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MICROFLORA AND PLANT PATHOGENIC FUNGI AFFECTING BACTERIA IN GRAPE PLANTATIONS IN UZBEKISTAN

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SUMMARY

In the presented study, isolation of bacterial strains, viz., *Pantoea agglomerans, Priestia megaterium* and phytopathogenic micromycetes that cause damage and eventually death of grape crops, came from a 10 to 15-year-old vine plantation. A *Pantoea agglomerans* gram-negative bacillus facultative and anaerobic bacterium strain achieved isolation from grape plants, with its morphological characteristics studied. Bacterial strains with antifungal activities against phytopathogenic micromycetes succeeded in their identification. Bacterial isolates collected from the vines underwent screening for their growth properties. It was apparent that *Pantoea agglomerans* actively grew wheat coleoptiles by 2.6 mm and maize coleoptiles by 2.3 mm compared with the control. Observable evidence also showed that sorghum coleoptile actively grew by 1.7 mm compared with the control treatment by 2.9 mm. The 26 *Aspergillus* sp., 23 *Penicillium* sp., 25 *Fusarium* sp., 30 *Alternaria* sp., and five *Curvularia* sp. phytopathogenic micromycetes belonging to the genus were notable. Bacterial strains isolated from the vine showed the highest antifungal activity against micromycetes belonging to the genus *Penicillium* and reduced the radius of phytopathogenic growth to 47–54 mm. Compared with micromycetes belonging to the genus *Fusarium*, it was also apparent that the pathogen reduced the growth radius to 27–35 mm. Isolation of phytopathogenic micromycetes from the vine allows early detection and prevention of grape diseases. Based on these studies, the identification of antifungal activity of the bacterial strains isolated from the vine and the presence of phytohormones in the culture fluid indicated that it is an essential and environmentally friendly biological tool in the cultivation of grapes for human consumption.

Key words: *Vitis vinifera* L., grape, *Pantoea agglomerans, Priestia megaterium, Lactobacillus plantarum, Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Alternaria* sp., *Curvularia* sp., microflora, phytohormone, antagonist, phytopathogen

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Key findings: Disease-spreading phytopathogenic micromycetes belonging to the genera *Fusarium*, *Penicillium, Alternaria, Curvularia,* and *Aspergillus* spp. reached identification from infected grape organoids in the territory of Uzbekistan. Strains of *Priestia megaterium* and *Pantoea agglomerans* isolated from healthy grapevine gained genetic recognition and showed high antifungal activity against grapevine phytopathogens.

INTRODUCTION

Currently, grape diseases are common in Uzbekistan and worldwide, severely damaging the vine, spoiling the quality of the product, and reducing the fruit yield by 25%–70%. Powdery mildew (*Uncinula necator*), anthracnose (*Gloeosporium ampelophagum*), cercosporiosis (*Cercospora vitis*), gray rot (*Botrytis cinerea*), black rot (*Phoma lenticularis*), cypress necrosis (*Rhacodiella vitis*), and bacterial root collar cancer (*Agrobacterium tumefaciens*) diseases are prevalent (Vafaie *et al.,* 2018; Turaeva *et al.,* 2020; 2021). Increasing the export potential of Uzbekistan to meet the population's needs for environmentally friendly products is one of the crucial issues.

The study of prospects for developing environmentally friendly products showed that, based on world market requirements, crop production in farms, especially viticulture, encounters a decrease in export opportunities (Suirta *et al*., 2021; Osman *et al*., 2023). A decline in the growing products might be due to insufficient attention to producing and applying intensive technologies. "Toifi," "Rizamat," "Kishmish sugdiyona," "Khusaini," "Kelin barmok," "Shohonak," and "Mercedes" are the existing grape cultivars in Uzbekistan. "Kishmish Black" is a local grape cultivar with a high bush, large branches, and fast-growing characteristics. Also resistant to diseases and pests, it is easily affected by cercosporiosis and powdery mildew. Grape bunches are large, with an average weight of 172 g. The shape of the cylinder is conical, porous, sometimes dense, and black.

Midseason raisin variety in August-September, the leaf is medium, five-lobed, dark green, and the flower is bisexual. The head is large, 400–450 g, with many scales, and the scattering is medium. Clusters are large, oval, black, and covered with wax (4–5 g). The skin is thin, ripe, and fleshy. Sugar content and acidity are 25%–26% and 6 g/l, respectively. Productivity is 180–200 t/ha, and the bush grows sturdily. Rizamat ota – an early-midseason cultivar, the shrub is medium, the berries are large, and the average weight is 350–400 g. It is cylindrical, pink, large, and hard. The fruits fully ripen on July 20–25, and the productivity is 200–250 t/ha.

Grapes are one of the valuable crops of the Mediterranean region. Major grape-growing countries are Italy, France, Spain, Turkey, Greece, and Portugal. Turkey and Greece are significant exporters of dried grapes. Many biotic and abiotic factors cause quantitative and qualitative losses to grapes every year. The vine is a type of perennial plant that annually absorbs large amounts of nutrients from the soil and also sustains infection of about 700 diseases. The most dangerous diseases are *Alternaria*, gray mold, *oidium*, and bacterial cancer (Turaeva*,* 2019). In perennial plants, the foremost source of infections is the field soil and its stem (Qin *et al.,* 2020) and the damage caused by phytopathogenic microorganisms (Berto *et al.,* 1999; Turaeva *et al.,* 2023). Phytopathogens causing plant damage include bacteria, viruses, micromycetes, and nematodes (Zhou and Li, 2020; Shavkiev *et al.,* 2022).

The fungus *Alternaria alternata* is a phytopathogen that parasitizes various crops, including citrus fruits and grapes. Alternaria disease develops on the plant's leaves and damages the leaf plate and stems, causing the plant to dry out (Bazioli *et al.,* 2019). In protecting vineyards from phytopathogenic microorganisms, using up to 100 kg of pesticides per hectare of land annually is necessary. Yet, these pesticides also cause numerous environmental problems that harm the environment and human health. Regularly using chemicals against pathogens leads to an increase in the adaptability and viability of

phytopathogens, as well as, a high level of damage to vineyards (Turaeva *et al*., 2020; 2021; Zhou and Li, 2020). High humidity and warm temperatures favor developing phytopathogenic fungi species *Alternaria* and *Aspergillus* (Signaboubo *et al*., 2016; Romain *et al*., 2020).

Beneficial microflora of plants are helpful microorganisms that form the basis of bioprotectors, biocontrol agents, or biostimulants and are environmentally friendly and promising pesticide alternatives. The need in the population for ecologically friendly products that can be stored safely for a longer time without chemical preservatives is increasing. Therefore, it is imperative to isolate local strains of microorganisms with aggressive properties and use them as biological control agents (Silva-Valderrama *et al*., 2021). The generally recognized mechanisms of control of biocontrol agents of phytopathogenic micromycetes are the loss of competition for nutrients, space, and resistance, which comprise indirect interaction of the plant and pathogen. It refers to the formation of short rings and the introduction of cell wall-destroying enzymes into pathogenic hyphae (Köhl *et al*., 2019).

Potentially antagonistic microorganisms, the mechanism of action of biocontrol agents through the production of antibiotics that interact directly with mycoparasites and phytopathogens is the most effective method of biological control that reduces the risk of toxic damage to humans, crop plants, and the environment (Zang *et al*., 2020). The phytopathogenic micromycete *Plasmopara viticola* causes rot disease and damage to viticulture. The isolate, SY286 *Ochrobactrum* sp., has been identified as a species and described as a biological control agent for grape root rot and to reduce leaf spot by 93.18% (Burovinskaya and Yurchenko, 2021). The grape leaves with necrotic spots result from several species of fungi belonging to the genus *Alternaria*. In addition, phytopathogenic species of *Alternaria* kill the plant tissues, reducing the photosynthetic potential of plants (Millan *et al.,* 2021).

The influence of the phytopathogenic micromycete *Botryosphaeria* in the spring helped determine less development of branches and bud formation and damage to the xylem tissues. The phytopathogenic micromycete *Botryosphaeria* causes grape cancer, and the severe damage triggers the death of younger vine plants (Markus *et al.,* 2021; Muntean *et al.,* 2022). *Botrytis cinerea, Plasmopara viticola,* and *Erysiphe necator* initiate gray rot and downy mildew in grapes fruit, and vegetable crops (Tsalgatidou *et al.,* 2022). The phytopathogenic micromycete *Penicillium digitatum's* isolation had its yeast used as a biological control agent (Katrijn *et al.,* 2020). The micromycete belonging to the genus *Penicillium* causes blue mold rot, which is also dangerous for the stems and fruits of grapes (Kim *et al.,* 2007). Under the influence of this pathogen, the berries soften and change color, causing crop losses and further producing mycotoxins, which are harmful to the human body (Habib *et al.,* 2021).

The fungi Aspergillus, Penicillium, and Alternaria have reached identification as representative isolates of the major toxigenic genera affecting grapes in Lebanon. By studying the microflora of grapes affected by blue mold, it was evident that phytopathogenic micromycetes *Penicillium digitatum, Alternaria solani,* and *Fusarium oxysporum* were dominant (Gobbi *et al.,* 2020). Biological control with high potential is an essential and safe tool to reduce dependency on chemical pesticide uses for disease control. However, limited effective and commercially available biocontrol agent is one of the chief hurdles to the widespread use of biocontrol agents. The antagonist strain formed a ring 11.67 mm in diameter against the phytopathogenic micromycete *Colletotrichum gloeosporioides* and 14.67 mm against *Colletotrichum acutatum* and stopped the mycelium growth.

The antifungal activity of the four strains of bacteria *Lactobacillus plantarum* against phytopathogenic and ochratoxygenic strains of *Aspergillus carbonarius* isolated from grape plantations and fruits underwent studies. The bacterial strains inhibited the growth of pathogens by 88% and significantly reduced the production of toxins by 100%. Using these bacteria as biological agents for plant pathogens has declarations as safe by the

European Food Safety Authority (Habib *et al*., 2021; Millan *et al*., 2021). By studying the antifungal activity of more than 7000 bacteria against the phytopathogens *Fusarium*, *Aspergillus*, *Rhizopus*, *Alternaria*, *Phytophthora*, *Glomerella*, *Sklerotium*, *Rhizoctonia*, and *Sclerotinia*, a high activity of some bacterial strains was revealed (Miguel *et al.,* 2018). The purpose of the research is to isolate phytopathogenic micromycetes from diseased grape plant samples in some regions of Uzbekistan, to study vine diseases, identify antagonistic strains, and develop biological control measures.

MATERIALS AND METHODS

Sampling and culture of microorganisms

Samples of local "Kishmish" and "Rizamat" cultivars grown in the foothills of Urgut District of Samarkand region and Altiariq District of Fergana region, Uzbekistan comprised specimens taken from the stem, leaves, fruit, and root rhizosphere of infected grape cultivars. The stem, fruit, and leaf samples incurred 10 times cleaning with 3% hydrogen peroxide, alcohol, and distilled water. The research maintained that up to 70% of plants sustained phytopathogenic micromycete infections.

The infected plants developed round, dark brown spots 5 mm in diameter. Cutting small pieces (5 mm^2) from infected plant leaves continued washing with 70% ethanol for 30 s and with sterile water, then planted on a potato dextrose agar medium and incubated in a thermostat at 25 °C. The study of morphological features proceeded, isolating three isolates (Alimova, 2010).

Breaking the samples using crushed glass ensued in the laboratory, with the broken specimens cultured under sterile conditions. By the classical microbiological (smear) method, the prepared sterile cotton swabs took samples and placed them under 0.9% saline. Rhizosphere root specimen at 1 g incurred diluting with 0.5 ml in 10 test tubes, each containing 5 ml of sterile water (Felšöciová *et al.,* 2017). The samples in diluted test tubes 4-3 and 5-6 continued sowing on nutrient media. The samples prepared for microbiological analysis acquired planting on meat-peptone agar (MPA), potato dextrose agar (PDA), and oatmeal agar (OA), then inoculated on Czapek medium with agar and placed in a thermostat with a temperature from 20 °C to 38 °C. Pure cultures of phytopathogenic micromycetes reached isolation by subculture on nutrient media.

Identification of bacteria

Growth properties of *Bacillus* sp.1, *Azotobacter* sp.5, *Pseudomonas* sp.1, *Pseudomonas* sp.2, *Bacillus* sp.2, *Azotobacter* sp., *Pantoea* sp., *Bacillus* sp.3, and *Pseudomonas* sp.3 strains isolated from vines gained screening by the hypocotyl method. For this, growing 10 different bacterial strains continued in a liquid (pH 6.2) DeMan-Rogosa-Sharpe (MRS) medium at 37 °C for two days on a shaker with a rotation speed of 200–220 rpm. The observed growth effects of wheat coleoptiles (0.5 cm long) received measuring for 24 h in culture fluids filtered through a 0.22 μκ filter. The morphological characteristics study under the XSP-136 B and NLCD-307B light microscopes (400 times magnification) helped to identify microbial species. Microbiological indicators' utilization aids in identifying microorganisms (Pidoplichko and Milko*,* 1971; Litvinov, 1967). Microorganisms' identification also employed mass spectrometry (MALDI TOF) in the sanitary and hygienic laboratory of the Ministry of Health, Uzbekistan (Bilay, 1982).

Antagonistic properties of isolated bacterial strains

Determining the antagonistic properties of bacterial strains isolated from grapes grown in Uzbekistan followed the agar block method. The bacterial strains growing in Petri dishes on a DeMan-Rogosa-Sharpe (MRS) HiMedia agar medium (pH 6,2) ensued for two days. On Czapek's agar (g/l: KH2PO4 – 1,0; MgSO4-0,5; NaNO3 - 3,0; KCl - 0,5; FeSO4 - pieces; sucrose -2; microelements - 1 ml; [mixture of microelements: 500 mg FeSO4; 156 mg

MnSO4 4H2O; 167 mg ZnCl2; 200 mg CoCl2; 1 mg 19% HCl, and 100 ml distilled water]) had the phytopathogenic micromycetes grow for six days in a thermostat at 28 °C. Selected bacterial strains' cultivation engaged the streak method. Blocks' preparation with a diameter of 6 mm from phytopathogenic fungi grown on Czapek's nutrient medium with agar for six days progressed. The experiments in triplicate had the antifungal activity of the selected bacteria determined by reducing the pathogen growth radius.

Statistical analysis

All experiments, performed in triplicate, had the mean values and their standard error (SE) calculated in Microsoft Excel (Microsoft Corporation, USA). The analysis of significant differences among the various treatments, including control, used the analysis of variance (ANOVA) program (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

The cultural characteristics of bacterial strains isolated from grapes have undergone study. *Bacillus* sp.1, *Azotobacter* sp.5, *Pseudomonas* sp.1, *Pseudomonas* sp.2, *Bacillus* sp.2, *Azotobacter* sp., *Pantoea* sp., *Bacillus* sp.3, and *Pseudomonas* sp.3, bacterial strains, based on the culture test, incurred evaluation on the high culture properties of *Bacillus* sp.3 and *Pantoea* sp., determining the active growth properties of bacterial isolates. The experiments carried on with 10 replications. The experimental variants consisted of the native *Bacillus* sp.1, *Azotobacter* sp., *Pseudomonas* sp.1, *Pseudomonas* sp.2, *Bacillus* sp.2, *Azotobacter* sp., *Pantoea* sp., *Bradyrhizobium* sp., *Bacillus* sp.3, and *Pseudomonas* sp.3 bacterial strains and the distilled water as control (Figure 1).

Growth characteristics have shown in some bacterial strains, with the highest activity of the bacterial strains (*Bacillus* sp.3 and *Pantoea* sp.) identified in variants using a liquid culture. Observations also noted that in the genus *Pantoea* sp., wheat coleoptile grew by 2.6 mm, corn coleoptile by 3.5 mm, and in variants using *Bacillus* sp.3 liquid culture, the growing wheat coleoptile enhanced by 2.5 mm and corn coleoptile by 2.9 mm. Compared with the control, the strain *Pantoea* sp. fluid culture caused active growth by 2.3 mm in the maize coleoptile, and *Bacillus* sp.3 fluid culture enhanced growth by 1.7 mm in the maize coleoptile. These two variants were options for further studies. In addition, the research and identification of morphological characteristics of bacterial strains with active growth properties isolated from the grape cultivars "Kishmish" and "Rizamat" (*Vitis vinifera* L.) took place. The morphological characteristics' scrutiny of bacterial isolates, i.e., *Pantoea* sp. and *Bacillus* sp.3, isolated from grapes, also progressed (Figure 2).

The strain *Pantoea* sp. belongs to the gram-negative and rod-shaped type of bacteria. The rods were 1.3 μм and 3 μм wide and long, respectively. This type of bacteria also belongs to the facultative group of anaerobic bacteria. The nutrient grows in a circle on the surface of the medium. Also producing a yellow pigment in some nutrient medium, the colony grows to a round, slightly raised, and smooth shape. The pH values of four and nine develop at an optimum temperature from 35 °C to 40 °C (Figure 2A). The *Bacillus* sp. gram-positive belongs to the group of aerobic spore-forming bacteria. The rod-shaped spores were 1.5 μм wide and 5–6 μм long. Bacteria of this group constitute the largest group living in the soil, and their cells can form pairs or short chains. In nutrient media with pH values of five and nine, it develops at an optimum temperature from 28 °C to 45 °C. (Figure 2B). In achieving high precision, identifying bacterial strains, viz., *Pantoea* sp. and *Bacillus* sp., provided 16S RNK sequences and presented in a phylogenetic tree.

Evolutionary relationships of taxa

Evolution history inferred used the Neighbor-Joining method, and the optimal tree has emerged (Aristovskaya *et al.,* 1962). Above the branches, the percentage of replicate trees with linked taxa appears based on the Bootstrap test (500 replicates) (Hsuan *et al*.,

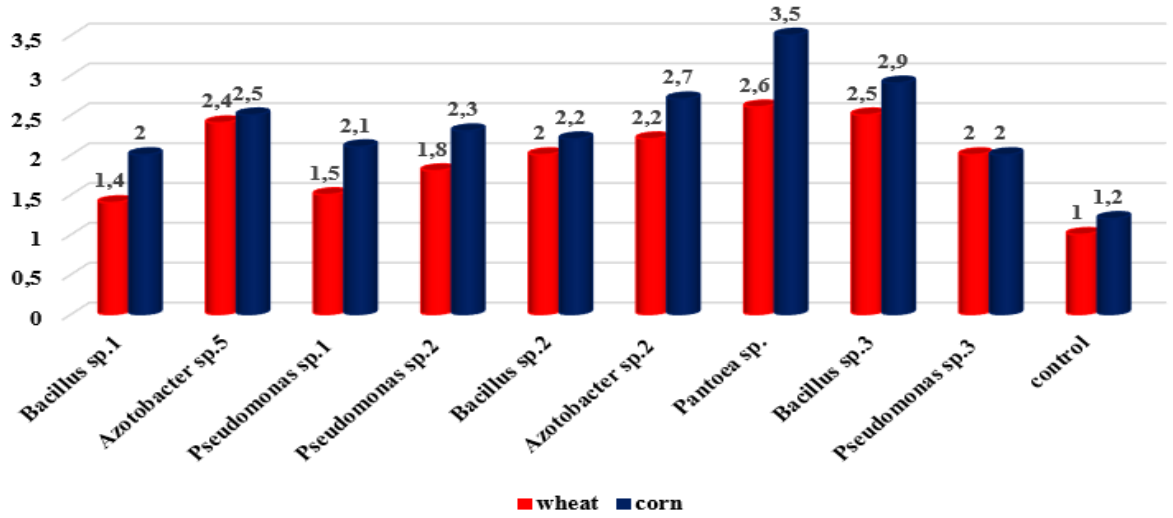

Figure 1. Cultivation properties of bacterial isolates isolated from grapes on coleoptiles of wheat and corn.

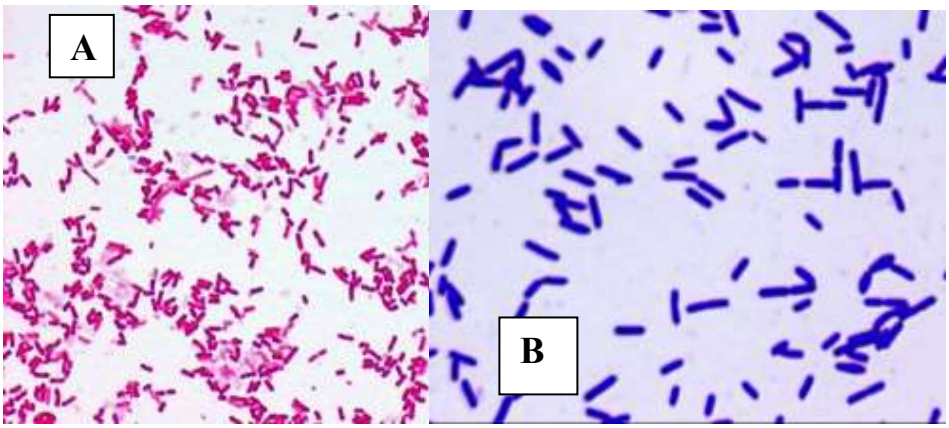

Figure 2. Microscopic view of bacteria of the *Pantoea sp*. (A) and *Bacillus sp.* (B) isolated from grapes.

2011). The tree drawn to scale had the branch length in the same units as the evolutionary distances used to define the phylogenetic tree. Evolutionary distance formulation followed the Maximum Composite Likelihood method, and the sections of the number of base substitutions per site were given (Garibova and Lekomtseva, 2005). The analysis included 12 nucleotide sequences, removing all the ambiguous positions for each pair of series. The final dataset contained 1511 total positions, with the evolutionary analysis performed on МЕГА X (Kazakov, 2017).

In the course of this research, the influence of the liquid cultures of the isolated strains of bacteria on the root formation of grape cuttings was definite. The experiment ran for 40 days. Bacterial strains *Priestia megaterium* and *Pantoea agglomerans'* incubation in optimal nutrient media continued for three days and carried out in triplicate. The study had bacterial strains of *Priestia megaterium* and *Pantoea agglomerans* diluted in a ratio of 1/50 and 1/100 of a 3-day bacterial fluid. It was visible in the rooting of grape cuttings. Water served as the control (Figure 3).

Pantoea agglomerans BDI-1 16S ribosomal RNA https://www.ncbi.nlm.nih.gov/nuccore/OP727725

GGCGTAACACATGCAAGTCTGACGGTAGCACAGAGGAGCTTGCTCCTTGGGTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGGATCTGCCCGATAGAGGGGGATAACC
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TGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATGGGGTTAATAACCTTGTCGATTGACG
TTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTT
AAGTCAGATGTGAAATCCCCGGGCTTAACCTGGGAACTGCATTTGAAACTGGCAGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAG
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GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCACGGAATTTGGCAGAGATGCCTTAGTGCCTTCGGGAAC
CGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGATTCGGTCGGGAACT
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GGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTATCCACTTTGTGATTCA
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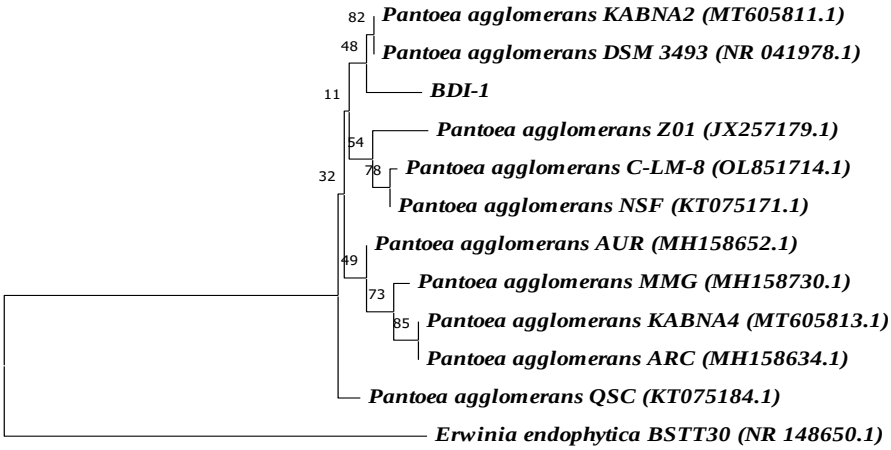

Priestia megaterium BDI-2 16S ribosomal RNA. https://www.ncbi.nlm.nih.gov/nuccore/OP782582

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CCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTCATGGGAGATGATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGC
GGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG
GAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGT
ACAAGAGTAACTGCTTGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGG
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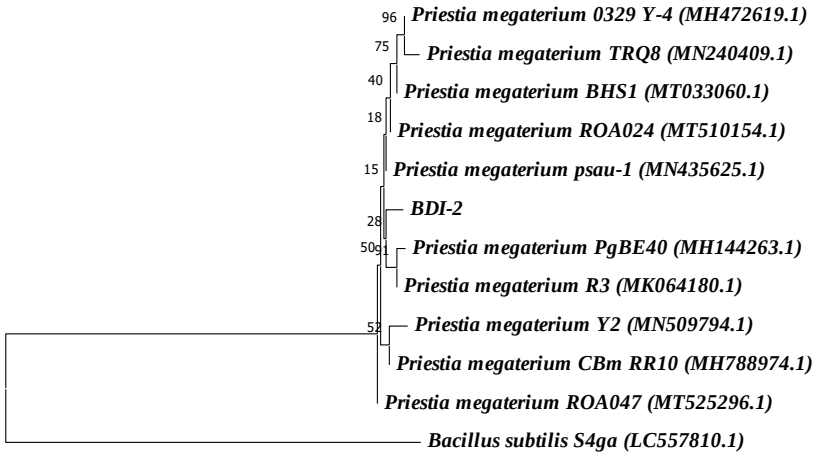

Figure 3. Molecular identification of *B. megaterium* and *P. agglomerans* bacterial strains.

The leaf formation, along with the rooting of grape cuttings, showed in experimental variants where bacterial strains of *P. megaterium* and *P. agglomerans* gained diluting in a ratio of 1/100. Further research on a diluted 1/100 version of the selected bacterial strains proved to be the optimum concentration for vine root formation. In the study of the microflora of grape stems, leaves, and fruits in a 10 to 15-year-old grape plantation, the isolation of phytopathogenic micromycetes transpired.

The samples of 10- and 15-year-old Black "Kishmish" and "Rizamat" cultivars grown in the District Urgut, Samarkand region and District Altyarik, Fergana region, Uzbekistan (Figure 5), underwent a microbiological analysis. The obtained samples sustained purification with 3% hydrogen peroxide, alcohol, and distilled water, then sown under sterile conditions on pre-prepared nutrient media and placed in thermostats at 27 °C (Figure 4). Microbiological analysis employed the wet chamber method. The samples taken from the organelles and fruits of grapes attained plating on meat-peptone agar, starch-ammonia agar, potato-dextrose agar, Mandel agar, and Czapek agar. All these variants also incurred heat exposure on thermostats with a temperature from 20 °C to 38 °C (Saitou and Nei, 1987). On the third day of the study, the formation and development of micromycetes colonies were evident in cultural samples.

Evaluating the microflora of grape organelles had plant samples placed on nutrient media, planted, and incubated for six to seven days. By isolating pure cultures, adding PDA to the nutrient medium continued (potato dextrose agar with 1000 ml of water, 200 g of washed and peeled potatoes and boiling solution, 20 g of dextrose, 20 g of agar powder, the pH value at 5,6 ± 0,2) (Felsenstein, 1985). The replanting method used is generally accepted in microbiology (Figure 6).

The conducted studies had three replications. Subculturing the PDA medium 7-8 times obtained the pure microorganism isolates. The isolates grown in three replicates on a nutrient medium incurred placing in thermostats at 26 °C. The next stage of the research studied the morphological characteristics of microorganisms to determine the types of isolates. Based on the results, the conidia appeared in a chain arrangement, the apex of the conidia has a spherical or spherical swollen shape, with an average diameter of 3.12 ± 2.24 μм, from dark brown to black, and the cell walls were uneven (Figure 7). It has been distinguishable that conidiophores with smooth walls and flat spherical spores belong to *Aspergillus Micheli* ex Fries. These characteristics of micromycetes were descriptive of filamentous fungi belonging to the genus *Aspergillus* (Litvinov, 1967).

Based on the results, it was apparent that the development of phytopathogenic micromycetes belonging to the genus *Aspergillus* also emerged in the experimental variants (Figure 8A). Some species of fungi belonging to the genus *Aspergillus* (*Aspergillus niger, Aspergillus flavus*) spread powdery mildew (powdery rosa) in crop plants. It was evident that 12%–17% of the grape (*Vitis vinifera*) crop sustained powdery mildew bearings in the 5th region of Pakistan. Relating to the presented research, infected fruits of grapes, covered with black spores and inky, incubated in a nutrient medium with PDA for 14 days at 25 °C, isolated and identified phytopathogenic micromycetes belonging to the genus *Aspergillus* (Tamura *et al*., 2004). Phytopathogenic micromycetes spread to vineyards through the water and air. Also, the perennial host hibernates in plants, and since the remainder of the previous year, infected plants developed in the soil, damaging and destroying the crop.

Assessing the morphology of a purified colony of micromycetes on an agar medium also happened. Formation of white-gray, from blue-green to dark-green colonies, with spherical spores, uniform, 200–500 μм long, mostly 2–3 and four-layered, thin, smooth-walled candidophores, 7–14 μм in size, cylindrical, smoothly separated, and spherical conidia protruded. These characteristics were of micromycetes belonging to the genus *Penicillium*, with the isolates identified as *Penicillium* sp. (Figure 8B). Also, micromycetes form fluffy white colonies at 1–2 days of

Figure 4. The influence of bacterial strains *P. megaterium* and *P. agglomerans* on the rooting of grape cuttings.

Figure 5. The samples taken in September and December from infected grape cultivars "Kishmish" and "Rizamat" in a 10 to 15-year-old plantation.

Figure 6. Samples planted in nutrient media for identification of cassava vine or microflora.

Figure 7. Pure isolates of micromycetes isolated from grape stems, leaves, and fruits.

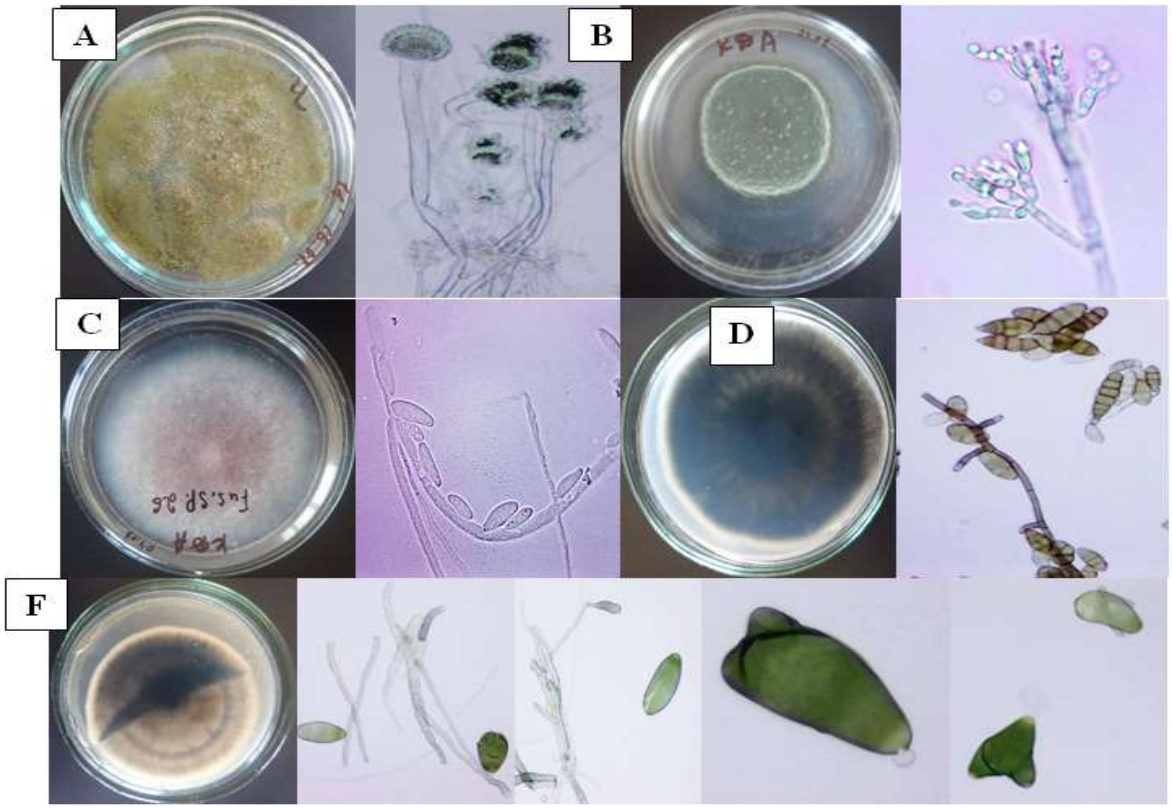

Figure 8. Microscopic view of isolated pure micromycetes.

development; on the 4–5 days, the back of the calyx becomes dark purple, and the upper part is light purple.

Micromycetes did not produce microconidia; however, they formed numerous thick macroconidia. Macroconidia were 2.5 to 5 µm thick and 20 to 30 µm long, divided into three or five. Chlomidospores with a diameter of 9 to 14 µм, thick-walled, spherical, singly, together, or in chains, emerged both in hyphae and in macroconidia. The viability of chlamydospores in macroconidia lasts longer. These morphological features of *Fusarium* sp. characteristic of fungi belonged to the genus (Figure 8C). By studying the morphological features of the micromycetes, it was notable that it forms a long chain and forms spores of various types from the ends of the hyphae of conidiophores, forming conidiophores in the form of a straight elongated chain or flexible, and light or dark brown. Conidiophores have proven to produce short-beaked, brown, smooth, flat-surfaced conidia with an average diameter of 10 ± 2.10 µm and have the characteristics of the genus *Alternaria*. *Alternaria* sp. has been distinctive as fungi belonging to the genus (Figure 8D). Curvularia is a worldwide group of micromycetes that contain pathogens, which also transmit diseases to crops. Curvularia species are crucial pathogens of foremost crops, such as, rice, corn, wheat, and sorghum. The said species members have been markedly the cause of respiratory, skin, brain, and cornea infections in immunocompromised patients.

In the presented research, isolates of micromycetes belonging to the genus *Curvularia* came from infected grape samples (Figure 8F). Micromycetes of this species grow slowly; the hyphae change color from gray to light brown and form a white ring around them. Conidiophores can be single or multiple, branched, or septate, with a wall thickness of 2–4.5 µм. Conidiogenous cells were smooth-walled, terminal, or intercalary; their reproduction is sympodial, pale brown,

Table 1. Phytopathogenic micromycetes isolated from infected grape plantations in viticultural areas of Samarkand and Fergana regions, Uzbekistan.

No.	Phytopathogenic micromycetes	Samarkand region		Fergana region	
		District Urgut	District Kattakurgon	District Altyarik	District Yozevon
1	*Aspergillus sp.*	7	10	3	6
2	*Penicillium sp.*	9	4	5	5
3	*Fusarium sp.*	11	7	2	5
4	*Alternaria sp.*	8	9	4	9
5	*Curvularia sp.*	3	2	-	-

subcylindrical to swollen, and 6–17.5 µm × 3–6 µm. Conidia warty, geniculate, ellipsoid, oval, middle cells disproportionately enlarged, light brown to dark brown, apical and basal cells light, (3)4-distoseptate, 20–27.5 µm × 8–12 µm, darkened, slightly thickened, 1.8–3.2 µm wide. Chlamydospores and microconidia showed no formation, and the ends of the mycelium protruded, making the individual warty conidia.

The width of the conidia ranges from 12–17 µм to 14–18 µм, the length is from 20–27.5 µм to 20–35 µм, and it may form various sizes of spores (Kumar *et al*., 2018). According to the study results, micromycetes belonging to the genera *Fusarium*, *Penicillium*, *Alternaria*, *Curvularia*, and *Aspergillus* attained isolates from plant organelles and fruits of 10 to 15-year-old grape plantations in the regions of Samarkand and Fergana, which spread diseases to grapes (Table 1). Agreeing with the prevailing research, phytopathogenic micromycetes (*Alternaria alternata*, *Penicillium expansum*, *Botrytis cinerea*, *Verticillium dahlia*, and *Fusarium oxysporum*) notably remained viable in the soil even after harvest (Ghuffar *et al*., 2021).

Throughout the research, 26 isolates of micromycetes belonging to the genera *Aspergillus*, *Penicillium* sp., 18 isolates of phytopathogenic micromycetes, 25 isolates of *Fusarium* sp., 30 micromycetes of the genera *Alternaria* and *Curvularia* sp., and five isolates of micromycetes of the genus had their morphological features scrutinized with the microscope. Methods of Litvinov (1967), Bilay (1982) detectors, and Pidoplichko N.M. helped determine in the traditional way using atlases. The systematic position of phytopathogenic micromycetes' determination ran the site http://www.mycobank.org. About the classification, it was also well-defined that Fusarium isolates belonged to the Nectriaceae family, the Hypocreales order, the Sordariomycetes class, and the Ascomycota division and the Penicillium and Aspergillus isolates belonged to the Aspergillaceae family, the Eurotiales order, the Eurotiomycetes class, and the Ascomycota division.

The studied grape cultivars "Kishmish" and "Rizamat" are also suitable for human consumption. Therefore, it is vital to analyze the grape microbiome. Given that grapes are perennials, phytopathogenic microorganisms live in the soil and damage next year's crop (Table 1). The isolation of phytopathogenic micromycetes from diseased samples of grape plants used for human consumption is critical for maintaining human health, detecting plant diseases, and developing biological control measures. Analogous to the latest research, representative isolates of common, major toxigenic genera that infect grapes for human consumption were the fungi *Fusarium*, *Penicillium*, *Alternaria*, *Curvularia*, and *Aspergillus* (Donets, 2000). Citing their toxic substances production, such as, ochratoxin A, fumonisins, and patulin were also contents in past scientific sources (Ghuffar *et al*., 2021).

The antifungal activity of bacterial strains *P. megaterium* and *P. agglomerans* were also verifiable against phytopathogenic isolates of micromycetes *Fusarium* sp., *Penicillium*, *Alternaria*, *Curvularia*, and *Aspergillus*, collected from the vine. The antagonistic properties of the isolated bacterial strains' confirmation used the agar block method. Reducing the growth radius of the pathogen determined the antifungal activity. The highest antifungal activity of bacterial

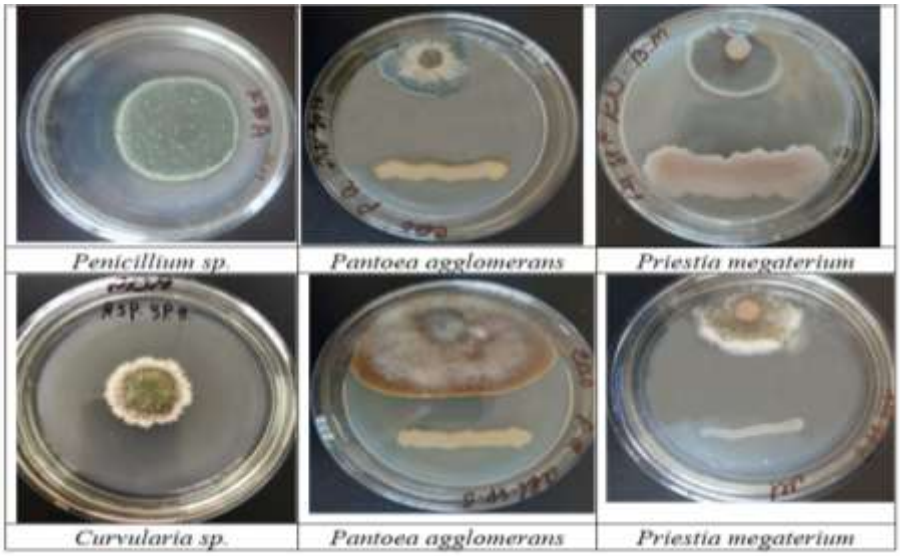

Figure 9. Antifungal activity of bacterial strains *P. megaterium* and *P. agglomerans* against phytopathogenic micromycetes isolated from grapes.

strains *P. agglomerans* and *P. megaterium* against micromycetes belonging to the genus *Penicillium* has been positive. The phytopathogen reduces the growth radius to 47–54 mm. Antifungal activity against the genus *Fusarium* was notable, decreasing the observed growth radius (27–35 mm) of the pathogen (Figure 9).

It was evident that the bacterial strain *P. agglomerans* showed the highest antifungal activity against micromycetes belonging to the genus *Alternaria*, recorded as the dominant species in grapes. It has also been crystal-clear that the phytopathogen reduces the growth radius to 27–33 mm. The strain *Priestia megaterium* showed somewhat lower activity and proved to have a decrease in the radius of pathogenic growth to 21–27 mm (Figure 7). Compared with isolates of micromycetes belonging to the genus *Curvularia*, isolated strains of bacteria revealed the highest antifungal activity. It was also noticeable that *P. agglomerans* reduced the growth radius of the pathogen to 33–42 mm and *P. megaterium* to 39–43 mm. Some past studies have established that antagonist strains exhibited the highest antagonistic property (54.47%) against the phytopathogenic micromycete *Aspergillus flavus* (Ahmad *et al*., 2008).

CONCLUSIONS

Vitis vinifera L., being a large-scale commercial product of high economic value, is one of the most valuable fruit crops consumed by humans worldwide. Various biotic stress agents, including pathogenic fungi, cause severe diseases in different plant organs and affect the grapes. In viticulture regions, the most dangerous grape diseases affect wood tissues, causing significant crop losses and, eventually, the death of grapes. However, diverse taxonomically unrelated phytopathogenic fungi cause these complex diseases. With the colonization by pathogenic micromycetes on the leaf plate, stems, and young branches of grapes, general wilting and complete death of the plant were visible.

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