PROBIOTIC MICROORGANISMS: METABOLITES, MECHANISMS OF ACTION, AND APPLICATIONS

G.Sh. Abdulaxadova[1], N.Yu. Zukhritdinova[2], G.J. Qutliyeva[3]
Senior lecturer, Tashkent Pharmaceutical Institute, Tashkent[1]
PhD in Biological Sciences, Senior Researcher, Institute of Zoology, Academy of Sciences of the Republic of Uzbekistan, Tashkent[2]
PhD in Biological Sciences, Senior Researcher, Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan, Tashkent[3]
https://doi.org/10.5281/zenodo.19660335

Abstract. *In this study, the amounts of organic acids produced in the culture liquids of various lactic acid bacteria strains belonging to the genera Lactobacillus and Pediococcus were determined. According to the results obtained, the Lactobacillus plantarum 3G strain was distinguished by higher levels of acetic (0.44 mg/mL) and succinic (0.32 mg/mL) acids, while Lactobacillus fermentum LF1 demonstrated the highest production of lactic acid (22.79 mg/mL). In Lactobacillus brevis 4G, Pediococcus acidilactici 1G, and Pediococcus acidilactici 2G strains, the concentrations of succinic, acetic, and lactic acids were 0.18, 0.28, and 11.37 mg/mL, respectively. In addition, the L. plantarum 3G strain exhibited a superior level of 3-phenyllactic acid production (1.85 mg/mL) compared to the other strains. The findings indicate that L. plantarum 3G and L. fermentum LF1 strains are promising probiotic microorganisms with a high capacity for organic acid production and pronounced antifungal properties and may be recommended for application in the pharmaceutical field.*

Keywords: *lactic acid bacteria, 3-phenyllactic acid, antifungal activity, antimicrobial activity.*

Introdcution

The primary probiotics are lactic acid-producing microorganisms, which are typical representatives of the normal human intestinal microflora [1].

Lactobacilli have been studied for their ability to produce acetic acid, lactic acid, reuterin, pyrrolidone-5-carboxylic acid, and 3-phenyllactate [2]. Lactic acid bacteria exhibit antagonistic activity against pathogenic and opportunistic microorganisms, as lactobacilli synthesize a very broad spectrum of antimicrobial compounds. Among these, lactic and acetic acids are of particular importance [3]. Another of their metabolites is hydrogen peroxide; in the presence of oxygen, lactic acid bacteria can produce through the action of NADH oxidase and superoxide dismutase. The antimicrobial property of hydrogen peroxide is associated with its strong oxidizing effect. By producing hydrogen peroxide, it increases oxidative stress and exerts an effect against pathogenic bacteria. This process targets the membranes of harmful microorganisms, leading to their destruction [4]. 3-phenyllactate is one of the metabolites of phenylalanine metabolism, produced from p-hydroxyphenyl-pyruvate in LAB cells. This end-metabolite demonstrates antibiotic activity against both Gram-positive and Gram-negative bacteria, and also affects a wide range of microscopic fungi [5]. 3-phenyllactate is an antimicrobial compound with a broad spectrum of activity against yeasts such as *Candida pulcherrima* and *Rhodotorula mucilaginosa*, as well as mycotoxigenic fungal species including *Aspergillus ochraceus*, *Penicillium verrucosum*,

and *Penicillium citrinum*. In the course of the research, *L. acidophilus*, *L. fermentum*, *L. brevis*, *L. plantarum*, *L. casei*, *L. delbrueckii*, and *L. jensenii* were isolated from the vaginas of a group of infected and healthy women. The synthesis of lactic acid, hydrogen peroxide, and diacetyl by these *Lactobacillus* strains was found to be 1.46 mg/l, 1.36 mmol/l, and 1.72 mg/l, respectively. In patients with vaginosis, the maximum levels were identified as 1.08 mg/l for lactic acid, 1.36 mmol/l for hydrogen peroxide, and 0.86 mg/l for diacetyl [6]. Quantitative analysis of lactic acid in the *L. fermentum* LF1 strain using the spectrophotometric method revealed a synthesis capacity of 40.91 g/l. It was confirmed that the ability to produce lactic acid depends on their genetic characteristics and cultivation conditions [7].

Lactic acid bacteria and their metabolites hold great significance in modern medicine as agents for prevention, treatment, and biotherapy. Their safety, natural origin, and broad biological activity make them a promising direction for the development of probiotic and postbiotic preparations.

Objectives: Modern microbiology aims to identify new types of probiotics, study their properties and antimicrobial metabolites, and explore the prospects for their practical implementation.

Materials and Methods. *Quantitative analysis of antimicrobial metabolites in the culture liquid was performed using High-Performance Liquid Chromatography (HPLC).*

Quantitative analysis of volatile fatty acids in the culture liquids of the strains was performed using an Agilent Technologies-1200 chromatograph (USA). Uninoculated MRS (de Man, Rogosa, and Sharpe) broth was used as a control. 50 µl of reconstituted Lactobacillus cultures were inoculated into 5 ml of MRS broth and incubated at 37°C for 18 hours. Standard samples of organic acids were weighed and dissolved in 1 ml of distilled water in specialized test tubes for quantitative analysis. To prevent column contamination and a rapid decrease in efficiency caused by mechanical and undissolved particles, the solutions were filtered through a 0.2 µm pore size filter. The sample solutions were then placed into the chromatograph's autosampler and analyzed according to the developed method. Centrifugation was carried out at 8,000 rpm for 10 minutes. The injection volume for each analysis was 5 µl.

Identification of metabolites using the Chip-Q-TOF mass spectrometry method. Antimicrobial metabolites of lactic acid bacteria were identified using an Agilent 6520B Series Chip-Q-TOF mass spectrometer. The culture liquid was fractionated using an Agilent Technologies 1200 series chromatograph. The following mobile phases were applied: Phase A – 0.1% formic acid + 5% acetonitrile; Phase B – acetonitrile + 0.1% formic acid + 10% deionized water. The sample was introduced at a flow rate of 4 µl/min. The gradient concentration for Phase B was measured as follows: 0% at 3 minutes, 60% at 12-18 minutes, and 0% at 20 minutes. Samples were injected into the column at a volume of 2 µl using a Micro WPS device. The obtained results were analyzed via mass spectrometry, accounting for the ionization source, drying gas flow and temperature, fragmentor voltage, ionization method, and mass range.

Analysis of the Results. The quantitative composition of fatty acids, such as succinic, acetic, propionic, and lactic acids, as well as 3-phenyllactate, in the culture liquids of the five studied strains was determined using the HPLC method. The acetic acid content in the culture liquid of *L. plantarum* 3G was the highest at 0.44 mg/ml, while the succinic acid content was 0.32 mg/ml. The *L. fermentum* LF1 strain was the leader in lactic acid production, with its culture liquid containing 22.79 mg/ml of lactic acid. In the culture liquid of *L. brevis* 4G, the succinic acid content was 0.18 mg/ml; for *P. acidilactici* 1G, the acetic acid was 0.28 mg/ml; and for the *P.*

acidilactici 2G strain, a lactic acid content of 11.37 mg/ml was identified. The *L. plantarum* 3G strain recorded the highest level of 3-phenyllactate at 1.85 mg/ml (Table 1).

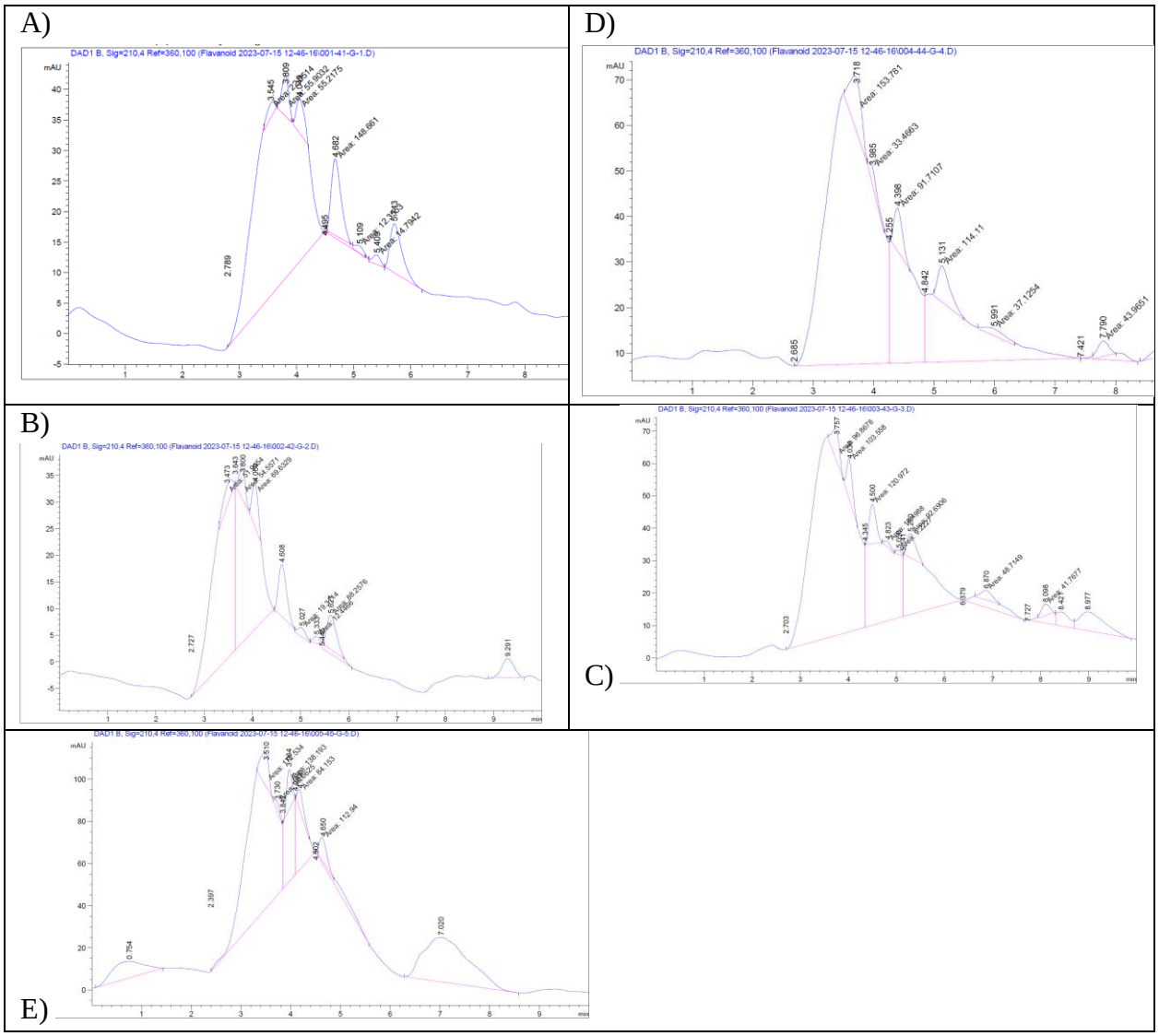


Figure 1. Quantitative analysis of antimicrobial metabolites in the culture liquid of LABs using the High-Performance Liquid Chromatography (HPLC) method.A) L.brevis 4G, B) L.fermentum L.F1, C) L.plantarum 3G, D) P.acidilactici 1G, E) P.acidilactici.

Table 1.

Content of organic acids in the culture liquid of the strains

Organic acids	*L.brevis 4G*	*L.fe rmentum L.F1*	*L.plantarum 3G*	*P.acidilactici 1G*	*P.acidilactici 2G*	Control MRS broth
	Concentration, mg/ml					
Succinic acid	0,18	0,23	0,32	0,11	0,10	0,31±0,05

Lactic acid	1,49	22,79	2,80	4,17	11,37	0,62±0,02
Acetic acid	0,005	0,04	0,44	0,28	0,03	0,34±0,03
Propionic acid	0,006	0,005	0,0070	0,002	0,006	0,72±0,09
3-phenyllactic acid	0.69	0.82	1.85	0.84	1,28	0.39±0,03

During the research, a qualitative analysis of antimicrobial metabolites, specifically succinic acid and 3-phenyllactate, found in the culture liquid of local LAB (Lactic Acid Bacteria) strains was performed using an Agilent 6520B Series Chip-Q-TOF mass spectrometer. In the analysis of metabolites from the *P. acidilactici* 1G strain, succinic acid was identified within the retention time range of 1.8–1.9 minutes. Given that the base peak in the spectrum was [m/z] = 118.11, it is highly probable that this represents the molecular ion ($M+H^+$) of succinic acid. During the fragmentation process (MS/MS), succinic acid dissociates into various fragments: m/z = 72, representing the fragment after the loss of a CO_2 group, and m/z = 102, a fragment characteristic of the carboxylic acid moiety (Figure 2).

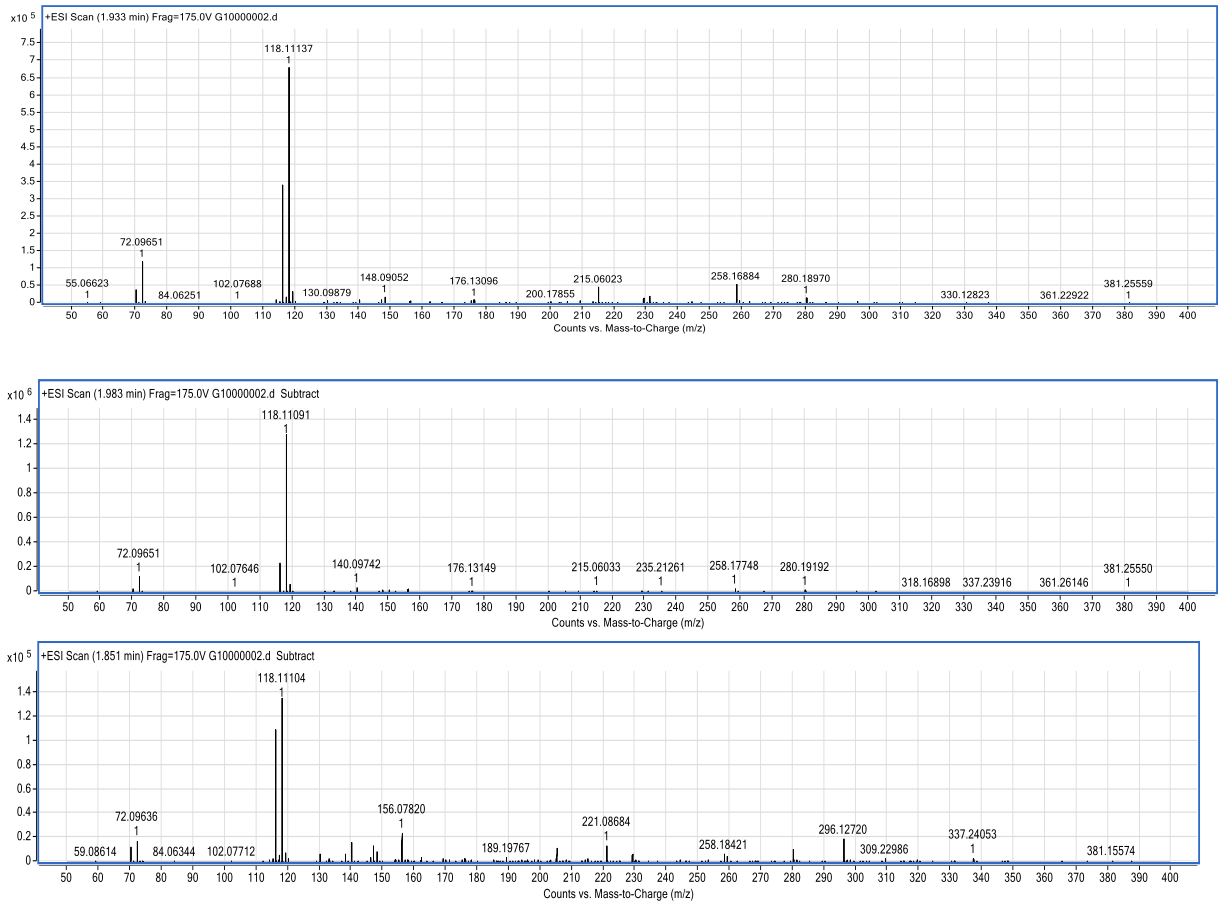


Figure 2. Qualitative analysis of succinic acid in the P. acidilactici 1G strain culture liquid using Chip-Q-TOF mass spectrometry.

Taking into account the presence of peaks at m/z 72.09651 and 102.07688, succinic acid was identified by comparison with the **HMDB (Human Metabolome Database)** online database

(https://hmdb.ca/). In the process of identifying the **3-phenyllactate** metabolite of the *P. acidilactici* 2G strain, one of the main peaks detected between 2.5 and 2.8 minutes was found to be [m/z] = 166.12. Comparison with the HMDB database confirmed its consistency with 3-phenyllactate (Figure 3).

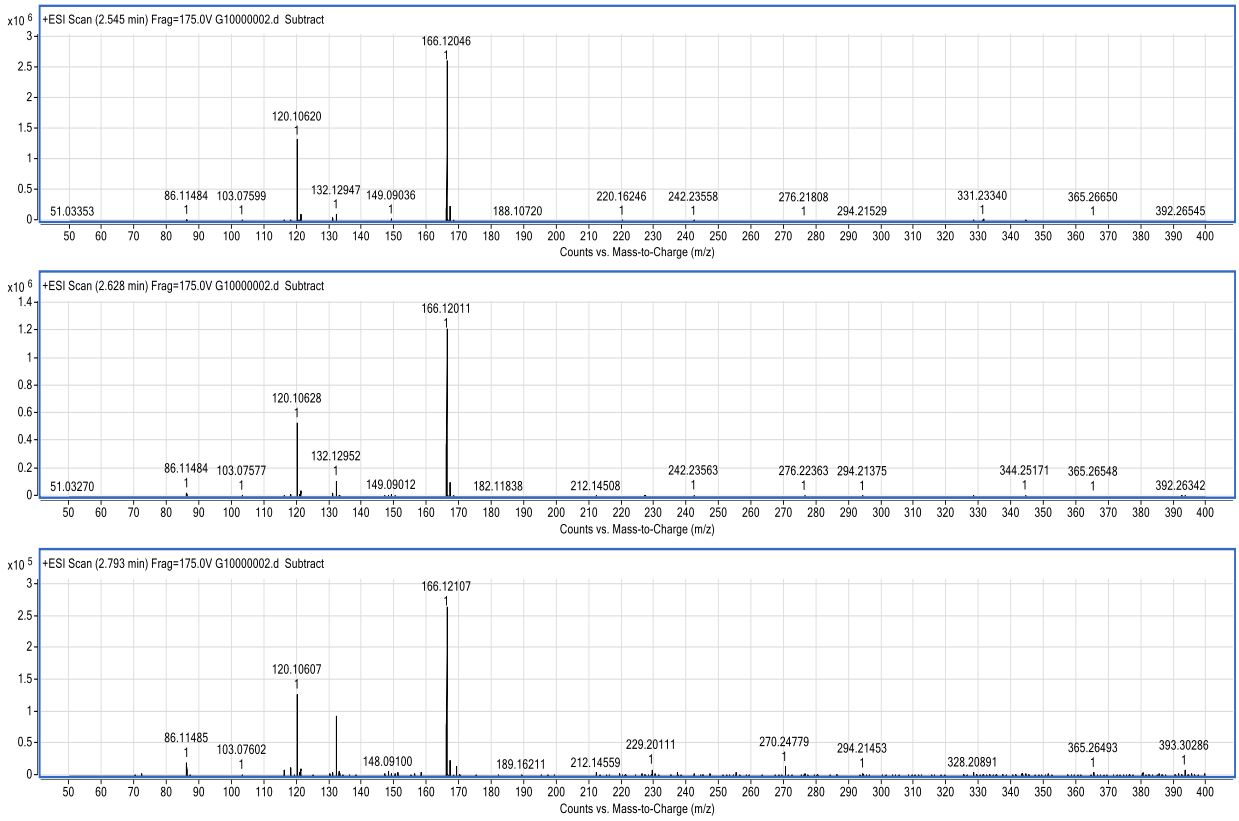
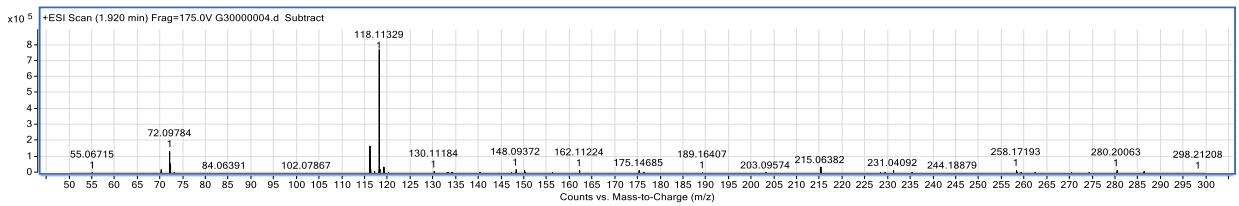


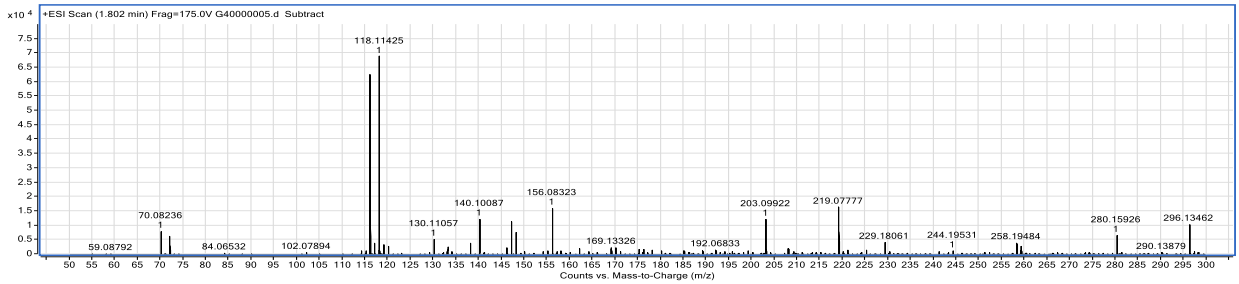
Figure 3. Chip-Q-TOF mass spectrometric analysis of 3-phenyllactate in the culture liquid of the P. acidilactici 2G strain.

Qualitative analysis of *L. plantarum* 3G, *L. brevis* 4G, and *L. fermentum* LF1 strains revealed a major spectral peak at [m/z] = 118.11 at a retention time of 1.9 minutes, similar to the *P. acidilactici* 1G strain. Based on the presence of mass fragments at m/z 72.09651 and 102.07688, the formation of succinic acid was confirmed. The results were verified by comparison with the **HMDB (Human Metabolome Database)** online database (https://hmdb.ca/) (Figure 4)

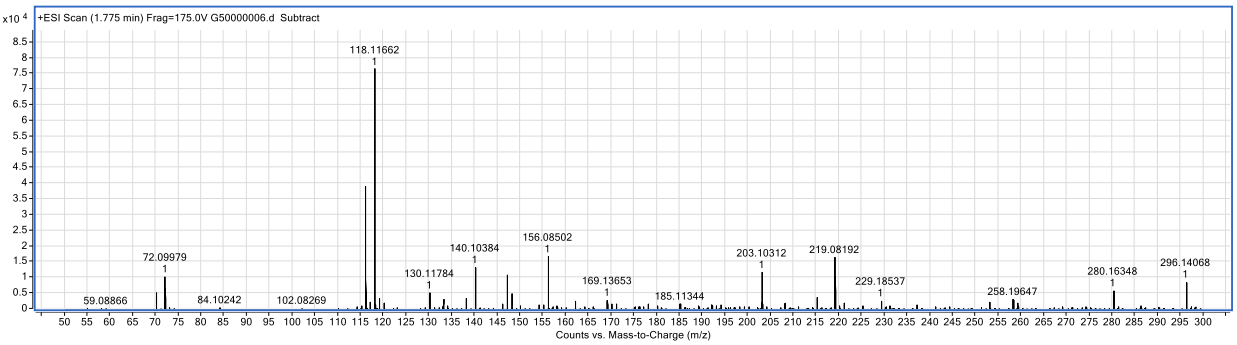
A)



B)



D)



4-pic. CHIP-Q-TOF mass spektrofotometr usulida A) L.plantarum 3G, B) L.brevis 4G, D) L fermentum LF1 shtammining qahrabo kislotasi sifat tahlili

Based on these findings, we concluded that all the studied strains produce high amounts of succinic acid, and the *P. acidilactici* 2G strain, in particular, contains 3-phenyllactic acid.

Conclusion.

As probiotics, these strains can be effective tools in maintaining the balance of human intestinal microbiota, eliminating dysbacteriosis, and restoring microflora after antibiotic use. Lactic and acetic acids create a natural barrier against pathogenic bacteria, specifically *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and fungi/yeasts such as *Candida albicans*, which is crucial in preventing infectious diseases. Furthermore, 3-phenyllactic acid exhibits potent antifungal and antioxidant effects, possessing the ability to reduce inflammatory processes, neutralize free radicals, and support cellular stress defense mechanisms. In this regard, these strains may also hold therapeutic significance in treating skin diseases (e.g., dermatitis, eczema), oral cavity infections, and maintaining women's urogenital health.

Additionally, the metabolites of *L. plantarum* 3G and *L. fermentum* LF1 exert immunomodulatory effects, activating the body's natural defense system, reducing allergic reactions, and enabling their use as a complex therapeutic agent in chronic inflammatory diseases (e.g., irritable bowel syndrome, metabolic syndrome). Consequently, the development of next-generation probiotic preparations, biotherapeutic agents, functional foods, and pharmaceutical dietary supplements based on these strains holds great practical importance in medicine, serving as a natural and safe alternative to antibiotics. The *L. plantarum* 3G and *L. fermentum* LF1 strains stood out as promising probiotic microorganisms producing high amounts of organic lactic and acetic acids. It was determined that their metabolites, especially 3-phenyllactic acid, along with the antimicrobial and antifungal properties of lactic and acetic acids, possess significant biotechnological value.

REFERENCES

1. Borges S, Barbosa J, Silva J, Teixeira P. Evaluation of characteristics of Pediococcus spp. to be used as a vaginal probiotic. J Appl Microbiol. 2013 Aug;115(2):527-38. doi: 10.1111/jam.12232. Epub 2013 May 16. PMID: 23611355.
2. Vazquez-Munoz R, Dongari-Bagtzoglou A. Anticandidal activities by Lactobacillus species: an update on mechanisms of action. Front Oral Health. 2021;2:689382.
3. Hoxha R, Todorov D, Hinkov A, Shishkova K, Evstatieva Y, Nikolova D. In vitro screening of antiviral activity of lactic acid bacteria isolated from traditional fermented foods. Microbiology Research. 2023;14(1):333-342.

4. Jaleel S, Kiliç AO. Antimicrobial action of isolated probiotic Lactobacillus plantarum from different fermented dairy products from Trabzon city. Research Journal of Pharmacy and Technology. 2020;13(5):2445-2451.
5. Stoyanova LG, Ustyugova EA, Netrusov AI. Antimicrobial metabolites of lactic acid bacteria: diversity and properties. Applied Biochemistry and Microbiology. 2012;48(3):259-275. (Original Russian: Л. Г. Стоянова, Е. А. Устюгова, А. И. Нетрусов. Антимикробные метаболиты молочнокислых бактерий: разнообразие и свойства. Прикладная биохимия и микробиология, 2012, том 48, № 3, с. 259–275.)
6. Cortés-Zavaleta O, López-Malo A, Hernández-Mendoza A, García HS. Antifungal activity of lactobacilli and its relationship with 3-phenyllactic acid production. International Journal of Food Microbiology. 2014;173:30-35.
7. Abdulakhadova GS, et al. Analysis of lactic acid content in lactobacilli using spectrophotometric method. Science and Innovation. 2025;4:182-186.