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Microbiome of centenary trees growing around historical monuments

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Abstract

This study investigated the microflora of ancient trees growing around two historical monuments in Samarkand city — the Ulugbek Observatory and the Sherdor Madrasah. The morphological characteristics of pure cultures of microorganisms isolated from the collected samples were examined. The results showed that 21 micromycete and 16 bacterial isolates were obtained from trees near the Ulugbek Observatory, and 17 micromycete and 16 bacterial isolates from trees near the Sherdor Madrasah. Analysis of the microbiome composition revealed the presence of pathogenic and potentially harmful species that may affect the trees and nearby historical monuments.

Keywords: *trees, microbiome, endophyte, epiphyte, pathogen, factors.*

Introduction

Trees are one of the main components of the Earth's biosphere and play an essential role in maintaining ecosystem structure, stability, and biodiversity [1]. They provide important ecosystem services such as nutrient retention and water filtration [2]. In parks, recreational areas, and historical sites, trees are valued for their health benefits and aesthetic importance. Ancient trees, being physiologically active throughout the year, play a significant role in enriching the atmosphere with oxygen through photosynthesis [3].

The tree microbiome consists of microorganisms (bacteria and micromycetes) inhabiting the roots, stems, and leaves, along with their genetic material. Some microorganisms enhance the physiological processes of trees and increase their resilience to environmental stresses such as drought and extreme temperature [4]. The composition of the microbiome is influenced by factors such as tree age, soil properties, climate, and humidity [5].

In recent decades, infections of ancient and ornamental trees by various phytopathogenic microorganisms have been increasing due to adverse climatic and abiotic factors [6]. This not only poses a threat to trees but also contributes to the rise of allergic diseases in humans, thus representing a serious environmental risk [7]. Pathogenic microorganisms alter plant physiology and metabolism, leading to diseases. They may exist on plant surfaces as epiphytes or within tissues as endophytes, occupying intercellular spaces [8].

Endophytic and epiphytic microorganisms can be either beneficial and symbiotic or phytopathogenic, causing plant diseases [9]. Endophytic microorganisms include diverse ecological groups such as root and soil endophytes [10]. Some endophytes may shift between mutualistic and latent pathogenic stages during their life cycle [11, 12]. Therefore, endophytes exhibit multiple functional characteristics at different life stages.

The use of antagonistic endophytic microorganisms is one of the most effective biological control strategies against plant pathogens [13, 14]. Endophytes are also promising sources for developing biological preparations and bioactive compounds such as alkaloids, cytochalasins, polyketides, terpenoids, flavonoids, and steroids [15–17]. These substances have great potential for application in medicine, agriculture, and industry. Considering the vast plant diversity worldwide, endophytic micromycetes are a valuable source for discovering new natural biological control agents [18].

Microorganisms are dispersed through soil, water, air, and various anthropogenic factors. When soil conditions change, saprophytic microorganisms can multiply rapidly [19]. The phytopathogenic micromycete *Rhizoctonia solani*, which causes various plant diseases, was isolated and its morphological characteristics studied. In laboratory conditions, the pathogenic properties of most isolated phytopathogenic microorganisms were confirmed [20].

Soil microbial populations often include bacterial genera such as *Pseudomonas*, *Bacillus*, and *Pasteuria*, which have strong potential for biological control of nematode populations [21]. Promising results were also obtained from experiments using biological control agents against *Pantoea agglomerans*, the causal agent of fire blight. Following these studies, the mechanisms of antagonist activity were examined in detail [22].

To develop effective biological control methods and create environmentally friendly biological agents, it is necessary to isolate antifungal and antibacterial antagonist strains from ancient trees [23]. However, current research on endophytic microorganisms remains limited, leading to the spread of diseases caused by them. Therefore, studying the diversity of endophytic and epiphytic microorganisms and their potential effects on tree species and human health is of great scientific importance.

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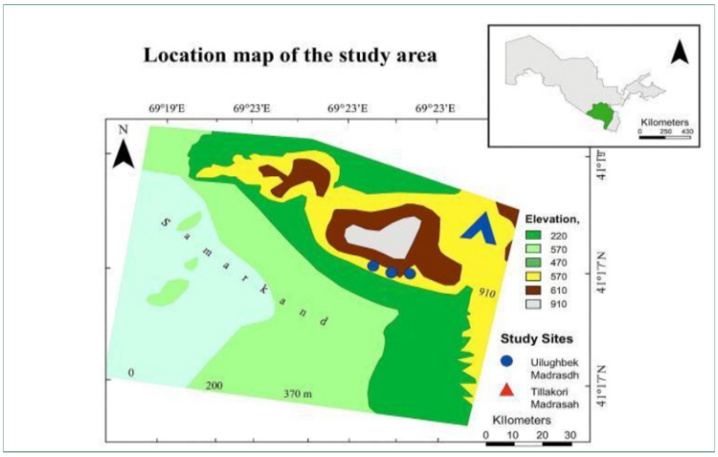
Research Area and Methods

The research was conducted in the area surrounding the Ulugbek Observatory and Sherdor Madrasah in Samarkand, Uzbekistan.



The Ulugbek Observatory, built in 1428–1429 on Choponota Hill near the Obirakhmat stream, is one of the rare examples of 15th-century architecture. Around the observatory, ancient plane trees (*Platanus orientalis*) are found.

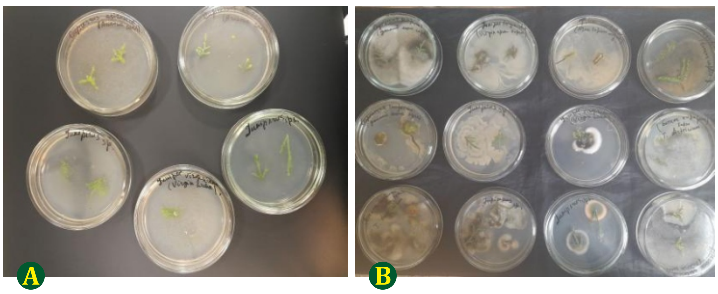
The Sherdor Madrasah, located on Registan Square, dates to the 17th century and was included in the UNESCO World Heritage List in 2001. Around this monument grows an ancient *Juniperus virginiana* (Virginia juniper), also known as pencil cedar, which was planted in 1873 and is notable for its evergreen foliage.

Plant samples were collected aseptically from the bark, branches, leaves, flowers, and immature fruits of several ancient trees near these monuments — including *Pinus nigra*, *Picea pungens* Engelm., *Pinus eldarica* Medw., *Aesculus* sp., and *Morus* sp. (near the Ulugbek Observatory), and *Thuja orientalis*, *Juniperus virginiana*, *Pinus sylvestris*, *Juniperus* sp.1, and *Morus* sp. (around the Sherdor Madrasah). Samples were taken from a total of six trees near the Ulugbek Observatory and eight trees near the Sherdor Madrasah.

A seasonal study revealed that microbial activity was highest in spring, while lower levels were observed in summer and autumn.

In the laboratory, all procedures were performed in a biological safety cabinet (BSC-1300IIA2-X). To isolate epiphytic microorganisms, plant material was first treated with 3% hydrogen peroxide for 15 minutes and then rinsed ten times with sterile distilled water. Small tissue sections were excised using sterilized scalpels and tweezers and inoculated onto potato dextrose agar (PDA) plates.

To isolate endophytic microorganisms, plant samples were covered with sterile quartz sand in porcelain dishes and inoculated into PDA medium. The inoculated samples were incubated at temperatures ranging from 20°C to 36°C (Figure 1)



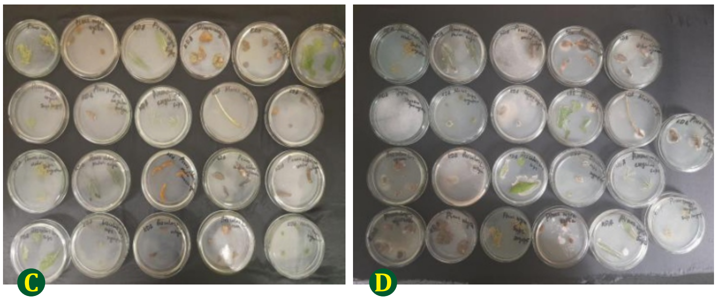


Figure 1. Samples taken from the Ulugbek Observatory and Sherdor Madrasah and colonies of microorganisms that developed in the samples

From the 2nd day of the study, the formation, that is, the development of bacterial and micromycete colonies was observed in the samples planted.

Data analysis: The results obtained were identified using generally accepted methods in microbiology, genetic and MALDI-TOF MS methods. Pure isolates were isolated by re-sowing microorganisms on nutrient media (Figure 2). When studying the developed microflora from samples taken from trees, the samples obtained were incubated on the above nutrient media for 6-7 days. To isolate pure cultures from the samples, the generally accepted method of re-sowing on PDA nutrient media was used. In the next stage of the study, studies were conducted on the isolation of pure cultures from the colonies of microorganisms that developed in the samples.

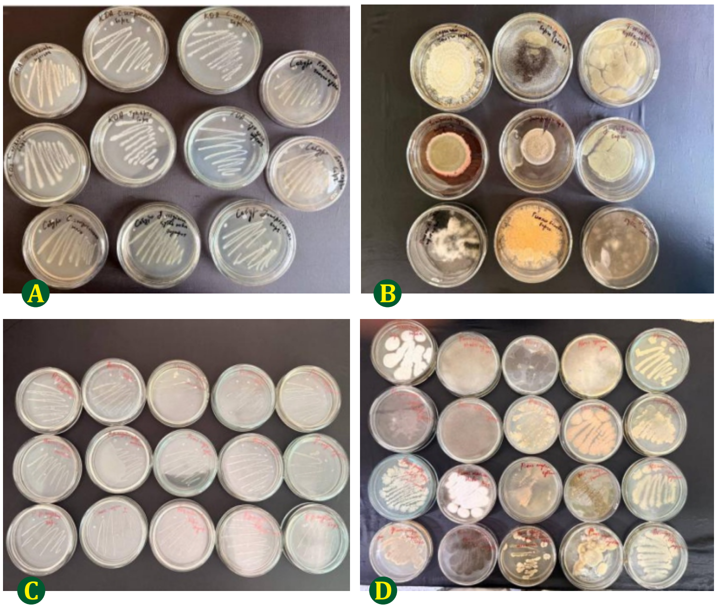


Figure 2. Results of the study conducted in laboratory conditions. A-Bacterial isolates isolated from samples taken from Sherdor madrasah; B-micromecit isolates; C-Bacterial isolates isolated from samples taken from Ulugbek Observatory; D-micromecit isolates

In order to identify the types of pure isolates, the morphological characteristics of the microorganisms were examined. The microscopic structure of the microorganisms was examined using XSP-136B and OLYMPUS BX41 light microscopes at 400× magnification. At the end of the research, the morphological characteristics of the pure isolates obtained from the samples were determined. According to the experimental results, a total of 17 micromycete and 16 bacterial isolates were obtained from samples collected from century-old trees around the Sherdor Madrasah. In particular, four bacterial and three micromycete isolates were obtained from Thuja orientalis samples. Of these, 2 types were isolated from leaves, 1 type from fruits, and 2 types of bacteria from young branches; 2 types of micromycete isolates were isolated from bark and 1 type from branches.

4 types of bacteria and 4 types of micromycete isolates were isolated from *Juniper Virginiana* samples. Of these, 3 bacterial isolates were isolated from young leaves and shoots of the plant, 1 from the bark of the plant. 3 types of micromycetes were found in both the shoots and bark of the plant, and 1 micromycetes isolate was isolated from the leaves. 3 bacteria and 6 micromycetes isolates were isolated from *Pinus sylvestris* tree samples. Among these, 2 bacterial isolates were isolated from young shoots and leaves of the plant. 3 of the isolated micromycetes isolates were isolated from the bark samples of the plant, 2 from the leaves and one from the pollen domes. 2 bacteria were isolated from the leaves and shoots of the *Morus* samples. A total of 4 micromycetes and 4 bacterial isolates were isolated from the *Juniperus sp1*. samples.

21 types of micromycetes and 18 types of bacteria were isolated from samples taken from century-old trees around the Ulugbek Observatory. In particular, 4 types of bacteria and 5 types of micromycetes were isolated from samples taken from *Pinus nigra*. Of these, 1 type of endophyte, 1 type of epiphyte bacteria, 1 type of immature fruit, 2 types of bacteria from young branches; 2 types of micromycetes were isolated from bark and 1 type of branch, and 1 type of flower. 2 types of endophytes, 1 type of epiphyte bacteria were isolated from leaves and 1 type of bacterial isolate from branches, 3 types of micromycetes were isolated from leaves and branches from *Picea pungens Engelm.,* tree samples. 4 types of bacteria and 5 types of micromycetes were isolated from *Pinus eldarica Medw.,* samples. Of these, 2 bacterial isolates were isolated from young leaves and shoots of the plant, 1 endophytic bacterial isolate from the leaf, and 1 from the bark of the plant. 3 micromycetes were found in both the shoots and bark of the plant, and 1 micromycetes were isolated from the leaves and 1 from the pollen domes. 3 bacteria and 6 micromycetes were isolated from the *Aesculus L.* sample. Among these, 2 bacterial isolates were isolated from young shoots and leaves of the plant and 1 endophytic bacterial isolate from the leaf. Of the isolated micromycetes, 3 were isolated from the bark samples of the plant, 2 from the leaves, and one from the shoot. Three types of bacteria were isolated from the leaves and shoots of the *Morus* samples, and two types of micromycetes were isolated from the infected leaves and bark of the plant. The isolated bacterial isolates were examined microscopically using the Bergy detector and identified using the MALDI-TOF method (1-2 figure), and their morphological characteristics were studied.

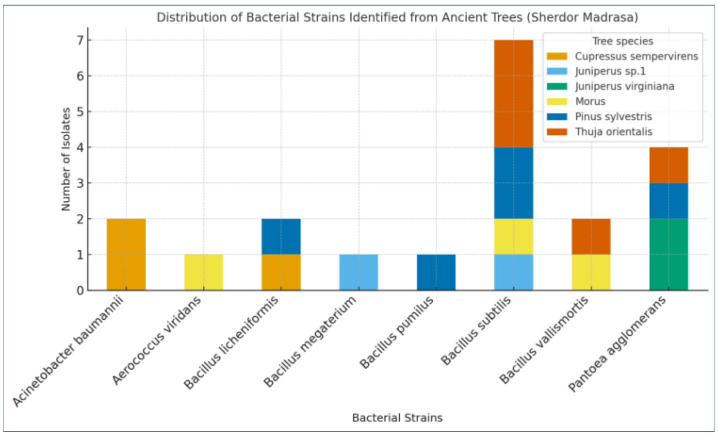
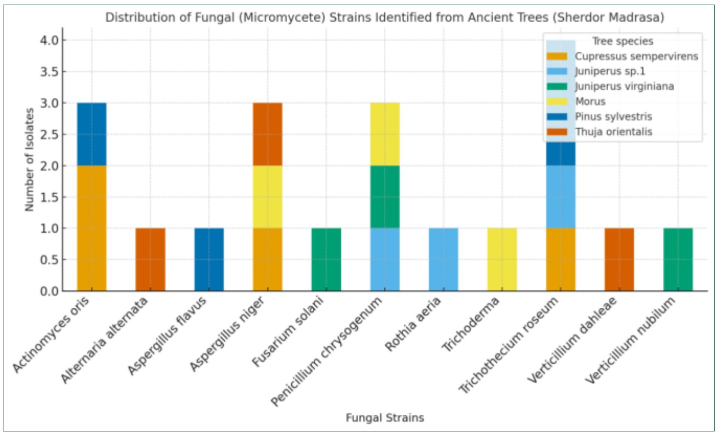


Figure 1. MALDI-TOF identification of bacterial isolates from ancient trees of Sherdor Madrasa

According to the results, among a total of 16 different bacterial isolates isolated from tree samples growing around the Sherdor Madrasah, strains belonging to the *Bacillus* genus dominated. *Bacillus subtillis, Bacillus megaterium, Bacillus licheniformis, Bacillus vallismotris strains,* and *Bacillus spp.,* bacterial isolates were identified.



Among the 17 different micromycete isolates presented in the figure, strains belonging to the genera *Penicillium* and *Aspergillus* prevailed. Among micromycete species such as *Penicillium spp., Fusarium, Alternaria,* and *Verticillium* cause diseases of various plant organs (root, stem, leaves). These species are resistant to chemical control. Biological control studies are of particular importance in combating diseases.

Conclusions

Pathogenic microorganisms negatively affect rare century-old trees, reducing their viability and causing their extinction. Studying the microbiome of century-old trees will allow identifying phytopathogens present in them and finding strains with high antagonistic properties, which may allow developing biological control measures and selecting environmentally friendly biological agents with high antifungal activity. According to the results of the study, it was found that the morphological characteristics of microorganisms isolated from samples taken from century-old trees differ. Based on information from scientific sources, newly isolated micromycetes have been recorded as disease-causing species for ornamental trees, softwood and hardwood trees, and century-old trees.

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