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Original Research

Isolation and Characterization of Lactic Acid Bacteria From Diverse Plant Sources With Evaluation of Antimicrobial Activity

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Abstract

Background: Excessive use of mineral fertilizers and pesticides in agriculture exacerbates environmental problems. In this context, the application of lactic acid bacteria (LAB) as an environmentally safe alternative has become increasingly relevant. **Methods:** In this study, bacteria were isolated from medicinal and food plants using morphological characterization, Gram staining, and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS); meanwhile, molecular-genetic identification was performed based on 16S rRNA gene analysis. Antimicrobial activity was assessed using several assays, and organic acids were quantified by high-performance liquid chromatography (HPLC). **Results:** In total, 30 LAB isolates were obtained from 30 medicinal and food plant species in Uzbekistan. Among these, *Lactiplantibacillus plantarum* M1, *Pediococcus pentosaceus* A2, and *Enterococcus faecium* A2 were studied in detail. All thirty isolates underwent preliminary screening, and strains exhibiting the strongest inhibitory effects against phytopathogenic fungi and bacteria were identified. Therefore, the three strains with the highest antifungal and antibacterial activities were selected for further study. The M1 strain produced 103.1 µg/mL of lactic acid, the *P. pentosaceus* A2 strain produced 139.2 µg/mL, and the *E. faecium* A2 strain produced 141.1 µg/mL. In addition, the production of propionic, acetic, succinic, and oxalic acids was confirmed. The selected strains also produced a compound with pronounced antimicrobial activity, 3-phenyllactic acid (PLA). **Conclusions:** This study identified three lactic acid bacterial strains with promising antimicrobial activity against phytopathogenic fungi and bacteria. Their production of organic acids, including lactic acid and 3-phenyllactic acid (PLA), may contribute to their inhibitory effects. These findings highlight the potential of plant-derived LAB as environmentally friendly agents for plant disease management.

Keywords: lactic acid bacteria; biocontrol; antibacterial activity; antifungal activity; agriculture

1. Introduction

Currently, conventional agriculture relies heavily on mineral fertilizers and pesticides to increase crop yields [1]. However, excessive use of these chemical agents disrupts ecological balance, contaminates soil and water resources, and may adversely affect human health [2,3]. In this context, the concept of sustainable agriculture, aimed at developing organic farming, enhancing soil fertility, and improving environmental quality, has gained increasing global relevance [4]. The development of pathogen resistance and the accumulation of pesticides in soil and plants are key factors limiting the widespread application of chemical agents. Sustainable agriculture can be defined not only in general terms but also through microbiological and biological approaches. For example, the use of beneficial microorganisms, biocontrol agents, and probiotic strains to maintain soil and plant health has emerged as an effective strategy for enhancing sustainability. Among these, lactic acid bacteria (LAB) offer several advantages over *Bacillus* and *Pseudomonas* strains due to the associated high safety (non-toxicity and generally recognized as safe (GRAS) status), mild and stable antagonistic activity, and potential applications in probiotic and functional foods.

Interactions between plants and microorganisms are integral to agroecosystem resilience. Agrobiotechnologies based on the use of beneficial microorganisms help improve soil fertility and protect plants from various biotic and abiotic stresses. In particular, probiotic microorganisms, including LAB, play an important role in stimulating plant growth, enhancing nutrient uptake, and strengthening plant defense mechanisms [5]. The metabolic simplicity of LAB, the associated ability of these microorganisms to utilize diverse carbon sources, and the high environmental safety make LAB promising candidates for widespread agricultural use. Indeed, by producing organic acids, hydrogen peroxide, and bacteriocins, LAB can improve soil health, optimize plant nutrition, and effectively suppress the development of phytopathogens [6]. In particular, the production of organic acids and 3-phenyllactic acid (PLA) plays an important role in enhancing soil biological activity, optimizing plant nutrition processes, and effectively suppressing the development of phytopathogenic microorgan-

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isms. Recent studies have reported the widespread presence of LAB in the rhizosphere, phyllosphere, and endosphere of plants [7]. For example, species of the genus *Lactiplantibacillus*, particularly *Lactiplantibacillus plantarum*, can function as epiphytes and endophytes at various stages of plant ontogeny, promoting growth and strengthening plant defense systems [8]. The antagonistic activity of LAB against phytopathogens is primarily attributed to the synthesis of biologically active compounds, including organic acids, bacteriocins, hydrogen peroxide, fatty acids, and other low-molecular-weight metabolites, which disrupt pathogen cell membranes and inhibit pathogen growth [9]. For instance, strains of the genus *Pediococcus*, particularly *Pediococcus pentosaceus* SK25, produce PLA, which effectively suppresses the mycelial growth of *Penicillium expansum* [10]. Similarly, the supernatants of *Lactobacillus plantarum* TR7 and TR71 strains exhibit pronounced antifungal activity against *Aspergillus flavus* and *P. expansum* [11]. Thus, LAB represent a promising biotechnological resource for sustainable agriculture, enhancing soil fertility and protecting crops from phytopathogens, and are important candidates in the development of environmentally safe biocontrol agents [12].

Therefore, this study aimed to investigate the potential of selected LAB strains as environmentally safe biocontrol agents in sustainable agriculture. Particular attention is given to their role in improving plant growth and soil health through their antagonistic activity against phytopathogenic microorganisms. Thus, we hypothesize that LAB strains, owing to their ability to produce biologically active metabolites, including organic acids and PLA, can effectively suppress phytopathogens and enhance plant resilience under agroecosystem conditions.

2. Materials and Methods

2.1 Plant Samples

Under the climatic conditions of Uzbekistan, samples were collected from the following plant species: *Mangifera indica*, *Vaccinium uliginosum*, *Crassula* spp., *Mentha piperita*, *Brassica oleracea*, *Elaeagnus angustifolia*, *Ribes nigrum*, *Ficus carica*, *Prunus armeniaca*, *Vitis vinifera*, *Anethum graveolens*, *Ginkgo biloba*, *Juniperus communis*, *Olea europaea*, *Valeriana officinalis*, *Olea* spp., *Amaranthus caudatus*, *Passiflora edulis*, *Folium cydonii*, *Solanum dulcamara*, *Ananas comosus*, *Calendula officinalis*, *Rheum officinale*, *Rosa canina*, *Cepa matura*, *Aloe vera*, *Taraxacum officinale*, *Magnolia grandiflora*, *Lactuca sativa*, and *Ficus* spp.

Samples were collected from both vegetative organs (roots, stems, leaves) and generative organs (flowers, fruits). Initially, several samples were taken from each plant species and organ. However, after isolation and pure-culture separation, only one pure strain from each sample was selected for subsequent analyses. Plant samples were collected from cultivated and wild species growing under various ecological conditions. The study area has a temperate continental climate, characterized by warm summers, relatively cold winters, and moderate precipitation. The research encompassed fruit trees, vegetable crops, medicinal plants, and wild species. The results showed that LAB were recovered primarily from vegetative organs, such as leaves and stems, rather than from generative organs such as flowers and fruits.

2.2 Culture Media

De Man, Rogosa, and Sharpe (MRS) medium is a widely used selective medium for cultivating and isolating LAB; the nutrient composition and vitamin content in MRS medium provide favorable conditions for LAB growth while partially inhibiting the growth of other microorganisms. The MRS medium had the following composition (g/L): peptone (10.0), meat extract (10.0), yeast extract (5.0), Tween-80 (1.06), sodium acetate (5.0), ammonium citrate (2.0), glucose (20.0), $MgSO_4 \cdot 7H_2O$ (0.2), $MnSO_4 \cdot 4H_2O$ (0.05), and L-cysteine (0.2); the pH was adjusted to 6.2–6.5. The medium was sterilized at 121 °C for 15 minutes at 1 atm. The MRS agar contained all components of MRS broth, with the addition of agar at 18.0 g/L.

Sabouraud agar medium was composed as follows (g/L): dextrose (glucose; 40.0), mycological peptone (10.0), and agar (15.0); the pH was adjusted to 5.6 ± 0.2. Sterilization was performed at 121 °C for 15 minutes at 1 atm. Bacteria inoculated into the bromocresol purple (BC) medium caused a color shift from blue to yellow due to lactic acid production. This color change was used as a criterion for identifying and screening LAB. A 1.6% BC solution was used as an indicator, adding 1.4 mL per 1 L of medium; carbohydrate fermentation by LAB was evaluated by a color change from purple to yellow. All culture media were sterilized at 121 °C for 15 minutes at 1 atm.

2.3 Preliminary Sample Treatment

During sample processing, aliquots were taken from disinfected plant surfaces and incubated in MRS medium to check for contamination. Collected samples were subjected to sequential surface treatments to reduce microbial contamination using 3% hydrogen peroxide, 70% ethanol, and sterile distilled water. From each plant, 1 g of material was aseptically collected and placed into liquid MRS medium (HiMedia, India).

2.4 Isolation of LAB

For bacterial isolation, MRS agar supplemented with 0.0025% neutral red indicator was used. Inoculated Petri dishes were incubated at 37 °C for 48 hours. After incubation, yellowish colonies with distinct morphological characteristics were selected and purified by repeated streaking to obtain pure isolates. A total of 3–5 colonies were randomly selected from each plate.

2.5 Bacterial Identification

The isolated strains were characterized based on the associated morphological and cultural features, examined microscopically, and assessed for physiological and biochemical properties. Selected strains were further identified using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). The morpho-cultural characteristics of the isolated bacterial strains, including nutrient preferences, optimal growth temperature, pH range, and salt tolerance, were determined through systematic laboratory experiments. Growth conditions for each strain are presented in tabular form to allow precise phenotypic identification and assessment of ecological adaptability. Carbohydrate fermentation was tested using Giss media containing arabinose, fructose, melibiose, trehalose, mannitol, galactose, adonitol, sorbitol, and Kligler's medium containing glucose and lactose. Fermentation was detected by the "color series" method, based on a yellow color change, and the results were compared with those reported in *Bergey's Manual of Systematic Bacteriology*. Subsequently, the nucleotide sequence of the 16S rRNA gene was determined [13]. Phylogenetic analysis was performed using MEGA version 6.06 (Molecular Evolutionary Genetics Analysis, Center for Evolutionary Medicine and Informatics, Tempe, Arizona, USA) with the maximum likelihood method based on the Tamura–Nei model [14].

2.6 Determination of Antimicrobial Activity

2.6.1 Antifungal Activity

The antagonistic activity of the isolated LAB strains against the phytopathogens *Alternaria alternata*, *Verticillium dahliae*, *Fusarium* spp., *Aspergillus flavus*, and *Aspergillus oryzae* from the laboratory collection was evaluated using the agar block method. LAB were cultured on MRS agar at 37 °C for 48 hours. Phytopathogenic fungi were grown on solid Czapek medium and incubated at 28 °C for 6 days. Agar blocks with a diameter of 6 mm, cut from LAB colonies, were placed on the surface of fungal colonies. Antifungal activity was assessed based on the inhibition of fungal mycelial growth. All experiments were performed in triplicate.

2.6.2 Antibacterial Activity

The antibacterial activity of LAB strains against pathogenic microorganisms was evaluated using the agar block method. Thus, LAB were spotted onto the surface of Petri dishes containing MPA (meat–peptone agar) and incubated under anaerobic conditions at 37 °C for 48 hours. Subsequently, laboratory test strains (*Pseudomonas syringae*, *P. viridiflava*, *Erwinia carotovora*, *Ralstonia solanacearum*) were prepared as suspensions at a concentration of 10^6 CFU (colony-forming units)/mL and added to a semi-solid (0.75%) agar medium containing heart–brain extract, which served as the indicator medium. Then, the medium with the indicator strains was poured over the bacterial spots and incubated at 37 °C for 24 hours. Antibacterial activity was assessed by measuring the diameter of the bacterial growth inhibition zones (mm); strains forming zones greater than 1 mm were considered positive.

2.7 Determination of Organic Acids

The concentrations of lactic, acetic, and propionic acids in the culture supernatants of LAB strains were determined using an Agilent Technologies 1200 Series high-performance liquid chromatography (HPLC) system (Agilent Technologies Inc., Santa Clara, CA, USA). Briefly, 50 µL of culture was inoculated into 5 mL of MRS medium and incubated at 37 °C for 18 hours. MRS medium without bacterial inoculation was used as a control. Analyses were performed on an Agilent Technologies 1200 chromatograph using a C18 column (5 µm, 4.6 × 250 mm) operated in isocratic mode. The mobile phase consisted of 0.1% orthophosphoric acid and acetonitrile (95:5). Detection was carried out at 210 nm, the column temperature was maintained at 30 °C, and the injection volume was 5 µL. Proteins were precipitated by centrifugation at 8000 rpm for 10 minutes.

2.8 Statistical Analyses

Statistical analyses and exponential curve fitting were performed using Origin 8.6 software (Microcal Software Inc., Northampton, MA, USA). Results are expressed as the mean ± standard error (SE). One-way analysis of variance (ANOVA) was performed to determine the statistical significance of the results among strains.

3. Results

3.1 Isolation of LAB From Medicinal and Edible Plants

LAB were isolated from various organs of medicinal and edible plants growing under the environmental conditions of Uzbekistan. Samples of leaves, stems, roots, flowers, and fruits were collected to characterize the microbiological composition and to identify potentially beneficial LAB strains (Table 1). Sampling multiple plant organs enabled assessment of the diversity and spatial distribution of LAB within plant environments, which is important for the subsequent selection of strains with probiotic potential.

A total of 30 bacterial isolates were obtained. Based on their morphological characteristics (Table 2) and Gram-staining results, three isolates associated with LAB from Mangifera indica, Juniperus communis, and Aloe vera were selected for further investigation (Fig. 1). These three isolates were chosen as the most promising candidates for subsequent analyses in accordance with the objectives of the study. Only the 16S rRNA gene sequences of these three selected isolates were deposited in the NCBI database.

Based on the associated morphological characteristics, isolates forming smooth, medium- to large-sized colonies that were nearly white in color were selected. Gram staining confirmed that the cells were Gram-positive,

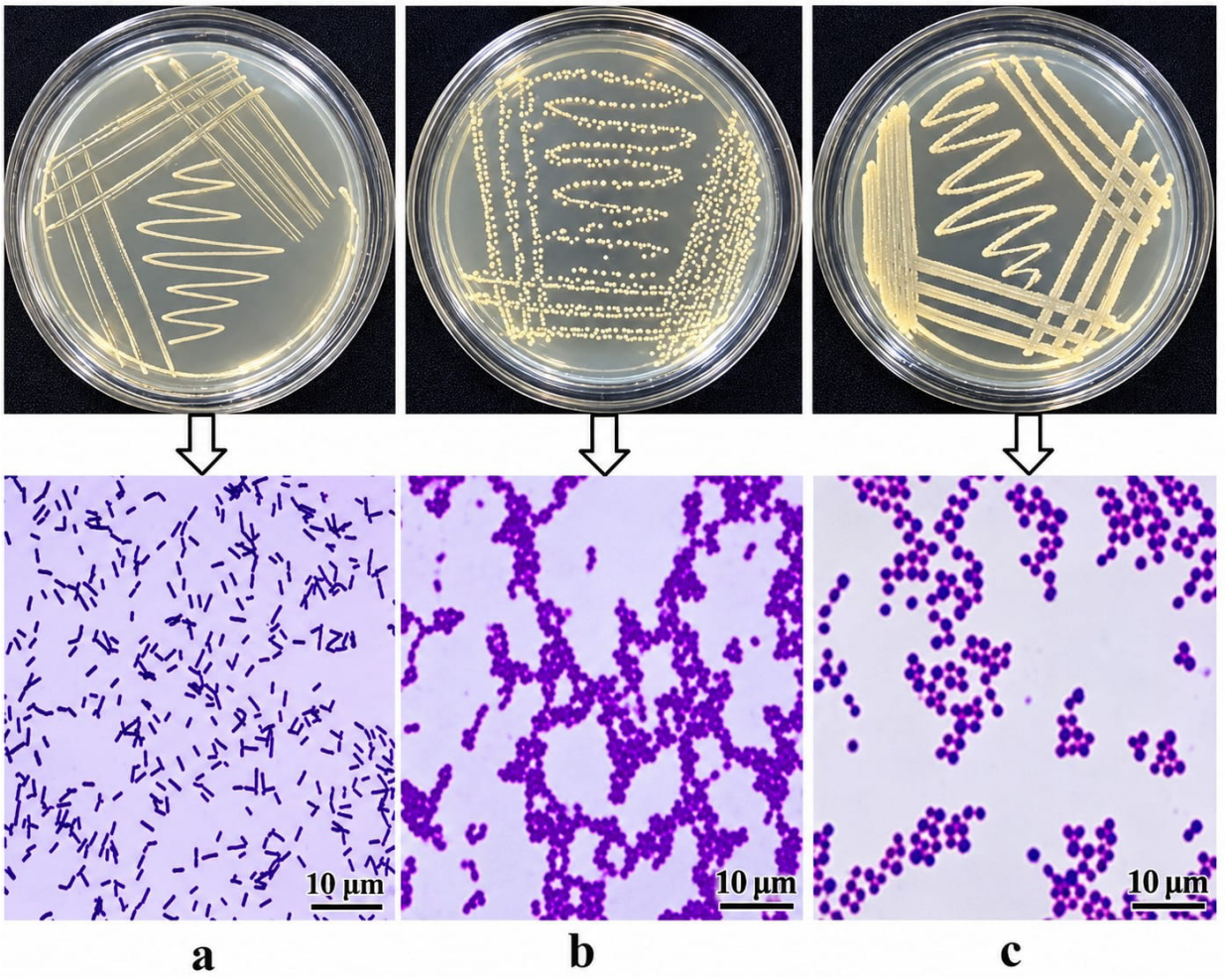


Fig. 1. Growth and Gram staining characteristics of isolated LAB strains in De Man, Rogosa, and Sharpe (MRS) medium. (a) *Lactiplantibacillus plantarum* M1; (b) *Pediococcus pentosaceus* A2; (c) *Enterococcus faecium* A2. Scale bars = 10 µm.

which is a key feature of LAB. Isolates belonging to the genus *Lactiplantibacillus* were short, rod-shaped, Gram-positive, catalase-negative, and non-motile. *Pediococcus* isolates were spherical, occurring singly or in tetrads, Gram-positive, and catalase-negative. *Enterococcus* isolates were ovoid, forming pairs or chains, did not produce spores or capsules, and exhibited facultative anaerobic growth.

To determine the optimal temperature and pH ranges, each strain was cultivated in MRS agar or broth at temperatures from 10 to 45 °C in 5 °C increments (10, 15, 20, 25, 30, 35, 37, 40, and 45 °C), and growth activity was monitored at each stage. pH tolerance was assessed over a range of 3.0 to 9.0 in 0.5-unit increments. Bacterial growth was evaluated quantitatively and visually based on colony formation, colony morphology, and optical density (OD) measurements. The optimal growth temperature and pH values were defined as the conditions under which the highest growth activity was observed. In addition, the morphological characteristics presented in the table, including Gram-staining results, cell shape, and colony morphology, were determined using standard microbiological identification methods.

The carbohydrate fermentation profiles of these strains were determined using Giss and Kligler media and compared with the corresponding descriptions in Bergey's Manual (Table 3).

The species-specific and stable biochemical characteristics of the three strains allowed for their precise identification.

3.2 Molecular–Genetic Identification of LAB Strains

For the molecular genetic identification of the LAB strains, universal 16S rRNA gene primers were used. Three selected isolates were subjected to 16S rRNA gene sequencing, and the resulting sequences were deposited in the NCBI database under the following accession numbers: *Lactiplantibacillus plantarum* M1 — MZ434948; *Pediococcus pentosaceus* A2 — OQ430813; and *Enterococcus faecium* A2 — OQ430814. In addition, phylogenetic analyses of these strains were performed (Fig. 2).

Table 1. Plants used for the isolation of lactic acid bacteria (LAB) strains.

№	Scientific (Latin) name	Plant part used	Isolated bacteria
1	*Mangifera indica*	Fruit	*Lactobacillus* sp.
2	*Vaccinium uliginosum*	Leaf, fruit	*Lactobacillus* sp.
3	*Crassula*	Leaf	*Lactobacillus* sp.
4	*Mentha piperita*	Leaf, stem	*Lactobacillus* sp.
5	*Brassica oleracea*	Leaf, flower	*Lactobacillus* sp.
6	*Elaeagnus angustifolia*	Leaf, fruit	*Lactobacillus* sp.
7	*Ribes nigrum*	Leaf, fruit	*Lactobacillus* sp.
8	*Ficus carica*	Leaf, fruit	*Lactobacillus* sp.
9	*Prunus armeniaca*	Leaf, fruit	*Lactobacillus* sp.
10	*Vitis vinifera*	Leaf, fruit	*Lactobacillus* sp.
11	*Anethum graveolens*	Leaf	*Lactobacillus* sp.
12	*Ginkgo biloba*	Leaf	*Pediococcus* sp.
13	*Juniperus communis*	Leaf (needle)	*Pediococcus* sp.
14	*Olea europaea*	Leaf, fruit	*Pediococcus* sp.
15	*Valeriana officinalis*	Root	*Pediococcus* sp.
16	*Olea*	Fruit	*Pediococcus* sp.
17	*Amaranthus caudatus*	Leaf, flower	*Pediococcus* sp.
18	*Passiflora edulis*	Leaf, fruit	*Pediococcus* sp.
19	*Folium cydonii*	Leaf	*Pediococcus* sp.
20	*Solanum dulcamara*	Leaf, fruit	*Pediococcus* sp.
21	*Ananas comosus*	Leaf, fruit	*Pediococcus* sp.
22	*Calendula officinalis*	Leaf, flower	*Enterococcus* sp.
23	*Rheum officinale*	Root, stem	*Enterococcus* sp.
24	*Rosa canina*	Leaf, fruit	*Enterococcus* sp.
25	*Cepa matura*	Leaf	*Enterococcus* sp.
26	*Aloe vera*	Leaf	*Enterococcus* sp.
27	*Taraxacum officinale*	Leaf, flower	*Enterococcus* sp.
28	*Magnolia grandiflora*	Leaf, flower	*Enterococcus* sp.
29	*Lactuca sativa*	Leaf	*Weissella* sp.
30	*Ficus*	Fruit	*Weissella* sp.

Note: The plants listed in the table have been adapted to the environmental conditions of Uzbekistan, and samples were collected from various parts of each plant.

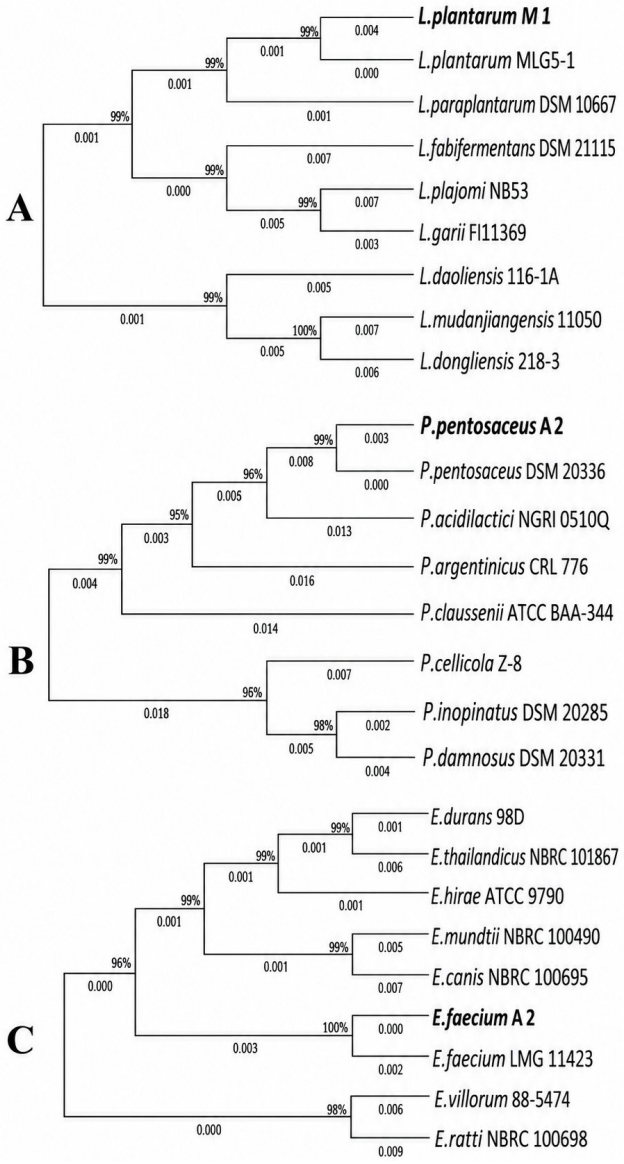


Fig. 2. The phylogenetic tree was constructed using the Maximum Likelihood method based on the Tamura–Nei model in MEGA version 6.06. (A) *Lactiplantibacillus plantarum* M1; (B) *Pediococcus pentosaceus* A2; (C) *Enterococcus faecium* A2.

3.3 Resistance of LAB strains to Different NaCl Concentrations

The salt tolerance of selected LAB strains was investigated. The growth of these strains was assessed in nutrient media containing 0%, 2%, 4%, and 6.5% NaCl (Table 4). The results showed that the *L. plantarum* M1 strain grew actively in media containing 2–4% and 6.5% NaCl. In a salt-free medium, the titer of this strain was 9×10^9 CFU/mL; in 2% NaCl, 7×10^9 CFU/mL; in 4% NaCl, 5×10^9 CFU/mL; in 6.5% NaCl, 3×10^8 CFU/mL. The *P. pentosaceus* A2 strain also showed tolerance to a certain level of salt: in a salt-free medium, 8×10^9 CFU/mL; in 2% NaCl, 7×10^9 CFU/mL; in 4% NaCl, 6×10^9 CFU/mL; in 6.5% NaCl, 4×10^8 CFU/mL. The *E. faecium* A2 strain was more sensitive to high salt concentrations: in a salt-free medium, the titer was 9×10^9 CFU/mL; in 2% NaCl, 8×10^9 CFU/mL; in 4% NaCl, 6×10^9 CFU/mL; in 6.5% NaCl, 4×10^7 CFU/mL.

The results show that salt tolerance in LAB strains varies among strains, with some remaining viable even at high NaCl concentrations. This characteristic is important for the application of LAB strains in various biotechnological processes, particularly in the food industry as probiotics.

3.4 Ability of LAB to Produce Enzymes Associated With Pathogenicity

An important criterion for selecting probiotic microorganisms is the ability to produce enzymes associated with pathogenicity. This is because microorganisms intended for use as probiotics must be safe for humans or animals; there-

Table 2. Morpho-cultural characteristics of the isolated bacteria.

Characteristic	Microorganisms		
	Lactiplantibacillus plantarum M1	*Pediococcus pentosaceus* A2	*Enterococcus faecium* A2
Gram staining	+	+	+
Cell shape	Rod-shaped (bacillus)	Spherical (coccus)	Spherical (coccus)
Cell arrangement	Single or short chains	Tetrads (pairs of pairs)	Pairs or short chains
Spore formation	Does not form spores	Does not form spores	Does not form spores
Motility	Non-motile	Non-motile	Non-motile
Growth medium	MRS agar/broth	MRS agar/broth	MRS agar/broth
Colony shape	Round	Round	Round
Colony edge	Smooth	Smooth	Smooth
Colony surface	Smooth, shiny	Smooth	Smooth, slightly granular
Colony color	Whitish–milky	Whitish	Whitish–milky
Temperature tolerance	15–45 °C	15–45 °C	10–45 °C
Optimal temperature	30–37 °C	30–37 °C	35–37 °C
Optimal pH	5.5–6.5	5.5–6.5	6.0–7.0
Minimal pH	3.2–3.5	3.8–4.2	4.0–4.5
pH tolerance	3.5–6.5	4.0–7.0	4.5–9.0
Oxygen requirement	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe

MRS, De Man, Rogosa, and Sharpe; (+), a positive result.

Table 3. Carbohydrate fermentation spectrum of LAB strains.

Carbohydrates	*L. plantarum* M1	*P. pentosaceus* A2	*E. faecium* A2
Glucose	+	+	+
Fructose	+	±	+
Mannose	+	+	+
Galactose	+	±	+
Ribose	+	+	+
Lactose	+	+	+
Cellobiose	+	±	+
Maltose	+	+	+
Sucrose	+	+	+
Mannitol	+	±	±
Sorbitol	+	+	+
Melicitose	+	–	+
Salicin	+	+	+
Trehalose	+	±	+
Melibiose	-	+	±
Raffinose	-	–	±
Arabinose	-	–	–
Rhamnose	-	–	±
Xylose	-	±	–

Note: (+), actively fermented; (±), slowly fermented; (–), not fermented.

Table 4. Resistance of strains to different NaCl concentrations.

Strains	NaCl concentrations (%)			
	0 (%)	2 (%)	4 (%)	6.5 (%)
L. plantarum M1	9×10^9	7×10^9	5×10^9	3×10^8
P. pentosaceus A2	8×10^9	7×10^9	6×10^9	4×10^8
E. faecium A2	9×10^9	8×10^9	6×10^9	4×10^7

Table 5. Production of pathogenicity-associated enzymes by LAB strains.

Enzymes	Strains		
	L. plantarum M1	*P. pentosaceus* A2	*E. faecium* A2
Lecithinase	–	–	–
Catalase	–	–	–
Gelatinase	–	–	–
Hemolysin	–	–	–

Note: (–), a negative result (absence of the tested enzymatic activity under the experimental conditions).

fore, the microorganisms should not possess pathogenic traits. In the present study, the production of pathogenicity-associated enzymes was evaluated in the strains *L. plantarum* M1, *P. pentosaceus* A2, and *E. faecium* A2. The enzymes tested included lecithinase, catalase, gelatinase, and hemolysin (Table 5). These enzymes are typically produced by pathogenic microorganisms and contribute to disease development by damaging cellular or tissue structures, weakening the immune response, or enhancing toxicity [15].

The results of the study showed that all of the above-mentioned strains are incapable of producing enzymes such as lecithinase, catalase, gelatinase, and hemolysin, confirming the absence of pathogenicity factors. These strains are considered safe for human health and are regarded as promising microorganisms for use in biotechnology and

Table 6. Antifungal activity of selected LAB strains against phytopathogenic fungi.

№	Strains	*A. alternata* (mm)	*V. dahliae* (mm)	*Fusarium* sp. (mm)	*A. flavus* (mm)	*A. oryzae* (mm)
1	*L. plantarum* M1	35.0 ± 1.6	33.0 ± 1.6	33.3 ± 0.6	29.6 ± 0.3	34.3 ± 0.6
2	*P. pentosaceus* A2	25.0 ± 0.6	25.0 ± 0.3	22.0 ± 0.0	26.6 ± 0.3	–
3	*E. faecium* A2	30.0 ± 0.3	25.0 ± 0.3	25.0 ± 0.3	28.0 ± 0.5	20.0 ± 0.3

the food industry as probiotics. Among probiotic bacteria, species belonging to the genera *Lactiplantibacillus*, *Pediococcus*, and *Enterococcus* are widely used. These species are characterized by the associated ability to survive in the gastrointestinal tract, resistance to acid and bile salts, antimicrobial activity, and capacity to modulate the immune system. The absence of enzyme production, such as lecithinase, gelatinase, and hemolysin, indicates that these species lack toxic or histolytic properties. Thus, these strains are considered safe microorganisms and approach GRAS status [16]. The inability of *L. plantarum* M1, *P. pentosaceus* A2, and *E. faecium* A2 to produce enzymes associated with pathogenicity further confirms the safety of these strains. Therefore, these strains can be selected as promising microorganisms for developing probiotic preparations or for inclusion in functional food products.

3.5 Antifungal Activity

Among the isolates obtained from plants, three selected strains, *L. plantarum* M1, *P. pentosaceus* A2, and *E. faecium* A2, exhibited high antagonistic activity against phytopathogenic fungi and bacteria. The block method was employed in the experiments, during which these strains effectively inhibited the growth of hazardous phytopathogenic fungi, including *Alternaria alternata*, *Verticillium dahliae*, *Fusarium* sp., *Aspergillus flavus*, and *Aspergillus oryzae* (Table 6). Organic acids, hydrogen peroxide, and bacteriocins produced by LAB were identified as the primary biologically active compounds that suppress the development of phytopathogens. In addition, these microorganisms activated plant immune responses and enhanced resistance to external stress factors. These results indicate that the selected strains have significant potential for agricultural applications as environmentally safe biocontrol agents.

According to the study results, the three selected LAB strains (*L. plantarum* M1, *P. pentosaceus* A2, and *E. faecium* A2) exhibited varying levels of antagonistic activity against phytopathogenic fungi. The strain *L. plantarum* M1 exhibited the highest antifungal activity against all tested phytopathogens. In particular, pronounced inhibition zones were recorded against *Alternaria alternata* (35.0 ± 1.6 mm), *Verticillium dahliae* (33.0 ± 1.6 mm), and *Aspergillus oryzae* (34.3 ± 0.6 mm), indicating the strong potential of this strain as an effective biological control agent. The strain *E. faecium* A2 also demonstrated broad-spectrum antagonistic activity, producing moderate to high levels of inhibition against all tested micromycetes (*A. alternata*, 30.0 ± 0.3 mm; *A. flavus*, 28.0 ± 0.5 mm), thus showing considerable promise for application as an environmentally safe bioprotective agent. Although *P. pentosaceus* A2 exhibited relatively lower overall antifungal activity, the strain was distinguished by a strong inhibitory effect against *A. flavus* (26.6 ± 0.3 mm), suggesting a potential use as a selective biological control agent targeting specific phytopathogens. Overall, the bioactivity confirms the potential of these three strains as promising probiotic bioagents for protecting plants against phytopathogenic microorganisms.

3.6 Determination of Antibacterial Activity

According to the study results, the antagonistic properties of *L. plantarum* M1, *P. pentosaceus* A2, and *E. faecium* A2 were also evaluated against the phytopathogenic bacteria *Pseudomonas syringae*, *Pseudomonas viridiflava*, *Erwinia carotovora*, and *Ralstonia solanacearum* isolated from infected plants. The agar block method was employed to assess antagonistic activity and to evaluate the potential of the strains as biological control agents. L. plantarum M1 exhibited inhibition zones of 26.0 ± 0.3 mm against P. syringae, 28.0 ± 0.6 mm against P. viridiflava, 25.3 ± 0.4 mm against E. carotovora, and 25.0 ± 0.3 mm against R. solanacearum (Table 7). Notably, *P. pentosaceus* A2 produced pronounced inhibition zones ranging from 22.5 ± 0.7 to 25.0 ± 1.3 mm against all tested phytopathogenic bacteria and stood out as one of the most effective strains with high antagonistic activity, further enhancing its prospects as a biological control agent. The strain *E. faecium* A2 also demonstrated high antagonistic activity, forming inhibition zones ranging from 20.0 ± 1.2 to 25.3 ± 0.3 mm against the phytopathogenic bacteria; this antibacterial effect can be attributed to the ability of the strain to effectively suppress the proliferation of phytopathogenic microorganisms in plants.

The three selected strains also showed strong antagonistic activity against phytopathogenic microorganisms. The antagonistic activity of these LAB strains, isolated from plant samples, was evaluated against both opportunistic and pathogenic microorganisms. The results indicated that all strains displayed antimicrobial and antifungal effects to varying degrees, with each strain exhibiting distinct selectivity. These findings confirm that LAB effectively inhibit the growth of opportunistic and pathogenic microorganisms through the production of bacteriocins, organic acids, hydrogen peroxide, and other secondary metabolites. Moreover, plant-derived lactobacilli, owing to the associated broad-spectrum antimicrobial properties, are considered promising, environmentally safe biocontrol agents.

Table 7. Inhibition zones (mm) of the isolated strains against phytopathogenic bacteria.

№	Strains	*Pseudomonas syringae*	*Pseudomonas viridiflava*	*Erwinia carotovora*	*Ralstonia solanacearum*
1	*L. plantarum* M1	26.0 ± 0.3	28.0 ± 0.6	25.3 ± 0.4	25.0 ± 0.3
2	*P. pentosaceus* A2	25.0 ± 1.3	24.0 ± 0.6	25.0 ± 0.3	22.5 ± 0.7
3	*E. faecium* A2	24.1 ± 0.6	25.3 ± 0.3	23.0 ± 1.0	20.0 ± 1.2

3.7 Determination of Organic Acids

The culture fluids of *L. plantarum* M1, *P. pentosaceus* A2, and *E. faecium* A2 were analyzed for organic acid content using HPLC (Fig. 3). The results indicated that among the tested strains, *P. pentosaceus* A2 (43.29 µg/mL) and *E. faecium* A2 (36.88 µg/mL) produced the highest amounts of acetic acid. All strains were dominant in lactic acid synthesis (Fig. 4), with an average concentration of 13.8 µg/mL in the culture fluid. Organic acids such as acetate and lactate lower the pH of the microbial environment, creating unfavorable conditions for the growth of many phytopathogenic organisms, including bacteria and fungi. This mechanism is recognized as a key aspect of the antagonistic activity of LAB. For example, *Lactiplantibacillus plantarum* strains effectively inhibit pathogenic fungal colonies through acidification of the environment and the action of other metabolites, while also modulating the LAB microbiome and competing with pathogenic microorganisms, thereby contributing to biological control.

A review of the scientific literature indicates that organic acids synthesized by LAB not only enhance their antagonistic properties but also serve as important biostimulants that promote plant growth [17,18,19]. Many *Lactiplantibacillus* strains predominantly produce lactic acid and, in some cases, also generate acetic acid [20]. Propionic acid is generally present at low concentrations; however, propionic acid has been reported in certain strains, such as *L. paraplantarum* B23115 and *L. paracasei* B4564 [21]. Thus, the ability of the LAB strains examined in this study to produce organic acids makes these strains a potentially promising microbiological resource for plants, providing dual benefits as biostimulants and biocontrol agents. Comparative data on LAB and selected organic acids produced are presented in Table 8 [22,23,24,25,26,27].

3.8 Quantitative Analysis of PLA Activity in LAB Strains

This study investigated the antagonistic activity of the selected LAB strains against pathogenic microorganisms, their potential to synthesize organic acids, and the phenolic antifungal and antibacterial compound 3-phenyllactic acid (PLA) (Fig. 5).

PLA is considered a natural antagonistic mechanism among microorganisms, effectively inhibiting the growth and development of pathogenic species by reducing their viability and reproductive capacity. This mechanism plays a critical role in microbial competition, limiting the spread of pathogens by decreasing metabolic activity, disrupting cellular structures, and impairing key physiological functions [28]. Numerous studies have shown that PLA, as a naturally occurring compound, exhibits inhibitory effects against pathogenic bacteria and biofilm-forming microorganisms. For example, the activity of foodborne pathogens such as *Salmonella enterica* and *Escherichia coli* has been effectively suppressed by PLA [29]. Other studies have further detailed the mechanisms through which PLA inhibits microbial growth and significantly reduces biofilm formation. Indeed, studies have demonstrated that PLA exerts antagonistic effects by damaging the cell membrane, causing leakage of intracellular contents, and altering signaling pathways [30]. According to our study, the strain *L. plantarum* M1 produced the highest PLA concentration, at 26.4 µg/mL. The strain *E. faecium* A2 produced 23.8 µg/mL, while *P. pentosaceus* A2 produced 19.2 µg/mL. Similar studies have demonstrated that various LAB strains play a significant role in PLA biosynthesis through their metabolic activity. For example, *Pediococcus acidilactici* La52 [31], *Lactiplantibacillus plantarum* MSC-C2, *Pe-*

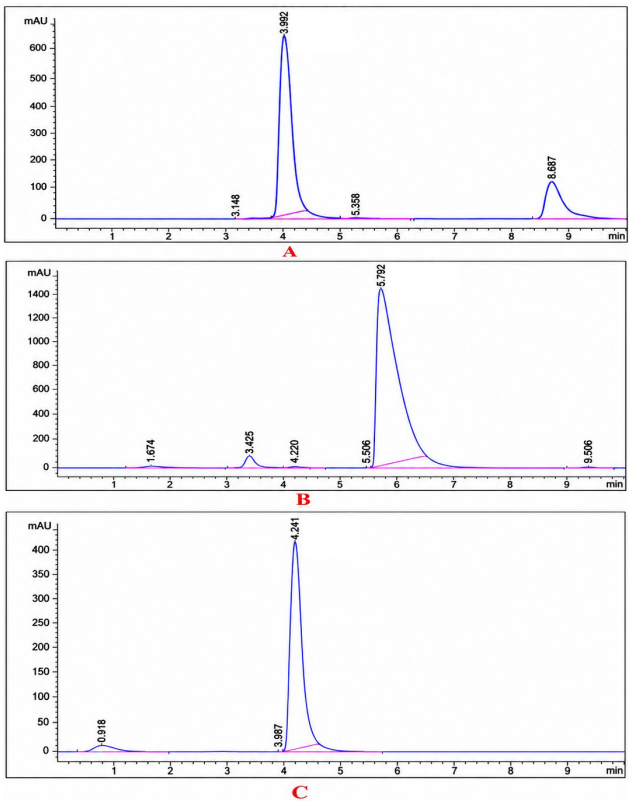


Fig. 3. High-performance liquid chromatography (HPLC) chromatogram of organic acids: (A) Lactic acid; (B) Propionic acid; (C) Acetic acid.

Table 8. LAB strains and the synthesized organic acids.

Strain	Identified bacterium	GenBank accession number	Main fermentation product	Additional organic acids	References
SBB-HI4	*Lactiplantibacillus plantarum*	PV959994	Lactic acid	Acetic, propionic, butyric acid	[22]
SBB-HI8	*Lactiplantibacillus pentosus*	PV959993	Lactic acid	Acetic, propionic, butyric acid	[22]
SBB-GT4	*Lacticaseibacillus paracasei*	PV959995	Lactic acid	Acetic, propionic, butyric acid	[22]
ChK-11	*Weissella paramesenteroides*	OQ860237	Lactic acid	Acetic acid	[23]
ChK-7	*Leuconostoc pseudomesenteroides*	OQ860222	Lactic acid	Acetic acid	[23]
FPL	*Lactobacillus plantarum*	KY883188	Lactic acid	Not determined	[24]
S.156	*Enterococcus faecium*	KM875593	Lactic acid	Not determined	[25]
s6	*Enterococcus faecium*	No data available	Lactic acid	Acetic acid, Formic acid	[26]
PA204	*Pediococcus acidilactici*	No data available	Lactic acid	Acetic acid	[27]

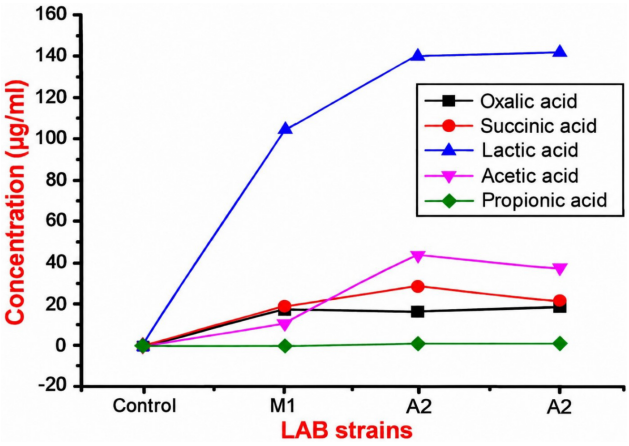


Fig. 4. Concentrations of organic acids produced by the selected LAB strains (Control, M1(*L. plantarum* M1), A2(*P. pentosaceus*), and A2(*E. faecium*)). Concentrations are expressed in µg/mL.

diococcus pentosaceus K40 [30], and other *Pediococcus acidilactici* strains [32] can produce PLA due to specific metabolic features. In these strains, PLA is synthesized from aromatic amino acids via transamination, L-lactate dehydrogenase activity, and other fermentative pathways. Thus, the metabolic activity of different LAB strains represents a key determinant of their PLA productivity, making these strains promising candidates for biotechnological processes aimed at producing high-value metabolites. The PLA synthesized by these strains effectively inhibits the growth of phytopathogenic microorganisms, limiting the development of harmful microflora in the plant rhizosphere, thereby promoting plant growth and enhancing stress tolerance. These results highlight the potential practical application of the studied strains in agriculture as both biological control agents and biostimulants.

4. Discussion

In this study, the highest PLA production observed in *Lactiplantibacillus plantarum* M1 underscores the associated strong biological activity, antibacterial potential, and significance as an environmentally safe microbial inoculant for plants.

The results indicate that LAB strains isolated under conditions in Uzbekistan exhibit high antagonistic activity against plant pathogens. In particular, *L. plantarum* M1 formed significant inhibition zones against pathogens such as *Pseudomonas syringae* and *Erwinia carotovora*, confirming its biotechnological potential for plant protection. The antimicrobial properties of LAB are primarily associated with the production of organic acids, hydrogen peroxide, PLA, and bacteriocins [33]. These metabolites limit the growth of pathogenic microorganisms by lowering environmental pH, disrupting cell walls, and impairing intracellular metabolism [34]. The high antagonistic activity observed in our study, especially for *L. plantarum* M1, can be attributed to the increased production of PLA and lactic acid. Previous studies also support these findings. In semi-field and field trials, the biocontrol efficacy of strains PM411 and TC92 was comparable to that of Bacillus amyloliquefaciens D747, B. subtilis QST713, chitosan, acibenzolar-S-methyl, copper-based products, and kasugamycin. Under *in vitro* conditions, their inhibitory activity was reported to be associated with a decrease in medium pH and the production of lactic acid [35].

The moderate antimicrobial activity of *E. faecium* A2 identified in our study may be associated with enterocin-type bacteriocins characteristic of this species. The primary mechanism of action for these species involves the formation of pores in the cell membrane, leading to electrolyte leakage [36]. Furthermore, enterocin-producing *E. faecium* strains play an important role in enhancing plant resistance to abiotic and biotic stresses by increasing the abundance of beneficial microorganisms in the rhizosphere, limiting

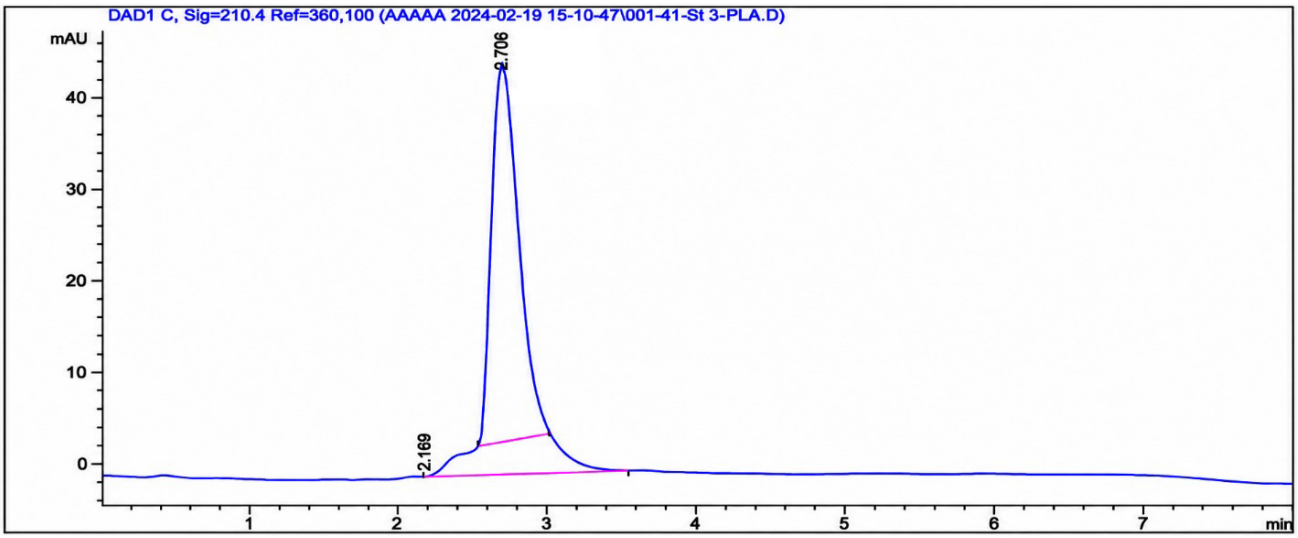


Fig. 5. Chromatogram of 3-phenyllactic acid (PLA).

pathogen proliferation, and maintaining microbiome balance [37].

Overall, these results indicate that LAB are effective in both food and probiotic applications and as biocontrol agents against plant diseases. Importantly, the environmental safety, lack of toxic effects, and ready biodegradability provide LAB-based agents with clear advantages over conventional chemical pesticides. Therefore, these strains hold promise for applications such as seed treatment, rhizosphere colonization, and the production of biofertilizers.

5. Limitations

This study has several limitations that should be acknowledged. First, only 3 of the 30 isolated strains were selected for detailed investigation, which may limit the broader representation of the microbial diversity obtained. Second, the antimicrobial and antifungal activities of the isolates were evaluated only *in vitro*, without validation under greenhouse or field conditions. Finally, salt tolerance was assessed only up to 6.5% NaCl, which may not fully capture the tolerance potential of the isolates at higher salinity levels.

6. Conclusion

Notably, this study comprehensively investigated LAB isolates from Uzbekistan with respect to their biological characteristics, safety, and biocontrol potential. The selected strains, *L. plantarum* M1, *P. pentosaceus* A2, and *E. faecium* A2, were confirmed through molecular–genetic and biochemical analyses to ensure safety and non-pathogenicity. These strains exhibited strong antagonistic activity against phytopathogenic fungi and bacteria, effectively inhibiting pathogen growth through the production of organic acids and PLA. In addition, the tolerance of these strains to salt stress, metabolic flexibility, and ability to produce organic acids enable their application as biocontrol agents and as plant biostimulants. Overall, these LAB strains isolated from the flora of Uzbekistan demonstrate significant biotechnological potential for agriculture, environmentally safe plant protection, and functional food production.

Availability of Data and Materials

All experiments and results obtained during this study are presented in this article. Additional information is available on request from the corresponding author.

Author Contributions

HK, GK, ZA, BT and DK contributed to this study. HK participated in the isolation, selection and morphological characterization of bacteria in plants; GK in their molecular identification. BT and ZA identified phytopathogenic fungi and bacteria and DK analyzed the results, worked with figures and tables. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Plant materials were collected in accordance with the generally accepted botanical field research and sampling guidelines described by Skvortsov (1977). No human participants or experimental animals were involved in this study. The plant materials were identified and authenticated by Dilafruz Jamalova, Junior Researcher at the Botanical Research Institute.

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Conflicts of Interest

The authors declare no conflicts of interest.

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