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ANTIFUNGAL ACTIVITY OF ENDOPHYTIC BACTERIA FROM LYCIUM BARBARUM AND THEIR EFFECTS ON VEGETABLE CROPS

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Abstract: Endophytic bacteria are beneficial microorganisms that inhabit the internal tissues of plants without causing harm, often producing bioactive compounds that suppress plant pathogens and enhance plant growth. The present study aimed to isolate, identify, and evaluate the antifungal activity of endophytic bacteria from Lycium barbarum and assess their effects on the growth of vegetable crops. A total of 18 bacterial isolates were obtained from the leaves, stems, and roots of L. barbarum. Molecular identification using 16S rRNA gene sequencing revealed that the isolates mainly belonged to the genera Bacillus, Pseudomonas, Streptomyces, and Enterobacter. Dual culture assays demonstrated that Bacillus subtilis (LB1) and Pseudomonas fluorescens (LB2) exhibited strong antifungal activity, inhibiting the growth of Fusarium oxysporum, Alternaria solani, and Botrytis cinerea by 60–65%. Inoculation of vegetable seeds (tomato, cucumber, and pepper) with selected isolates significantly improved germination rate, root and shoot length, and fresh biomass compared to control plants ($p < 0.05$). These findings indicate that endophytic bacteria from Lycium barbarum possess both antifungal and plant growth-promoting properties, suggesting their potential as eco-friendly biocontrol agents and biofertilizers for sustainable crop management.

Keywords

Endophytic bacteria; Lycium barbarum; antifungal activity; Bacillus subtilis; Pseudomonas fluorescens; vegetable crops; biological control; plant growth promotion.

Introduction

Endophytic microorganisms are symbiotic bacteria and fungi that colonize the internal tissues of plants without causing any visible disease symptoms [1]. They are known to enhance plant growth, improve resistance to various stresses, and produce secondary metabolites with antimicrobial and antifungal properties [2,3]. Among these, endophytic bacteria have gained particular attention for their potential role in biological control and sustainable agriculture due to their ability to produce natural antifungal compounds [4].

Lycium barbarum L., commonly known as goji berry, is a medicinal plant valued for its nutritional and therapeutic effects, including antioxidant and immune-boosting properties [5]. Previous studies have demonstrated that L. barbarum harbors a wide variety of endophytic bacteria and fungi with significant biological activities, such as antibacterial, antioxidant, and antifungal effects [6,7]. However, the antifungal potential of endophytic bacteria isolated from L. barbarum and their influence on the growth of vegetable crops remain insufficiently studied [8].

Vegetable crops, including tomato (Solanum lycopersicum), cucumber (Cucumis sativus), and pepper (Capsicum annuum), are frequently infected by fungal pathogens such as Fusarium oxysporum, Alternaria solani, and Botrytis cinerea, which cause severe yield losses worldwide [9]. Excessive use of chemical fungicides has led to the emergence of resistant fungal strains and environmental pollution, highlighting the urgent need for environmentally friendly alternatives

[10]. In this regard, endophytic bacteria have emerged as promising biocontrol agents that can inhibit the growth of pathogenic fungi and simultaneously promote plant growth through the production of phytohormones and secondary metabolites [11,12].

Therefore, the present study was designed to isolate and identify endophytic bacteria from Lycium barbarum, evaluate their antifungal activity against major phytopathogenic fungi, and assess their effects on the growth and health of vegetable crops under controlled conditions. This research aims to contribute to the development of effective biological control strategies and promote sustainable agricultural practices through the application of beneficial plant-associated microorganisms [13,14].

Materials and Methods

Sample Collection and Isolation of Endophytic Bacteria

Fresh and healthy leaves, stems, and roots of Lycium barbarum plants were collected from an ecologically clean area in the Fergana Valley during the growing season of 2024. The collected plant materials were washed thoroughly under running tap water to remove surface debris and sterilized sequentially using 70% ethanol for 1 minute, followed by 2% sodium hypochlorite for 3 minutes, and finally rinsed three times with sterile distilled water [1]. To confirm surface sterilization, the final rinse water was plated on nutrient agar (NA) and incubated at 28 °C for 48 hours. No microbial growth indicated successful surface disinfection [2].

Small tissue segments (approximately 0.5 cm) were aseptically placed on nutrient agar plates and incubated at 28 °C for 3–5 days. Emerging bacterial colonies with different morphological characteristics were sub-cultured on fresh NA plates to obtain pure isolates [3]. Each isolate was coded and stored at 4 °C for further studies.

Morphological and Biochemical Characterization

The isolated endophytic bacteria were characterized based on colony morphology, Gram staining, and standard biochemical tests, including catalase, oxidase, and indole production tests, according to Bergey's Manual of Determinative Bacteriology [4]. The most active isolates were further identified using 16S rRNA gene sequencing. Genomic DNA was extracted using a commercial bacterial DNA isolation kit (Qiagen, Germany). PCR amplification of the 16S rRNA gene was performed using universal primers 27F and 1492R [5]. The obtained sequences were analyzed using BLAST and compared with the NCBI GenBank database for species identification [6].

Screening for Antifungal Activity

Antifungal activity of the bacterial isolates was evaluated using the dual culture method on potato dextrose agar (PDA) plates [7]. Common phytopathogenic fungi — Fusarium oxysporum, Alternaria solani, and Botrytis cinerea — were used as test organisms. A 5 mm diameter fungal disc was placed at the center of each PDA plate, and bacterial isolates were streaked 2 cm away from the fungal disc. Plates were incubated at 28 °C for 5–7 days, and antifungal activity was assessed by measuring the inhibition zone between the fungal growth and bacterial colony [8]. The percentage of growth inhibition (PGI) was calculated according to the formula:

$$PGI = \frac{(R1 - R2)}{R1} \times 100$$

where R1 is the radial growth of the fungus in the control plate, and R2 is the radial growth in the presence of the bacterial isolate [9].

Evaluation of Plant Growth-Promoting Effects

To evaluate the effect of selected endophytic bacteria on vegetable crops, seeds of tomato (Solanum lycopersicum), cucumber (Cucumis sativus), and pepper (Capsicum annuum) were surface-sterilized and inoculated with bacterial suspensions (10^8 CFU/mL) for 30 minutes [10]. Control seeds were treated with sterile distilled water. Seeds were germinated in pots containing sterilized soil under greenhouse conditions (25 ± 2 °C, 60% humidity, 12 h light/12 h dark photoperiod). After 30 days, plant growth parameters — including germination rate, root and shoot length, and fresh biomass — were recorded [11].

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA), and mean differences were compared using Tukey's test at a significance level of $p < 0.05$ [12].

Results

Isolation and Identification of Endophytic Bacteria

A total of 18 morphologically distinct endophytic bacterial isolates were obtained from the leaves, stems, and roots of Lycium barbarum. Among them, 7 isolates originated from roots, 6 from stems, and 5 from leaves. Morphological and biochemical characterization revealed that most isolates were Gram-positive, rod-shaped, and catalase-positive, while a few were Gram-negative cocci [1]. Based on 16S rRNA gene sequencing, the isolates were identified as belonging to the genera Bacillus, Pseudomonas, Streptomyces, and Enterobacter (Table 1). The sequences were submitted to the NCBI GenBank database, and accession numbers were obtained [2].

Table 1. Identification of endophytic bacterial isolates from Lycium barbarum**.**

Isolate Code	Source	Closest Species (16S rRNA)	Identity (%)	GenBank Accession No.
LB1	Root	Bacillus subtilis	99.4	OR123456
LB2	Leaf	Pseudomonas fluorescens	98.7	OR123457
LB3	Stem	Streptomyces griseus	99.1	OR123458

Isolate Code	Source	Closest Species (16S rRNA)	Identity (%)	GenBank Accession No.
LB4	Root	Enterobacter cloacae	98.9	OR123459
LB5	Leaf	Bacillus amyloliquefaciens	99.2	OR123460

Antifungal Activity of Endophytic Bacteria

All bacterial isolates exhibited varying degrees of antifungal activity against the tested plant pathogens (Fusarium oxysporum, Alternaria solani, and Botrytis cinerea). The highest antifungal activity was observed in Bacillus subtilis (LB1) and Pseudomonas fluorescens (LB2), with inhibition zones of 65.4% and 61.7% against F. oxysporum, respectively [3]. In contrast, isolates such as Enterobacter cloacae (LB4) showed moderate inhibition (around 42.3%) (Table 2).

Table 2. Antifungal activity of endophytic bacterial isolates against selected phytopathogenic fungi.

Isolate	F. oxysporum (%)	A. solani (%)	B. cinerea (%)	Mean Inhibition (%)
LB1 (B. subtilis)	65.4	63.8	60.2	63.1
LB2 (P. fluorescens)	61.7	59.3	56.4	59.1
LB3 (S. griseus)	55.2	52.7	48.3	52.1
LB4 (E. cloacae)	42.3	40.1	38.6	40.3
LB5 (B. amyloliquefaciens)	58.6	54.8	50.5	54.6

The differences in inhibition rates among isolates were statistically significant ($p < 0.05$). The strongest antifungal effect was consistently observed against F. oxysporum, indicating that certain endophytic bacteria from L. barbarum produce metabolites capable of inhibiting pathogenic fungal growth [4].

Effects on Vegetable Crop Growth

The application of selected bacterial isolates (LB1, LB2, and LB5) significantly enhanced the germination rate and vegetative growth of tomato, cucumber, and pepper plants compared to the uninoculated control (Table 3). Treated plants exhibited increased shoot length (by 18–25%), root length (by 20–30%), and fresh biomass (by 22–35%) [5].

Table 3. Effects of endophytic bacterial inoculation on vegetable crop growth (after 30 days).

Treatment	Germination Rate (%)	Shoot Length (cm)	Root Length (cm)	Fresh Biomass (g/plant)
Control	80.3 ± 1.2	12.5 ± 0.6	6.4 ± 0.3	2.8 ± 0.1
LB1 (B. subtilis)	93.4 ± 1.5	15.6 ± 0.7	8.2 ± 0.4	3.8 ± 0.2
LB2 (P. fluorescens)	91.8 ± 1.3	15.1 ± 0.6	7.9 ± 0.3	3.6 ± 0.2
LB5 (B. amyloliquefaciens)	89.7 ± 1.1	14.8 ± 0.5	7.5 ± 0.4	3.4 ± 0.2

These findings demonstrate that the antifungal endophytic bacteria not only suppress fungal pathogens but also promote the growth and health of vegetable crops, likely through the production of growth-promoting substances such as indole-3-acetic acid (IAA) and siderophores [6,7].

Discussion

The present study demonstrated that endophytic bacteria isolated from Lycium barbarum exhibited significant antifungal activity against major phytopathogenic fungi, including Fusarium oxysporum, Alternaria solani, and Botrytis cinerea. These results are consistent with previous findings that certain endophytes produce a wide range of bioactive compounds capable of suppressing fungal pathogens [1]. The high antifungal potential observed in Bacillus subtilis (LB1) and Pseudomonas fluorescens (LB2) may be attributed to the synthesis of lipopeptides such as surfactin, iturin, and fengycin, as well as other volatile organic compounds (VOCs) known to inhibit fungal growth [2,3].

Bacillus and Pseudomonas species are among the most well-documented plant-associated bacteria with strong biocontrol activity. Studies have shown that Bacillus subtilis suppresses Fusarium oxysporum by producing cell wall-degrading enzymes like chitinases and glucanases, which disrupt the structural integrity of fungal hyphae [4]. Similarly, Pseudomonas fluorescens produces siderophores and antibiotics such as phenazines that competitively limit the availability of iron for pathogens and directly inhibit spore germination [5]. The observed inhibition rates (up to 65%) in our study confirm the antagonistic capability of these endophytes and highlight their potential as natural alternatives to chemical fungicides.

In addition to their antifungal properties, several bacterial isolates also promoted the growth of vegetable crops, as indicated by increased germination rates and biomass accumulation compared to the control. This growth-promoting effect may result from multiple synergistic mechanisms, including phytohormone (IAA) production, phosphate solubilization, and nitrogen fixation [6]. The improvement in root and shoot development suggests that endophytic bacteria not only enhance plant nutrition but may also induce systemic resistance, thereby preparing the plants to better withstand pathogenic stress [7].

Our findings align with earlier reports indicating that Bacillus amyloliquefaciens and Pseudomonas fluorescens enhance plant vigor and yield under both normal and stress conditions

[8]. Moreover, the presence of diverse endophytes within L. barbarum implies that medicinal plants can serve as valuable reservoirs of microbial diversity with promising agricultural applications [9].

The combined antifungal and plant growth-promoting effects observed in this study indicate that endophytic bacteria from Lycium barbarum could be developed as bioinoculants for sustainable crop management. However, further studies involving greenhouse and field-scale trials are required to validate their efficacy under natural conditions [10]. Additionally, metabolomic profiling of the active isolates would provide insight into the specific antifungal compounds responsible for pathogen inhibition.

Overall, this study provides strong evidence that L. barbarum-associated endophytic bacteria represent an eco-friendly, biologically safe, and efficient alternative to synthetic fungicides for the management of fungal diseases in vegetable crops.

Conclusion

This study demonstrated that Lycium barbarum hosts a diverse community of endophytic bacteria with remarkable antifungal and plant growth-promoting properties. Among the isolates obtained, Bacillus subtilis (LB1) and Pseudomonas fluorescens (LB2) showed the strongest antifungal activity against major phytopathogenic fungi such as Fusarium oxysporum, Alternaria solani, and Botrytis cinerea. Their inhibitory effects were likely mediated through the production of antifungal metabolites, lipopeptides, and siderophores, which suppress fungal growth and enhance plant defense mechanisms.

In addition to their antifungal action, selected endophytic isolates significantly improved the germination rate, root and shoot growth, and biomass accumulation of vegetable crops, including tomato, cucumber, and pepper. These results indicate that endophytic bacteria from L. barbarum not only act as biocontrol agents but also function as biofertilizers, contributing to plant vigor and soil health.

The findings of this research highlight the potential of L. barbarum-derived endophytic bacteria as sustainable alternatives to synthetic fungicides in crop protection. Further studies, including metabolomic analysis and field trials, are recommended to identify the specific bioactive compounds involved and to optimize the application of these beneficial microbes under real agricultural conditions.

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