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Antibacterial and Antifungal Activity of Certain *Lactobacillus* Species Against Conditional Pathogenic and Pathogenic Microorganisms

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Abstract According to the results of the study, 4 strains showed high antifungal activity against the selected phytopathogenic micromycetes *Alternaria alternate, Verticillium dahlia, Aspergillus flavus, Aspergillus oryzae and Fusarium specius. The antifungal activity of the bacterial strain L. fermentum No. 1 against the phytopathogenic Alternaria alternate, Verticillium dahlia, Aspergillus oryzae and Fusarium specius* was determined in the zones of 27±0.3, 15±0.3, 19.6±1.5 and 18±0.6 mm. The antifungal activity of lactic acid bacteria strains was determined by the growth radius of the phytopathogens and it was found that they form a ring from 15 to 35 mm.

Keywords *Lactobacillus plantarum, L. brevis, L. fermentum, Pediococcus pentosaceus,* Phytopathogen, Antibacterial, Antifungal property, Phytopathogen, Strain, Lactic acid bacteria

1. Introduction

The anticipated population growth in the coming decades and the increasing demand for livestock products necessitate the production of larger quantities of food products. Concerns about food quality in industrialized countries and the need to enhance soil fertility are among the major challenges facing global agriculture today [1]. The isolation and study of probiotic bacterial strains, as well as the development of new applications in this field, hold significant importance. The use of probiotics in crop production is gradually gaining traction [2]. The isolation of local microorganism strains with high antagonistic properties and the identification of their biologically active compounds are of great significance [3]. When discussing the biological activity of bacteria, it can be noted that they are completely safe for humans and the environment. Their culture supernatant has the ability to produce signaling molecules, immunoactive proteins, and biologically active compounds, including not only peptide antibiotics but also hormone-like substances that play a special role [4,5]. Additionally, probiotics produce substances (e.g., organic acids, peptides, cyclic dipeptides, fatty acids, volatile compounds, lactic acid, bacteriocins, hydrogen peroxide, and antibiotics) that exhibit activity against pathogenic and conditionally pathogenic microorganisms [6]. At present, plant fungal diseases are primarily controlled using synthetic fungicides. However, their excessive and improper use harms the environment and increases the resistance of phytopathogens to these fungicides [7].

Under the conditions of Uzbekistan, four strains of lactic acid bacteria were isolated from locally sourced cow milk yogurt and pickled cabbage: *Lactobacillus plantarum №1, Lactobacillus brevis №1, Lactobacillus fermentum №1, Pediococcus pentosaceus №1.*

These strains were identified based on their morphological and cultural characteristics using Bergey's Manual of Determinative Bacteriology, as well as by MALDI-TOF mass spectrometry. The antagonistic properties of the lactic acid bacteria isolated from pickled cabbage and kefir were determined using the agar block method. Selected lactic acid bacteria were cultivated on MRS agar medium (HiMedia, pH 6.2) in Petri dishes for 48 hours at room temperature. Phytopathogenic micromycetes were cultured on solid Czapek-Dox agar medium (g/l: KH_2PO_4 – 1.0; $MgSO_4$ – 0.5; $NaNO_3$ – 3.0; KCl – 0.5; $FeSO_4$; sucrose – 2.0; trace elements – 1 ml; trace element mixture: 500 mg $FeSO_4$, 156 mg $MnSO_4 \cdot 4H_2O$, 167 mg $ZnCl_2$, 200 mg $CoCl_2$, 1 ml 19% HCl in 100 ml distilled water) for 6 days at 28 ℃. 6 mm wells were made in the Petri dishes containing the lactic acid bacteria cultures. A spore suspension of phytopathogenic fungi was prepared at a concentration of 10^7 spores/ml from 4-day-old cultures grown on Czapek agar. *Lactobacillus plantarum* demonstrated strong antifungal activity against phytopathogenic micromycetes from the genera *Aspergillus*,

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Fusarium, *Penicillium*, and *Rhizopus* [8]. To assess bacteriocinogenic activity, the cultures were inoculated into MRS medium and incubated at 37 ±1 ℃ for 24 hours. Then, 1.5% agar-containing Petri dishes were prepared, and marked according to the number of test strains. 2–5 µl of 12-hour bacterial cultures were spotted on each mark. The inoculated dishes were incubated at 37 ±1 ℃ for 4–12 hours. Subsequently, the cultures were exposed to ultraviolet radiation for 30 seconds, followed by an additional 3–12 hours of incubation under the same conditions. The cultures were grown for 48 hours and then treated with chloroform. The Petri dishes were ventilated for 15 minutes. Thereafter, 3–5 ml of test cultures with a titer of 10^7 CFU/ml were inoculated into a soft agar overlay (0.7%) and incubated at 37 ±1 ℃ for 4–12 hours. The antimicrobial activity of the lactic acid bacterial cultures was studied against the following seven opportunistic pathogens: *Aeromonas veronii, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Candida albicans.*

2. Materials and Methods

Lactobacillus brevis №1 showed strong antibacterial activity against *St. aureus*, *B. subtilis*, *E. coli*, and *C. albicans*, but had *no activity against P. aeruginosa*. *Lactobacillus fermentum №1* exhibited the highest antimicrobial activity against *St. aureus*, *E. coli*, *Pr. mirabilis*, and *C. albicans*. *Pediococcus acidilactici P1* showed strong activity against *E. coli*, *P. aeruginosa*, and *C. albicans*. *L. plantarum* and *L. fermentum* showed no antimicrobial activity against *A. veronii*. (Figure 1)

One of the most widely distributed lactic acid bacteria, *Lactobacillus plantarum*, exhibits strong antagonistic effects against a wide range of microorganisms. To evaluate its antagonistic potential in vitro for postharvest decay control caused by phytopathogenic fungi such as *Aspergillus flavus*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, and *Fusarium avenaceum*, the strain *L. plantarum* (DSM 20174) was tested. The results showed that *Lactobacillus plantarum* exerted a stronger inhibitory effect on spore germination than on mycelial growth across all tested fungi.

3. Results and Analysis

To determine the antibiotic sensitivity of *Lactobacillus* strains, the disk diffusion method was employed. Selected *Lactobacillus* strains were grown in MRS nutrient medium at 37 ℃ for 24 hours. The cell suspension was adjusted to a concentration of 10^7 CFU/ml**,** and uniformly spread on the surface of MRS agar using a sterile spreader.

Antibiotic discs were placed on the surface of the inoculated Petri dishes, containing the following antibiotics: Rifampicin (5 µg), Erythromycin (15 µg), Oxacillin (1 µg), Cefotaxime (30 µg), Gentamicin (10 µg), Cefazolin (30 µg), Tetracycline (30 µg) The plates were incubated at 37 ℃ for 24 hours. The diameter of the inhibition zone around each antibiotic disc was measured to assess the susceptibility of each strain. All experiments were conducted in triplicate. Further research was carried out to assess the antifungal activity of the isolated bacterial strains: *Lactobacillus plantarum №1, Pediococcus pentosaceus №1, Lactobacillus fermentum.* These were tested against the following phytopathogenic micromycetes: *Alternaria alternate, Verticillium dahlia, Fusarium spp., Aspergillus flavus, Aspergillus oryzae.* The antagonistic properties of the bacterial strains were evaluated using the agar block method (Table 1).

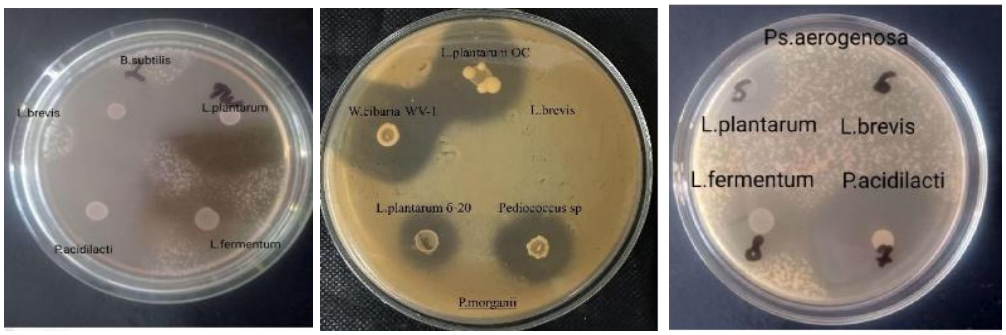

Figure 1. Antibacteriocinogenic Activity of Lactic Acid Bacteria

Table 1. Antifungal Properties of Lactic Acid Bacteria

№	Lactic Acid Bacteria Strains Used in the Study	Antifungal Activity of Lactic Acid Bacteria (Inhibition zone diameter, mm)				
		Alternaria alternate	Verticillium dahlia	Aspergillus flavus	Aspergillus oryzae	Fusarium specius
1	*L. plantarum №1*	35 ±1,6	33 ±1,6	29 ±0,3	34,3 ±0,6	25 ±0,3
2	*P. pentosaceus №1*	25 ±1,6	25 ±0	18 ±0,3	-	22 ±0,4
3	*L. fermentum №1*	27 ±0,3	15 ±0,3	-	19,6 ±1,5	18 ±0,6
4	*L. brevis №1*	22 ±1,6	15 ±0	19 ±0,3	-	21 ±0,4

"-" indicates the absence of a growth inhibition zone. Inhibition zone diameters are interpreted as follows: Less than 10 mm – No antagonistic activity 10–15 mm – Weak activity 15–20 mm – Moderate activity Greater than 20 mm – Strong (definite) activity

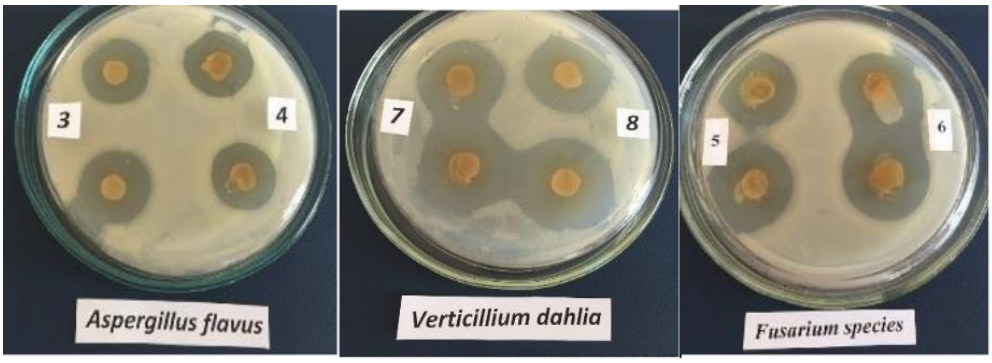

Figure 2. Antagonistic Properties of Lactic Acid Bacteria

According to the results of the study, all four selected strains demonstrated strong antifungal activity against the tested phytopathogenic micromycetes: *Alternaria alternata*, *Verticillium dahliae*, *Aspergillus flavus*, *Aspergillus oryzae*, and *Fusarium spp.*

The strain *Lactobacillus fermentum №1* showed the following inhibition zones (mean ± SD) against individual phytopathogens: *Alternaria alternata* – 27 ± 0.3 mm, *Verticillium dahliae* – 15 ± 0.3 mm, *Aspergillus oryzae* – 19.6 ± 1.5 mm, *Fusarium specius* – 18 ± 0.6 mm.

The antifungal activity of the lactic acid bacteria strains was determined based on the inhibition radius of the fungal growth, with clear inhibition zones ranging from 15 to 35 mm in diameter.

The highest antagonistic activity was observed in the strain *Lactobacillus plantarum №1*, showing inhibition zones of: 35 mm against *Alternaria alternate*, 33 mm against *Verticillium dahlia*, 29.6 mm against *Aspergillus flavus*, 25 mm against *Fusarium specius*, 34.3 mm against *Aspergillus oryzae*.The strain *Pediococcus pentosaceus №1* exhibited the following inhibition zones: 25 mm against *Alternaria alternate*, 25 mm against *Verticillium dahlia*, 18 mm against *Aspergillus flavus*, 22 mm against *Fusarium specius*. Meanwhile, *Lactobacillus fermentum №1* showed antifungal activity with inhibition zones of: 27 mm against *Alternaria alternate*, 15 mm against *Verticillium dahlia*, 18 mm against *Fusarium specius*, 19 mm against *Aspergillus oryzae*. These results clearly demonstrate the strains' ability to inhibit the growth of phytopathogens.

4. Conclusions

The antifungal activity of lactic acid bacteria strains was determined by the growth radius of the phytopathogens and it was found that they form a ring from 15 to 35 mm.

The isolated strains *Lactobacillus plantarum №1*, *L. brevis №1*, *L. fermentum №1*, and *Pediococcus pentosaceus №1* exhibited high antibacterial and antifungal activity against opportunistic and pathogenic microorganisms.

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