostoc genus, L. mesenteroides strain LDU-6 exhibited the highest resistance, while L. citreum strain LDU-31 exhibited the lowest. Leuconostoc mesenteroides LDU-6 persisted 2.0 and 1.3 times longer than L. citreum LDU-31 and L. lactis LDU-71, respectively. Strains from the Pedicoccus genus, namely P. pentosaceus LDU-8 and P. acidilactici LDU-142, exhibited high resistance, whereas P. halophilus LDU-59 exhibited poor resistance. Pedicoccus pentosaceum strain LDU-8 lasted a maximum of 100 minutes, which was 1.2-2.5 times longer than strains P. acidilactici LDU-142, P. cerevisiae LDU-19, P. cerevisiae LDU-158, and P. halophilus LDU-50. Peptococcus probiotics were most resistant to P. niger strain LDU-209 and least resistant to P. aerogenes strain LDU-44. Peptococcus niger LDU-209 strain is more resistant to bile than P. activus LDU-157, P. activus LDU-26 and P. aerogenes LDU-144 strains, with resistance levels of 1.1, 1.2 and 1.3 times respectively.

The study found that S. lactis LDU-155 and S. salivarus LDU-

164 and LDU-65 strains showed high resistance to *Streptococcus* probiotics, while *S. bovis* LDU-56 showed minimal resistance. The maximum persistence was observed in *S. salivarus* strain LDU-164, which was higher than that of *S. lactis* LDU-155, *S. aureus* LDU-171, *S. cremoris* LDU-34, and *S. bovis* LDU-56 strains by 1.2, 2.2, 2.9, and 3.2 times, respectively (graph 5.4).



Graph 5.4. Bile resistance of *Streptoccus* strains: 1 - S. *aureus* LDU-171; 2 - S. *bovis* LDU-56; 3 - S. *cremoris* LDU-34; 4 - S. *lactis* LDU-155; 5 - S. *salivarus* LDU-164; 6 - S. *salivarus* LDU-65.

The persistence of probiotics at the genus level was analysed. It was found that the genera *Lactobacillus* and *Streptococcus* had the highest persistence, while the genus *Leuconostoc* had the lowest. *Pedicoccus* and *Peptococcus* genera showed moderate persistence.

The study analysed the protease activity of various *Lactobacillus* strains. The results showed that *L. helveticus* LDU-9, *L. plantarum* strains LDU-136, and LDU-20 had high activity, while *L. acidophilus* LDU-127, *L. paracasei* strains LDU-9, and LDU-170 had weak activity. The maximum protease activity was recorded in *L. helveticus* LDU-159, *L. plantarum* strains LDU-136, and LDU-20, while the minimum activity was recorded in *L. acidophilus* strain LDU-127. The protease activity of *Lactobacillus helveticus* strain LDU-159 was compared to that of *L. plantarum* LDU-20, *L. brevis*

LDU-183, L. brevis LDU-60, L. paracasei LDU-9, L. paracasei LDU-170 and L. Acidophilus LDU-127 strains. The results showed that the protease activity of Lactobacillus helveticus strain LDU-159 was 1.2, 1.6, 1.5, 1.9, 1.7 and 2.2 times higher than that of the other strains, respectively. High proteolytic activity was found in Lactobacillus helveticus and L. plantarum at the species level. The Lactibacillus helveticus species showed the highest activity, while the L. acidophilus species showed the lowest activity. L. acidophilus was twice more active than L. helveticus. Among the strains tested, there was no significant difference in protease activity between Lactobacillus brevis strain LDU-183 and LDU-60, L. paracasei strain LDU-9 and LDU-170 of, and L. plantarum strain LDU-20 and LDU-136 (graph 5.5).



Graph 5.5. Protease activity of *Lactobacillu* bacterial strains: 1 - L. *acidophilus* LDU-127; 2 - L. *brevis* LDU-183; 3 - L. *brevis* LDU-60; 4 - L. *helveticus* LDU-159; 5 - L. *paracasei* LDU-9; 6 - L. *paracasei* LDU-170; 7 - L. *plantarum* LDU-20; 8 - L. *plantarum* LDU-136.

The Leuconostoc Leu. citreum LDU-31 strains showed the highest protease activity, while the L. mesenteroides strains LDU-6 showed the lowest. Among the Pedicoccus strains, P. cerevisiae LDU-19 and LDU-158 exhibited the highest protease activity, while

P. acidilactici strain LDU-42 showed the lowest. The protease activity of the first was 1.8 times higher than that of the second. The genus *Peptococcus* showed the highest protease activity. *Pep. niger* strain LDU-209 had the highest activity and *Pep. aerogenes* strain LDU-144 had the lowest. Among *Streptococcus* strains, *S. lactis* strain LDU-155 had the highest activity, while *S. salivaris* strain

LDU-15 had the lowest. The first had twice the activity of the second (see graph 5.6).

Probiotic genera can be divided into those with high protease activity, such as *Streptococcus* and *Lactobacillus*, those with minimal activity, such as *Leuconostoc* and *Pedicoccus*, and those with intermediate activity, such as *Peptococcus*. Strains of the genera *Streptococcus* and *Lactobacillus* have a maximum protease activity 20.6-23.1 and 2.3-2.5 times higher, respectively, than strains of the genera *Leuconostoc*, *Pedicoccus* and *Peptococcus*.

Therefore, it is established that *Lactobacillus* and *Streptococcus* have higher protease activity compared to strains of genera *Leuconostoc*, *Pedicoccus and Peptococcus*.



Graph 5.6. Protease activity of *Streptococcus* bacterial strains:1 – *S. aureus* LDU-171; 2 – *S. bovis* LDU-156; 3 – *S. cremoris* LDU-35; 4 – *S. lactis* LDU-155; 5 – *S. salivaris* LDU-164; 6 – *S. salivaris* LDU-15.

CONCLUSION

The study examined the distribution patterns of probiotics, specifically lactic acid bacteria, on fruit plants grown in homestead plots in Lankaran district of Azerbaijan. The results showed that leaves, flowers, and fruits had a high concentration of bacteria, while the green stem had a lower concentration. Notably, the highest concentration of probiotics was found in the fruits. This was 1.8 times higher than the concentration found on the surface of apple, pear, quince, cherry, white mulberry, and black mulberry plants, which had concentrations of 21%, 24.2%, 22.9%, 24.3%, 19.3%, and 18.5%, respectively. The study found that the highest concentration of probiotics was on the surface of blackberry plants, with 28.6%. This was 1.8 times higher than the concentration found on the surface of apple, pear, quince, cherry, white mulberry, and black mulberry plants, which had concentrations of 21%, 24.2%, 22.9%, 24.3%, 19.3%, and 18.5%, respectively. This was 1.8 times higher than the concentration found on the surface of apple, pear, quince, cherry, white mulberry, and black mulberry plants, which had concentrations of 21%, 24.2%, 22.9%, 24.3%, 19.3%, and 18.5%, respectively. It is important to note that these concentrations were only measured on the surface of the plants. Spherical bacteria outnumbered *bacilliform* bacteria 3.2 times and made up 76% of the probiotics. The selected strains demonstrated antibacterial activity against both Gram-negative and Gram-positive bacteria, as well as antifungal activity against opportunistic microorganisms Candida albicans and yeast C.tropicalis. Lactobacillus and Streptococcus probiotics have a broad spectrum of antibacterial and antifungal activity.

The bacterial strains showed higher antimicrobial activity (1.5and 1.2-1.3 times) compared to Leuconostoc, 1.8: 1.5-1.9 Pedicoccus, and Peptococcus strains. The strains with high antimicrobial activity belonged five genera. including to Leuconostoc, Pedicoccus, Peptococcus, Lactobacillus. and Streptococcus, and 20 species. Their resistance to acidity and bile, as well as protease activity, were studied.

The properties of probiotics, such as antibacterial and antifungal activity, acidity and acidity resistance, and protease activity, were analysed comparatively. This analysis allowed for the categorisation of probiotics into the following groups:

- 1) Only *Streptococcus lactis* strain LDU-155 belonged to the group with high antibacterial and antifungal activity, high acid and alkali resistance, and high protease activity. This strain exhibits exceptional properties that make it stand out from the rest.
- 2) The *Lactobacillus acidophilus* strains LDU-127, *L. brevis* LDU-183, *Streptococcus salivarius* LDU-65 and LDU-164 have high antibacterial and antifungal activity, are acidic, and have high bacterial resistance, but weak protease activity.
- 3) *Lactobacillus paracasei* strains LDU-9 and LDU-165, *Streptococcus aureus* LDU-171, *S. bovis* LDU-56, and S. cremoris LDU-14 have high antibacterial and antifungal activity, are highly acid-resistant, but have poor bacterial resistance and weak protease activity.
- 4) *Lactobacillus plantarum* strains LDU-20 and LDU-136 have high antibacterial and antifungal activity. They have low acidity resistance but high bacterial resistance and high protease activity.
- 5) *Lactobacillus helveticus* strain LDU-159 belong to a group with high antibacterial and antifungal activity. It has weak acidity resistance and high protease activity.
- 6) *Streptococcus bovis* strain LDU-56 belongs to a group with high antibacterial and antifungal activity, high acidity resistance, and high protease activity. However, it has weak bacterial resistance.
- 7) *Lactobacillus bovis* strain LDU-60 is included in the group with high antibacterial and antifungal activity, high bacterial resistance but weak acid resistance and weak protease activity.

MAIN RESULTS

- Probiotics were found on the surface of fruit plants, including leaves, green branches, flowers, and fruit. They accounted for 18.5-28.6% of the total number of bacteria on the plant surface, with the highest concentration on fruit (5.0-9.9x10⁴ CFU/g) and flowers (5.6-8.6x10⁴ CFU/g), and the lowest on green branches (1.6-6.0x10⁴ CFU/g) [2, 5, 6].
- 2. Spherical forms were the only ones present in the phyllosphere of apple, pear, and quince. However, cherry, blackberry, white and black mulberry had both spherical and bacilliform. Of the total probiotics, 76% were spherical and 24 % were bacilliform [5, 6, 8].
- 3. Eighty-one strains from the genera *Lactobacillus*, *Leuconostoc*, *Pedicoccus*, *Peptococcus*, and *Streptococcus* were isolated and tested for antibacterial and antifungal activity. Of these, 17 *Lactobacillus* strains, 15 *Streptococcus* strains, 5 *Pedicoccus* strains, and 2 *Leuconostoc* strains inhibited the growth of both Gram-positive and Gram-negative bacteria. The probiotics belonging to the genus *Lactobacillus* and *Streptococcus* exhibited the highest antibacterial activity. Gram-negative bacteria were found to be more susceptible to probiotics than Gram-positive bacteria [1, 3, 4, 7, 9, 10].
- 4. Ten strains of *Lactobacillus*, six strains of *Leuconostoc*, 11 strains of *Pediococcus*, 10 strains of *Peptococcus*, and 11 strains of *Streptococcus* exhibited antifungal activity against both yeast species. The highest antifungal activity was observed in probiotics of the genera *Pediococcus* and *Peptococcus*, which were 1.2-1.4 times more active than strains of the genera *Lactobacillus*, *Leuconostoc*, and *Streptococcus*. The maximum antibacterial activity in strains of *Lactobacillus* and *Streptococcus* was 1.7 and 1.8 times higher than the maximum antifungal activity [7, 9, 10, 13].
- 5. All 27 strains from 5 genera and 20 species with high antimicrobial activity demonstrated resistance to acid and

bile. The Lactobacillus acidophilus LDU-127, L. paracasei LDU-9, Streptococcus salivarius LDU-164, and LDU-65 exhibited the highest acid resistance, ranging from 82% to 84%. However, the strains Lactobacillus acidophilus LDU-127, L. brevis LDU-183, Streptococcus lactis LDU-155, S. salivarius LDU-164, and LDU-65 showed the highest resistance to both acid and bile [9, 11, 14].

6. Lactobacillus helveticus LDU-159 (8.2 V/mg protein), L. plantarum LDU-20 (7.0 V/mg protein) and LDU-136 (7.2 V/mg protein), as well as Streptococcus salivarius LDU-155 (8.1 V/mg protein), exhibited the highest protease activity (mg protein). However, most strains with high resistance to acid and bile (73-84% and 115-145 min) had low protease activity (3.8-5.1 V/mg protein). The probiotic Streptococcus lactis LDU-155 demonstrated high antibacterial, antifungal, and protease activity, as well as high resistance to acidity and bile [11, 12, 14].

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