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RESEARCH ARTICLE

Promising Biocontrol Agents Against the Main Pathogens of Apple Fruit Rot

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Abstract: Current study looked into how well microorganisms, both those already known and newly found, from the surface of plants in gardens in southern and southeastern Kazakhstan could fight against other harmful microbes. We evaluated the effectiveness of 16 strains of lactic acid bacteria, 11 strains from the Bacillus and *Pseudomonas* genera, and 7 strains of *Trichoderma* fungi (all sourced from SPC Microbiology and Virology LLP), along with 135 epiphytic microorganisms, against four major rot-causing pathogens: Penicillium chrysogenum, Monilinia fructigena, Alternaria alternata, and Fusarium sporotrichioides. Out of 16 lactic acid bacteria strains, 11 showed antagonistic activity, primarily against M. fructigena, and to a lesser extent, other pathogens. Lactobacillus casei 32 exhibited the strongest inhibitory effect. Among the bacterial cultures screened, only the Bacillus amyloliquefaciens strain was found to suppress the growth of specific pathogens. Among the 7 fungal strains of the genus *Trichoderma*, 4 had significant antagonistic activity. The maximum zone of pathogen suppression was found in the strain *T.* asperellum 30. Screening of potential antagonist microorganisms from microbial populations isolated from the surface of apple fruits revealed that only 1 isolate of the bacterial flora had inhibitory activity against the pathogen *M. fructigena*. The following microorganisms with maximum antagonistic activity are promising biocontrol agents against fruit rot: *Lactobacillus casei* 32, *B.* amyloliquefaciens and T. asperellum 30.

Keywords: Apple Fruits, Screening, Microorganisms, Fruit Rot Pathogens, Antagonists

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Introduction

Postharvest rot caused by fungal pathogens is one of the main factors limiting fruit shelf-life and leading to significant economic losses, especially in the fruit market supply chain [1-3]. The species composition and abundance of epiphytic microflora from the intact skin of fresh apple surfaces depend on many factors, namely, variety, conditions and area of cultivation (climatic, meteorological, soil composition, agrotechnical techniques, and others), as well as the timing and methods of harvesting and transportation.

The natural epiphytes on the surface of apples are bacteria, yeast and mold fungi. Damage to apples by phytopathogenic microorganisms can occur both during the growing season, that is, before harvesting, and during commercial processing and storage from containers, equipment, and storage facilities contaminated with fungal spores or bacteria. During storage, apples are affected by the following phytopathogenic microorganisms: Monilinia fructigena, Alternaria alternata, Fusicladium dendriticum, Penicillium expansum, Gloeosporium album, Fusarium avenaceum, and Phytophthora cactorum [4, 5].

While fungicides are commonly employed to control postharvest rot and extend fruit shelf-life, their efficacy is declining as pathogens develop resistance. This resistance often results in the overuse and misuse of fungicides, creating public health concerns [6, 7]. Consequently, there is a pressing need for alternative approaches to mitigate environmental contamination and health hazards associated with fungicide residues.

Given these challenges, biological control is emerging as an effective strategy to manage fruit spoilage resulting from phytopathogenic microorganisms during storage. Over the past decade, there has been a notable increase in the adoption of biological control agents as alternatives to synthetic fungicides for combating post-harvest fruit rot [8]. For apples, specifically, biological control agents have been suggested to mitigate rot occurring during storage [2, 9-12].

A promising method for reducing pathogen populations involves the application of formulations based on antagonistic microbes. This area of biotechnology is particularly relevant due to the global focus on organic farming and the demand for environmentally friendly food products. Currently, organic farming is practiced in 160 countries worldwide [13, and Kazakhstan is also actively developing this sector [5, 14].

The active agents of biological control are microbial antagonists, which are used as the basis of biological products. The use of biological products does not pose a threat to natural biocenoses, allowing the preservation of the beneficial microflora of apple fruit surfaces and the production of environmentally safe products.

Various microorganisms are used against various types of fruit rot as the basis for creating biological products: bacterial species, including Bacillus subtilis, Pseudomonas syringae, and Bacillus amyloliquefaciens, fungi of the genera Trichoderma, Epicoccum, Aureobasidium pullulans and Pantoea agglomerans. These microbes are highly effective in biologically controlling numerous pathogens responsible for fruit spoilage [10, 15-25].

The most active prokaryotic antagonists used to create biological products are representatives of the genera Bacillus and Pseudomonas. Among these common biocontrol microorganisms, B. subtilis is widely used in agriculture to prevent fruit rot [2, 10, 15, 26]. Certain species of actinomycetes have great antagonistic potential [27-30]. Some experimental studies using biocontrol agents have shown that yeast isolates such as Aureobasidium pullulans [31] and Epicoccum purpurascens [32, 33] and the fungus Trichoderma polysporum, [20, 34], Trichoderma harzianum [36]. provide good protection from M. fructigena used alone or in mixtures to injured apples.

In Kazakhstan, the biopreparation "Bioconservant MP-3" based on yeast (Metschnikowia pulcherrima) is widely used to control the rot complex of apple fruits developed for storage [5]. All known antagonists of pathogens have properties that allow them to delay their development or kill pathogenic microbes. These properties include the ability to synthesize substances that inhibit pathogen growth. The antagonistic capabilities of these agents form the foundation for developing bio-based products designed to safeguard plants against diverse infections. Therefore, biopreparations based on antagonistic microbes are widely used in agriculture worldwide.

The development of safe biological methods for protecting plants and fruits on the basis of antagonist strains may eventually completely replace chemical agents known as conventional ones. This prevents the spread of resistant strains, harming human health through the use of toxic substances and environmental pollution. Thus, it can be argued that the search for new promising biocontrol agents from various natural sources against apple fruit rot pathogens is an actual area.

This study aimed to investigate the biocontrol potential of both collected and naturally occurring epiphytic microorganisms against four primary pathogens responsible for apple fruit rot in Kazakhstan, both during the growing season and after harvest.

Materials

Fruits of various varieties of apples were selected during the growing season and stored in various natural and climatic zones of horticulture in Kazakhstan.

Microorganisms, including bacteria, fungi, and yeast, were sourced both from the "SPC Microbiology and Virology" LLP collection and isolated directly from the surfaces of healthy and diseased apple trees.

The pathogens used for testing were fungal cultures, specifically P. chrysogenum (blue mold), M. fructigena (moniliosis fruit rot), A. alternata (alternaria rot and black spotting), and F. oxysporum (fusarium rot), all isolated from infected apple trees.

Methods

Isolation of Epiphytic Microflora of Apple Fruits

To isolate epiphytic accompanying and pathogenic microflora, apple fruits were gathered from southeastern orchards and storage facilities of Kazakhstan. These collected fruits were then transported in paper bags to the "Scientific and Production Center of Microbiology and Virology LLP" laboratory, following strict protocols for biological material selection and transport to prevent contamination and preserve the native microflora. The method of isolation and the method of cumulative cultures were used to isolate the surface microflora (tou.edu.kz https://textbook.tou.edu.kz › books).

For culturing various types of bacteria, the following were used: Meat-Peptone Broth (MPB), Meat-Peptone Agar (MPA) and Sabouraud medium for yeast. Fungal flora was grown on potato-glucose agar (KGA), Chapek-Dox, and wort-agar [37].

Sowing on solid nutrient media from test tubes with dilutions was carried out by the surface seeding method. 0.01 ml of suspension was poured onto the surface of the solid medium and ground with a spatula. Then placed in a thermostat at a temperature of 24 -28°C. For 7 days, the number of grown colonies was observed and counted. Determination of the species composition of microorganisms isolated from the surface of apple fruits was carried out by studying the cultural characteristics of fungal isolates by microscopy after their initial growth on different media within a controlled incubator set between 24°C and 30°C for 7 days using various disease identifiers.

In vitro Screening of Microorganisms for Antagonistic Activity Against the Main Pathogens of Fruit Rot

The sterile water was used at the test tubes to reactivate the microbial cultures. Then, they were placed in a thermostat at 30–37°C (various types of bacteria) or at 28°C (fungi) for one day.

Upon completion of the experiment, cultures were filtered onto solid nutrient media, and 0.1 ml of the resulting suspension was plated onto agar media in Petri dishes, rubbed with a sterile spatula, and then placed in a thermostat at 37°C (for lactic acid bacteria), 28°C (for bacterial culture) and 22–24°C (for fungi). Bacterial growth was observed on day 2, and lactic acid bacteria and fungi growth were observed on days 5–7. The grown culture was then collected, surface was scraped with a sterile loop and then transferred to a test tube and gently mechanically stirred before the concentration was increased to 107. Microorganism fermentation occurred via deep cultivation in a "Biosan" incubator shaker. This process involved platform oscillation at 180 rpm and a temperature range of 30–37°C, sustained for 2–7 days, varying by microorganism type.

The collection of microorganisms and newly isolated apple fruits from the surface, aimed at selecting potential biocontrol agents against fruit rotting agents, was carried out via agar diffusion (well method) for mass screening. The main pathogens of fruit rot (Monilinia, Penicillium, Alternaria, and Fusarium) served as test cultures and were cultivated on plates with Chapek, PGA and Saburo media at 28°C for 7 days, varying with their rate of growth. Active bacterial strains were identified using a dense nutrient medium initially inoculated with a daily suspension of pathogens. Wells (8 mm diameter) were then made in this medium with a sterile cork borer, into which 0.1 ml of broth from the candidate microorganisms was added. Petri dishes were subsequently incubated at 28°C for 24 hours, after which the zones of pathogen growth inhibition around the wells were measured. Sterile water served as a control. Following two days of co-cultivation, the diameter of these growth suppression zones was measured in millimeters (excluding the well diameter). All experiments were performed in triplicate, with sterile distilled water used for control treatments.

Molecular Identification

The selected microorganisms were identified via molecular genetic analysis methods. The fungal flora were cultured on PGA and Saburo media in Petri dishes for 7–14 days, while bacterial isolates were cultivated on MPA and MRS media in test tubes for 1 day. The biomass was collected by scraping with a sterile scalpel.

DNA Extraction and PCR Amplification

Genomic DNA extraction was carried out using a Proba-GS kit (AgroDiagnostics LLC, Russia) following the manufacturer's instructions. Subsequently, the ITS and LSU regions were amplified using the universal ITS1 primers (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), LROR (ACC CGC TGA ACT TAA GC) and LR6 (CGC CAG TTC TGC TTA CC).

The PCR process began with an initial denaturation phase at 98°C for 30 seconds, followed by 30 cycles: denaturation at 98°C (10°C), primer annealing at 60°C (30°C), 57°C (30°C°C), and elongation at 72°C (60°C); and a final elongation at 72°C (10 min). The amplification products were separated via electrophoresis in a 1% ethidium bromide agarose gel and visualized via the Quantum-ST5 gel documentation system (Vilber Lourmat).

Sequencing and Phylogenetic Analysis

PCR products were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific). Subsequent sequencing was performed in both forward and reverse directions using the Sanger method, specifically with the BigDye™ Terminator v3.1 Cycle Sequencing Kit on an ABI 3500xL genetic analyzer (Applied Biosystems). The consensus sequences of the ITS and LSU fragments of the isolated fungi were obtained via the Clone Manager 9 program (Scientific & Educational Software, Cary, NC, USA). The obtained consensus sequences were compared with the ITS and LSU fragments available in the NCBI database via BLASTn. To ensure the accuracy of the analysis, BLASTn was limited to sequences from NCBI. After processing, up to 10 of the first obtained sequences with a percentage identity in the range of 99–100% were uploaded and used for evolutionary analysis. Multiple sequence alignment was performed via Clusta IW software as part of the MEGA11 program [38]. The evolutionary history of the taxa was inferred using a bootstrap consensus tree, generated from 1,000 replicas [39]. Evolutionary distances were determined using maximum complex likelihood method [38]. Bacterial identification was achieved by sequencing 16S rRNA gene fragments on an Applied Biosystems 3500 DNA Analyzer sequencer, utilizing the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) as per the manufacturer's instructions [40]. The resulting sequences were matched with the NCBI GenBank database to identify their most closely related strains (www.ncbi.nlm.nih.gov/gene) via the BLASTn program.

Statistical Analysis

This study utilized a randomized block design, with experiments replicated three times. Data analysis was performed using the General Linear Model (GLM) procedure within the Excel Stat software. Average values were differentiated by the Least Significant Difference (LSD) at 5% using the LSD test, and homogeneous groups were identified via the Newman–Keuls test.

Results and Discussion

Isolation of Epiphytic Microflora of Apple Fruits

To identify pathogenic and associated microflora during the growing season and storage, we selected 360 diseased and healthy apple fruits from various natural and climatic zones of apple cultivation. A total of 135 microorganisms were isolated via rinses from the surface of apple fruits. In the course of the conducted research, the dominant species were fungi, which accounted for 79% of the total number of microorganisms detected. In all samples of the fungal flora, representatives of the Ascomycetes department prevailed, accounting for 49% of the samples. Fungi of the genera Monilinia and Venturia, Cladosporium, Botrytis, and Mucor were attributed to these genera. Mold fungi of the Deuteromycete division of the genera Alternaria, Fusarium, and Penicillium were somewhat less common (30%). They mainly appeared on areas damaged by insects, birds, or mechanically injured fruits. The bacterial flora, depending on the variety, accounted for 12% of the bacteria. The percentage of yeast and unidentified microorganisms was 3% in each (Fig. 1) experiment. During the growing season, microbial cenosis is dominated by fungi of the genera Monilinia, Venturia and Alternaria, which cause significant economic losses and the production of low-quality apples. Fungi such as Penicillium, Fusarium, Botrytis, Mucor and Cladosporium caused the rot of apples mainly during storage. Some of these organisms are carried on fruits from gardens to storage and, after harvesting, can cause enormous economic losses if the storage conditions are such that they allow the growth of fungi. Moniliosis rot is the most destructive fungal disease during the growing season, while P. expansum causes the most damaging blue mold during storage.

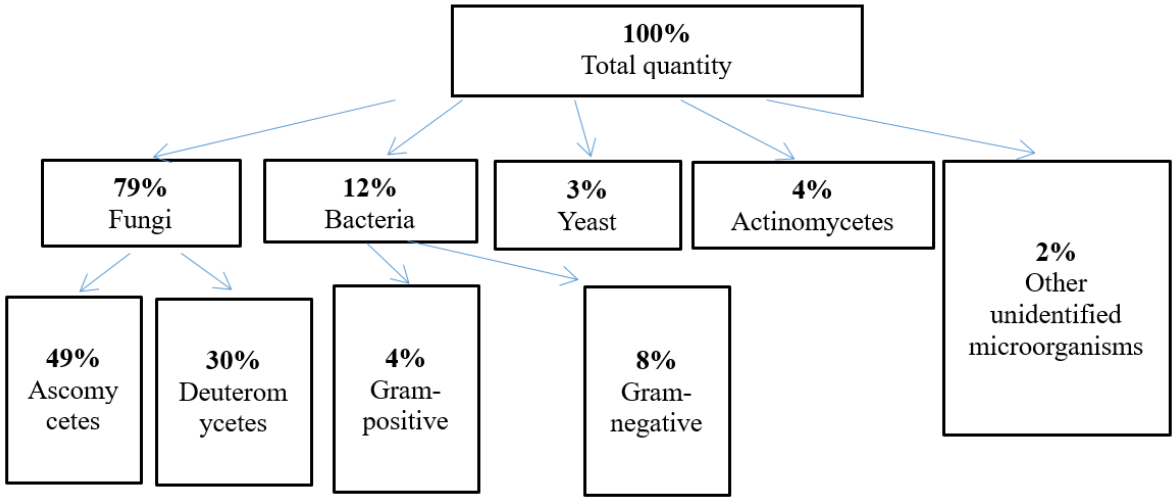


Fig. 1: The microbial community of apple fruit surfaces

Thus, for the growing period and storage, the main pathogens of the rotting fruit products were fungal microorganisms (Monilinia, Penicillium, Alternaria, and Fusarium), which were selected as test crops for further research.

In vitro Screening of Microorganisms as Inhibitors Against Causal Agents of Fruit Rot

To identify microorganisms with antagonistic activity against the main pathogens of apple fruit rot, a mass screening of collection crops from the Scientific and Production Center of Microbiology and Virology LLP was conducted.

According to an analysis of literature sources, the agents used for biological control against the rotting of apple fruits are fungi of the genus Trichoderma [34, 35]. Studies showed that treating wounded apples and pears stored at 20±1°C significantly reduced the size of mold lesions, with the effectiveness varying based on the type of mold and fruit. Additionally, fresh apple fruits, both injured and undamaged, were protected from mold infections for up to 2.5 months after being treated with the biopesticides under controlled and semicommercial conditions.

Therefore, our study involved assessing the inhibitory power of different Trichoderma strains available in the production center's collection.

It was found that 4 out of 7 Trichoderma strains had antagonistic effects, inhibiting the organism that causes moniliosis fruit rot. For these strains, the pathogen suppression zones ranged from 38.0±3.7 mm to 79.6±0.6 mm. A maximum antagonistic activity of 79.6±0.6 mm was observed in the T. asperellum 30 strain. This strain exhibits diverse antagonistic activity against pathogens like F. sporotrichioides, P. chrysogenum, and A. alternata. Their growth suppression zones ranged from 14.0± 5.0 mm–25.6±1.61 mm (Fig. 2).

Lactic Acid Bacteria (LAB) are deemed "Generally Recognized as Safe" (GRAS) due to their non-pathogenic nature, industrial applicability, and ability to produce antimicrobial substances. Their significant positive impact in food and agriculture [11] suggests a potential use as antagonists against apple fruit rot pathogens, especially the damaging moniliosis fruit rot that affects apples during both growth and storage.

P. chrysogenum

Control | *T. asperellum* 30 | *T. asperellum* 1 K

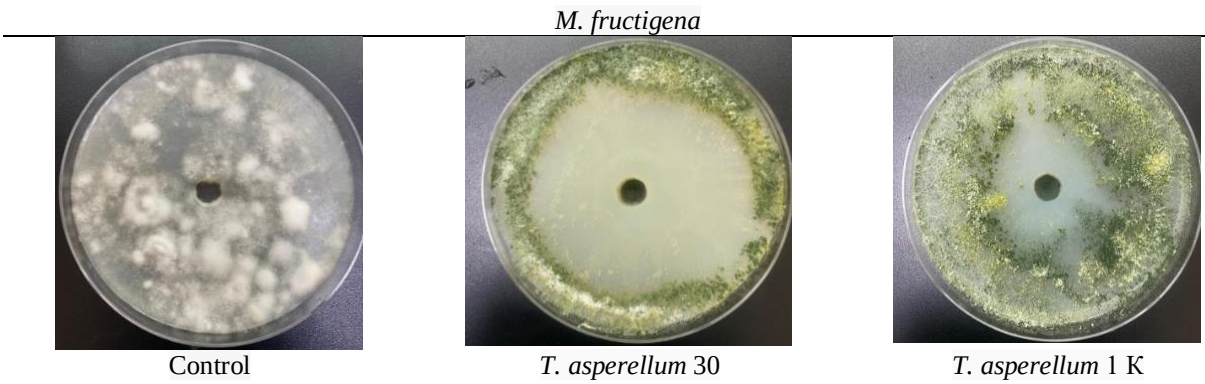


Fig. 2: Zones that suppress pathogen growth and cause rot in apple fruits

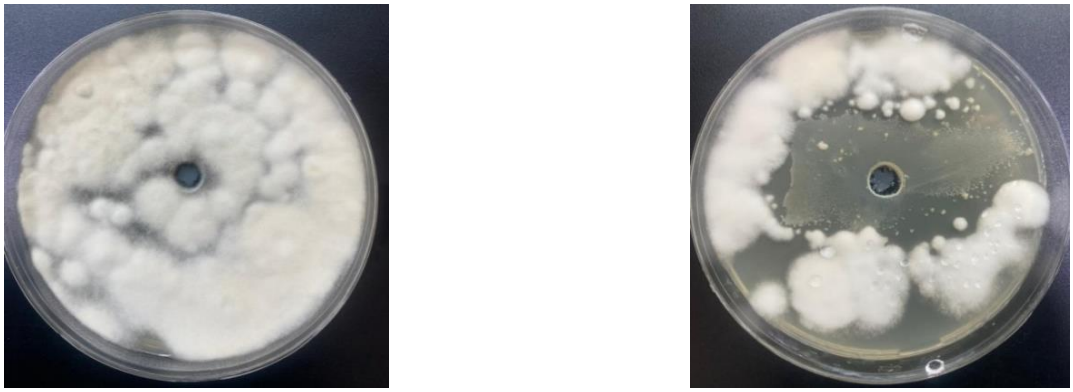
A review of existing literature indicates that information regarding the use of lactic acid bacteria to control apple fruit rot pathogens is limited and fragmented. In Kazakhstan, the use of biopreparations based on lactic acid bacteria against fire blight of fruit crops has been reported. The biocontrol effects of sixteen lactic acid bacteria strains were assessed. Eleven strains had varying degrees of inhibitory activity against the fungus M. fructigena. Their suppression zones ranged from 9.09.3±3.0 to 41.6±6.4 mm. The maximum inhibitory activity (41.6 = 6.4 mm) was detected in the L. paracasei 32 strain (Table 1, Fig. 3). The fungi M. fructigena, P. chrysogenum, A. alternata, and F. oxysporum were not inhibited by lactic acid bacteria.

Table 1: Screening of lactic acid bacterial strains from the collection against the main pathogens of apple fruit rot

№	Bacterial strains	The diameter of the pathogen growth suppression zone, mm			
		M. fructigena	P.chrysogenum	A. alternata	F. oxysporum
1	Lactobacillus paracei 44 m 26	26,7±5,1	0	0	0
2	Lactobacillus plantarum Wf 20	0	0	0	0
3	Lactobacillus plantarum 140/A 17	0	0	0	0
4	Lactobacillus cellobiosis 35/1	25,3±5,0	0	0	0
5	Lactobacillus paracei 33-4	0	0	0	0
6	Lactobacillus lactis K-1	0	0	0	0
7	Limosilactobacillus pontis Wf-6	11,0±3,3	0	0	0
8	Streptoccocus lactis 6	23,0±4,7	0	0	0
9	Lactobacillus carvatus 6	16,6±4,1	0	0	0
10	Lactobacillus cellobiosis 36	14,0±3,7	0	0	0
11	Limosilactobacillus pontis Wf-6	0	0	0	0
12	Lactobacillus plantarum 17 M	9,3±3,0	0	0	0
13	Lactobacillus paracei Wf-11	9,0±3,0	0	0	0
14	Pediococcus acidilactici Rf 26	13,6±3,7	0	0	0
15	Lacticaseibacillus paracasei 22	41,6±6,4	0	0	0
16	Lactobacillus cellobiosis 36-1	26,6±5,1	0	0	0
17	Control	0	0	0	0

Nine collection strains of the genus Bacillus and 2 strains of Pseudomonas were screened. Of these, B. brevis P2 96 (63.0±6.1 mm), B. corototanum P2 6 (48.6±7.7 mm), and B. albaratus (36.0±1.41 mm) had fungistatic inhibitory activity against A. alternata. A pure suppression zone of 32.0±1.61 mm was observed exclusively with the B. amyloliquefaciens MB 40 strain. [42] published comparable data, detailing this bacterium's in vitro antifungal action against fruit rot pathogens like Botrytis cinerea, M. fructicola, M. laxa, P. digitatum, P. expansum, and P. italicum. In the remaining bacterial cultures, the pathogen inhibition zones were insignificant or completely absent. Two strains, Micrococus lutens FSR (23.7± 4.5 mm) and P. arguate

CATTLE-184 (27.1±7.3 mm), had antagonistic activity against F. sporotrichioides (Fig. 4). No growth-suppressing bacteria were found against the fungi P. chrysogenum or M. fructigena.



Control Lacticaseibacillus paracasei 22

Fig. 3: Growth suppression zone of M. fructigena by the lactic acid bacterium Lactobacillus casei

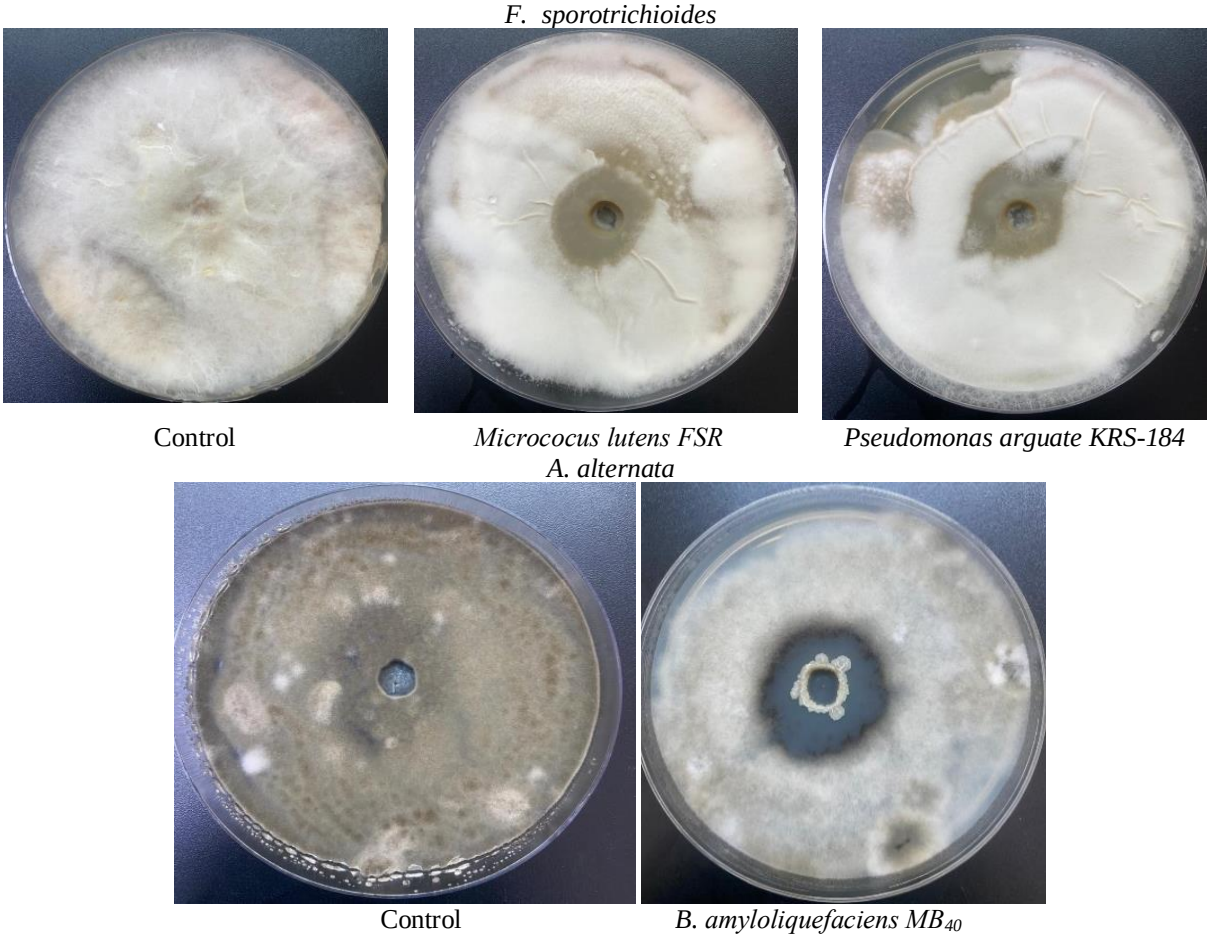
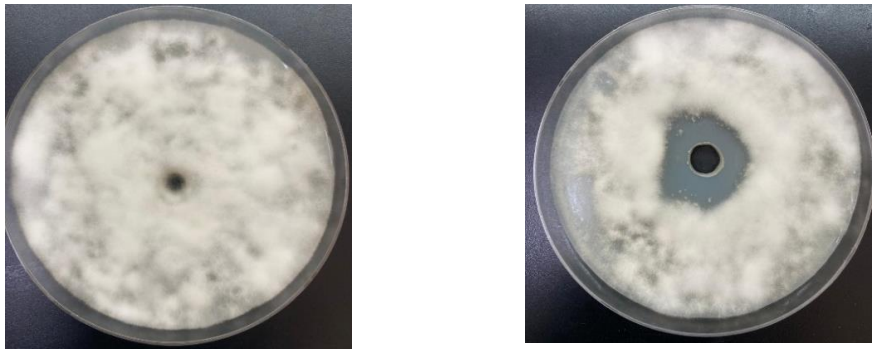


Fig. 4: Suppression zones of apple fruit rot pathogen activity under various bacterial cultures via the diffusion method

A selection of the most frequently occurring colonies, among 135 distinct epiphytic microorganisms isolated from apple trees, was evaluated for antagonistic activity against pathogens causing postharvest rot. Among the total number of epiphytic microflora, only 1 bacterial isolate had inhibitory potential against the pathogen M. fructigena (Fig. 5). The pathogen growth suppression zone was 25.0± 2.6 mm. Identification of the isolate via microbiological and molecular genetic methods revealed

that this microorganism is Leuconostoc mesenteroides M 1. This antagonist is a gram-positive, facultatively anaerobic, nonspore-forming immobile, heterofermentative lactic acid bacterium with a spherical or lenticular shape. The cell size ranged from 0.6 to 1.4 microns, and widely distributed in nature, including in soil and plants.



Control *Leuconostoc mesenteroides M 1*

Fig. 5: Growth suppression zone of the M. fructigena fungus by a newly isolated bacterium from the epiphytic microflora

The research led to the identification and selection of 11 lactic acid bacteria strains from the production center's collection that target M. fructigena, the prevalent and destructive pathogen responsible for fruit rot. Additionally, 3 strains of bacteria of the genus Bacillus with antagonistic activity against certain types of pathogens and 4 strains of fungi of the genus Trichoderma have been isolated. Among the epiphytic microflora isolated from the surface of the fruits, only 1 strain, Leuconostoc mesenteroides M 1, which is a lactic acid bacteria, was selected.

Among the selected antagonist microorganisms, the strain L. paracasei 22, which had the maximum growth inhibition zone, was chosen for further studies.

Molecular Identification

To improve the accuracy of the taxonomic identification of fungal isolates and to assess their evolutionary relationships, a comparative molecular phylogenetic analysis was performed using two markers: the internal transcribed spacer (ITS) and the large ribosomal RNA subunit (LSU, 28S rDNA). Based on the results obtained, phylogenetic trees were constructed. Figures 6 and 7 display partial sequences of the ITS and LSU genes according to their maximum likelihood of including both sequences of the studied isolates (indicated by red triangles) and reference sequences from the GenBank database. All the isolates were distributed into the corresponding cladistic groups, demonstrating a high degree of correspondence with the identified species. On the ITS tree, the Penicillium isolate clearly clustered with the reference strains of P. chrysogenum, forming a well-maintained monophyletic group. The LSU tree confirmed this relationship, but the support for the corresponding cluster was slightly lower (67%), which was probably due to the lower variability of the LSU region within the genus. Nevertheless, both markers indicate that the isolate belongs to the P. chrysogenum species, which reflects the reliability of the dual approach. Phylogenetic analysis of the Cladosporium isolate based on the ITS and LSU gene sequences revealed stable clustering within the C. herbarum complex, with the greatest similarity to the species C. allicinum, C. herbarum and C. cladosporioides. The high bootstrap values (100%) on both the ITS and LSU trees confirm the validity of this classification. The isolate of the genus Monilinia is located within the species M. fructigena according to the ITS and LSU sequences. In both trees, it forms a monophyletic clade with representatives of this species, with statistical support values reaching 100%. The phylogenetic position of the Alternaria isolate in the ITS and LSU trees clearly indicates that it belongs to the species A. alternata. In both reconstructions, it is included in a highly supported clade that includes various strains of this species. The Fusarium isolate in both phylogenetic reconstructions (by LSU and ITS regions) is steadily clustered with representatives of the species F. sporotrichioides, while the support of the corresponding nodes is 100%.

The research confirmed that molecular phylogenetic analysis, particularly with ITS and LSU DNA markers, is an effective strategy for the taxonomic classification of micromycetes. All the studied isolates were assigned to the corresponding species P. chrysogenum, C. herbarum complex, M. fructigena, A. alternata, and F. sporotrichioides which was confirmed by the high values of bootstrap support and the coincidence of tree topologies constructed via two independent markers. The data obtained demonstrate the absence of phylogenetic conflicts between the ITS and LSU regions, which indicates their consistency and applicability for a comprehensive taxonomic assessment of fungi (Fig. 6).

A screening of various strains from the collection site revealed that the lactic acid bacterium Lactobacillus plantarum 22 strain had the maximum growth suppression effect on the most harmful apple fruit disease, namely, moniliosis fruit rot. The sequence analyzed in GenBank showed 99.82% homology with Lacticaseibacillus paracasei 22 strains (Fig. 7).

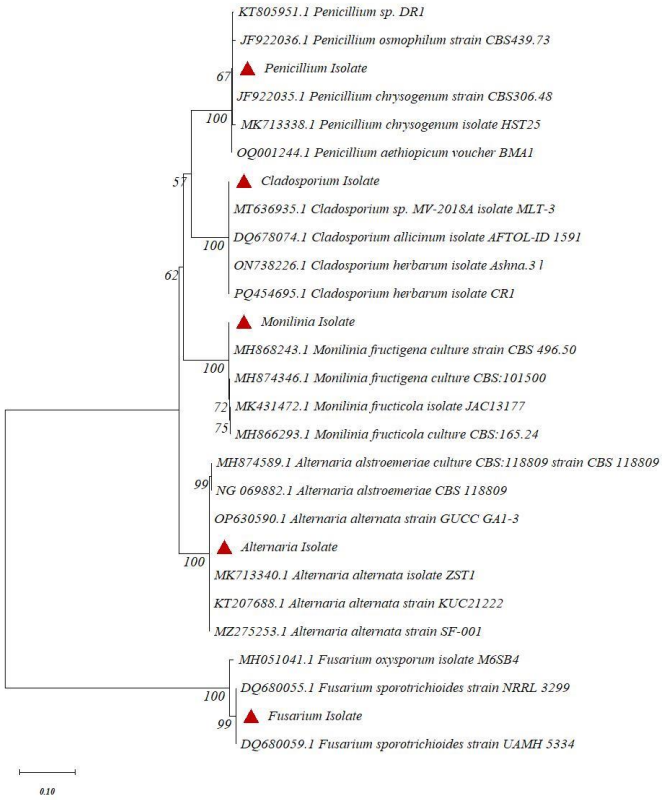


Fig. 6: A maximum likelihood phylogenetic tree, built from ITS and LSU rDNA gene sequences, illustrated the phylogenetic relationships between the isolates and database taxa

Homologous 16S rRNA gene nucleotide sequences were identified using the Basic Local Alignment Search Tool (BLAST) within the GenBank International Database (Table 2) of the USA's [41] National Center for Biotechnology Information (htpp://www.ncbi. nlm. nih.gov).

As depicted in Figure 7, strain L22 is identified as L. paracasei, exhibiting a 99.82% homology with the most closely related Lacticaseibacillus paracasei strains in the NCBI database.

Table 2: Results of bacterial nucleotide sequence identification in an international database

Bacterial strains	GenBank Inventory Number	Name of the strain	% of match
Lacticaseibacillus paracasei	NR_041054.1	Lacticaseibacillus paracasei subsp. tolerans strain NBRC 15906	99,82
	NR_025880.1	Lacticaseibacillus paracasei strain R094	99,82
	NR_113337.1	Lacticaseibacillus paracasei strain NBRC 15889	99,82
Lacticaseibacillus zeae	NR_037122.1	Lacticaseibacillus zeae strain RIA 482	99,46
Lacticaseibacillus chiayiensis strain	NR_179712.1	Lacticaseibacillus chiayiensis strain BCRC 81062	99,46
Lacticaseibacillus casei	NR_113333.1	Lacticaseibacillus casei strain NBRC 15883	99,28
	NR_041893.1	Lacticaseibacillus casei DSM 20011 = JCM 1134 = ATCC 393	99,28

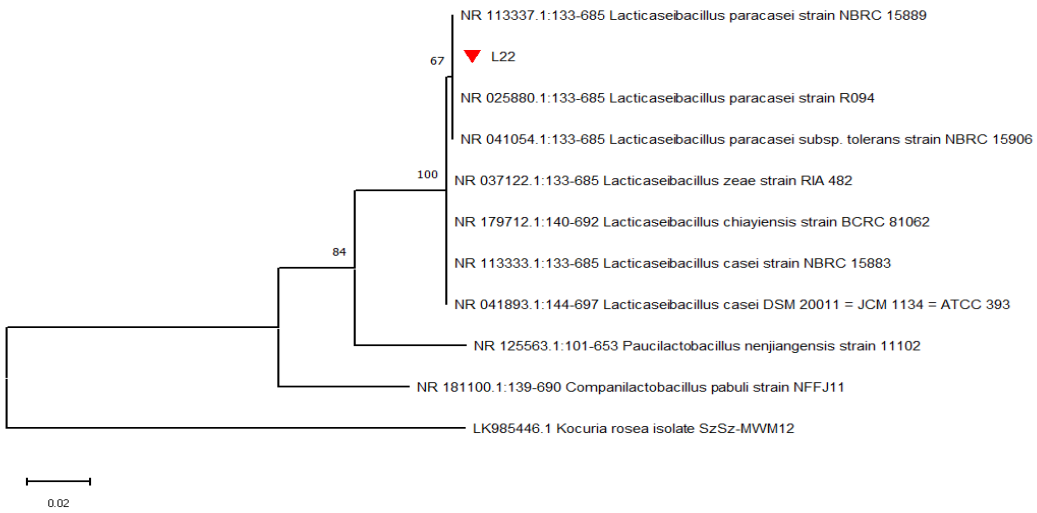


Fig. 7: The phylogenetic relationships between strain L22 and other related strains were illustrated in a tree, derived from their 16S rRNA gene nucleotide sequences

Discussion

The conducted research brought to light several important aspects requiring detailed discussion, particularly regarding recent progress in the biological control of post-harvest fruit diseases.

The effectiveness of various groups of antagonistic microorganisms. Our data demonstrate the pronounced specificity of the antagonistic activity of various microorganisms. Notably, the Lacticobacillus paracasei 22 strain exhibited strong efficacy against Monilinia fructigena, creating a suppression zone of up to 41.6±6.4 mm. This result is consistent with [43], who observed an 80% reduction in citrus fruit disease incidence following LAB treatment. However, the limited activity against other pathogens (Penicillium chrysogenum, Alternaria alternata) confirms the conclusions of [44] on the species specificity of antifungal metabolites LAB.

Problems with the use of Trichoderma spp. Despite the proven effectiveness of T. asperellum 30 [3], its practical application is complicated by intensive spore formation. This is consistent with the warnings of [4] about the technological difficulties of scaling biopreparations based on fungi. An alternative may be the use of cellular extracts or metabolites, as suggested by [45].

Epiphytic microflora as a source of antagonists. The fact that only one active bacterial isolate was found among 135 epiphytes confirms the results of [46], showing a limited number of microorganisms with pronounced antagonistic activity in the phyllosphere of horticultural cenoses. This highlights the need to improve screening methods, possibly using molecular genetic approaches [47].

Prospects for the application of L. paracasei 22. The prospects for using L. paracasei 22 as a basis for a biopreparation are due to its safety (GRAS status), stable activity against key pathogens, and technological cultivation, which is especially important for Kazakhstan, where autochthonous strains demonstrate better adaptation to local conditions [48]. For successful commercialization, further research is needed to optimize nutrient media, develop stable formulations, and conduct field trials in various agro-climatic zones [49].

Integration with other security methods. A promising direction is the development of integrated protection systems combining L. paracasei 22 with other biological agents: a combination with promising LAB strains [50] can expand the spectrum of action, combined use with endophytic yeast [51] can enhance the protective effect, and the use of nanotechnology delivery systems [42] - to increase the stability and effectiveness of the drug. Such integrated approaches will help overcome the limitations of single-strain drugs and create more effective biological protection systems for fruit crops.

Thus, the results obtained open up new opportunities for the creation of the first biopreparation in Kazakhstan against various types of rotting of apple fruits based on L. paracasei 22. However, as noted by [52], successful implementation requires an interdisciplinary approach combining microbiology, biochemistry, and agroengineering. Further research should be aimed at studying the mechanisms of antagonism, optimizing the production process and developing integrated garden protection systems.

Conclusion

Current monitoring surveys of horticultural cenoses in southern and southeastern Kazakhstan identified 135 epiphytes, which consisted mainly of fungal, bacterial and yeast morphotypes. Pathogenic microflora were isolated along with associated microorganisms. Fungi of the genera M. fructigena, P. chrysogenum, F. sporotrichioides, and A. alternata have been identified via molecular genetic analysis. The most widespread and harmful pathogen is the fungus M. fructigena, which causes moniliose fruit rot. This pathogen is found both during the growing season and during the storage of apple fruits.

The antagonistic activity screening of several fungal and bacterial strains allowed them to be differentiated into those that suppressed the fruit rot pathogen and those that only slowed its development. From all the epiphytic bacteria isolated from apple fruits, just one strain, L. mesenteroides M 1, demonstrated an inhibitory effect on the pathogen causing moniliosis fruit rot. In relation to other pathogens, its antagonistic activity was insignificant or completely absent.

An evaluation of the antagonistic activity of various Trichoderma species revealed that T. asperellum 30 has complex inhibitory activity against pathogenic microflora, causing rotting of fruit products. However, because this microorganism strongly sporulates during development, we have abandoned its use as a bioagent for the control of these diseases.

The screening of 11 bacterial strains (9 from the Bacillus genus and 2 from Pseudomonas) revealed that B. amyloliquefaciens MB40 was unique in displaying a complex suppression zone. In the remaining bacterial cultures, the pathogen inhibition zones were insignificant or completely absent.

Among 16 collection strains of lactic acid bacteria, 11 had inhibitory effects on the pathogen M. fructigena. The maximum suppression zone was in the L. paracasei 22 strain.

Thus, of all the selected antagonist microorganisms, we selected the strain L. paracasei 22, which has the maximum antagonistic potential, is the most environmentally friendly, and is convenient for developing a promising biofungicide against rotting apple fruits during the growing season and storage.

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Author’s Contributions

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Nurlan Kuldybayev: Translation.
Gul Baimakhanova: Writing review and editing, supervision.
Assel Molzhigitova: Investigation, methodology, resources, validation, and formal analysis.
Diana Tleubekova: Investigation, resources, validation.

Saule Daugalieva: Formal analysis, investigation.
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Aida Aidarkul: Investigation, resources.
Zhanna Issina: Writing review and editing, investigation, resources, validation.
Alnura Tursunova: Investigation, resources.
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Declarations

There are no conflicts of interest regarding the publication of this article. This article does not contain any studies involving humans or animals conducted by any of the authors.

Competing Interests

The authors declare that they have no competing interests.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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