# Isolation and characterization of Lycium barbarum endophytic bacteria

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**Abstract.** In the conditions of Uzbekistan, the endophytic bacteria isolated from the plant *Lycium barbarum* are considered among the first studies in the country. Using standard microbiological methods, a total of 18 types of endophytic bacteria were isolated from *Lycium barbarum*. All endophytic bacterial isolates were tested for their ability to produce plant growth-promoting hormones. Among the isolates with the highest enzymatic activity, 8 were selected, and their effects on pathogenic fungi were studied. As a result, nearly 60% of the endophytes demonstrated varying levels of activity against *Fusarium* species. The findings indicate that endophytic bacteria isolated from *Lycium barbarum* can be used to promote plant growth and combat their pathogens.

## Introduction

Goji berries are rich in carotenoids, which contribute to their characteristic orange-red color. The primary carotenoid is zeaxanthin, predominantly found in the form of zeaxanthin dipalmitate, accounting for one-third to one-half of the total carotenoids in the fruit. The total carotenoid content in dried goji berries ranges from 0.03% to 0.5%. Zeaxanthin plays a critical role in protecting the macula by shielding it from UV light damage and providing neuroprotective benefits to the retinas of diabetic animals. Other carotenoids present in smaller amounts include lutein, β-carotene, violaxanthin, and cryptoxanthin [1].

Goji plants are primarily cultivated in East Asia, particularly in the western regions of China, Tibet, Mongolia, Japan, Korea, and Taiwan. Recently, their cultivation has gained popularity in Europe as well. *Lycium* species have been grown for centuries in China's Ningxia region, which is renowned for its “daodi” status. Daodi products are recognized for their exceptional quality and effectiveness, attributed to the unique natural conditions of their geographical origins [2]. With the increasing global demand for goji berries and their ease of cultivation, new plantations are being established in several European countries, including Poland. Consequently, comparing the quality and health benefits of goji berries from Ningxia and Poland is valuable for both scientific research and consumer insight [3].

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In our previous research, we assessed the microbial quality and bioactivity of goji fruit extracts obtained using various solvents and green extraction methods. Our findings revealed a high risk of microbial contamination in dried goji berries, prompting us to investigate the predominant microorganisms present in these fruits [4]. Like most fresh fruits, goji berries are highly perishable due to their high-water content (over 80%), which provides a favorable environment for microbial growth, including pathogens [5]. Fresh goji berries have been shown to harbor harmful bacteria such as *Salmonella enterica*, *Escherichia coli* O157:H7, *Bacillus cereus*, *Listeria monocytogenes*, and *Pseudomonas* species. Drying the berries extends their shelf life by reducing water activity, thus inhibiting microbial growth. However, improperly dried fruits can still be contaminated with microorganisms. Dried goji berries may contain both harmful and harmless microbes, including bacteria such as *Streptococcus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Bacillus* spp., *Salmonella* spp., *Cronobacter* spp., *Staphylococcus aureus*, *Escherichia coli*, and *Proteus mirabilis*, as well as fungi such as *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. To ensure consumer safety, it is crucial to study not only contamination levels but also the overall microbial community structure in these dried fruits [6–8].

Goji berries are typically harvested in summer and autumn, traditionally dried in the shade and then under the sun, resulting in a soft pulp with a firm outer skin [9]. Today, goji berries are available in various forms, including freeze-dried, frozen, powdered, and tablet formats. The recommended daily intake for dried goji berries ranges from 6 to 18 grams. This highlights the importance of addressing consumer safety, particularly regarding microbial contamination and the microbiota composition in goji berries [10, 11]. The objective of this study was to analyze the microbiota diversity and the levels of bioactive compounds—such as polyphenols, flavonoids, and carotenoids—in dried goji berries from Poland compared to the highly regarded berries from the Ningxia region. To our knowledge, this is the first study to explore the microbiota composition of goji berries [12].

# Materials and methods

## 2.1 Isolation of Endophytic Bacteria

To isolate endophytic bacteria, *Lavandula* plants were collected from the botanical garden of NUUz, located in the Olmazar district of Tashkent city. Endophytic bacteria were extracted from various parts of the lavender plant, including the roots, fruits, stems, and leaves. The rhizosphere and epiphytes were rinsed under running water for 5 minutes to remove surface bacteria. Each plant part—roots, fruits, stems, and leaves—was weighed separately, with one gram of each sample prepared. The samples were initially immersed in 70% ethanol for 1 minute, followed by surface sterilization. The stems, fruits, and leaves were treated with 4% sodium hypochlorite for 4 minutes, while the roots were sterilized with 2% sodium hypochlorite for 10 minutes. Subsequently, all samples were rinsed five times with sterile distilled water to ensure sterility. The root, fruit, and stem samples were cut into smaller pieces and placed into petri dishes. The leaves were crushed in a sterile mortar and processed using the serial dilution method before being inoculated into separate beef extract agar and nutrient media. Each sample was plated in triplicate to ensure consistency. The plates were incubated at 28 ± 2°C for 2–3 days to allow the growth of endophytic bacteria. Distinct bacterial colonies were identified and subcultured onto three different nutrient media to isolate and select clones for further analysis [13].

## 2.2 Phosphate Dissolution

The phosphate mobilization properties of isolated endophytic bacteria were studied using Pikovskaya Agar nutrient medium. The composition of Pikovskaya medium (g/L) was as follows: yeast extract - 0.5; $Ca_3(PO_4)_2$ - 5.0; $(NH_4)_2SO_4$ - 0.5; KCl - 0.2; $MgSO_4$ - 0.1; $MnSO_4$ - 0.0001; $FeSO_4$ - 0.0001; Agar-Agar - 15.0; pH - 7.0. Additionally, 0.04% Bromocresol Purple was added as a pH indicator. The prepared nutrient medium, supplemented with the indicator, was poured into Petri dishes. Pure bacterial isolates were inoculated onto the medium and incubated for 7 days at 28 ± 2°C. Each inoculation was performed in triplicate to ensure consistency. During incubation, phosphate-dissolving bacteria capable of phosphorus assimilation produced enzymes that solubilized phosphate salts, leading to a change in the pH of the medium. This pH change caused a visible color shift in the medium, indicating phosphate solubilization activity [14].

### 2.3 Production of Phytohormones by Active Isolates

**Determination of Gibberellic Acid (GA):** Active bacterial strains were cultured in liquid Nutrient Broth (NB) medium at 28 ± 2 °C on a shaker set at 250 rpm for 7 days. The amount of gibberellic acid (GA) in the culture liquid was measured daily over the 7-day cultivation period. The methodology followed was based on the technique of Muromtsev and Nestyuk [15]. To determine GA concentration, 1.0 ml of the filtered culture liquid was placed in a 10 ml test tube. Then, 1.0 ml of Folin-Ciocalteu reagent (prepared with 100 g $Na_2O_4W$, 25 g $Na_2MoO_4$, 700 ml water, 50 ml 85% $H_3PO_4$, 100 ml concentrated HCl, 150 g $Li_2SO_4$, and 2–3 drops of bromine) and HCl were added. The mixture was thoroughly mixed and incubated in the dark for 40 minutes. Samples containing GA exhibited a color change to light or dark green. The GA content was quantified using a spectrophotometer by measuring the optical density of the supernatants at a wavelength of 750 nm through a red-light filter [16]. Antifungal Activity: The antifungal activity of endophytic bacteria was evaluated in vitro against common phytopathogenic fungi, including Fusarium moniliforme, Fusarium oxysporum, Fusarium solani, and Verticillium dahliae. Endophytic bacteria and pathogenic fungi were co-cultivated on Petri dishes at 28 °C for 3 days to assess the inhibitory effects of the bacteria on fungal growth [17].

## Results and discussion

### 3.1 Isolation of Endophytic Bacteria

Endophytes are a promising area of research, being an integral part of a plant's lifecycle as they coexist with the host throughout its development. These microorganisms provide numerous benefits to the plant, including improved growth and resilience. Particular attention should be given to their beneficial properties, especially in the context of isolating endophytic bacteria from medicinal plants.

In our study, we investigated the antibacterial activity of medicinal plants and isolated bacteria from the leaves, fruits, stems, and roots of the plant with high antibacterial activity. Specifically, we focused on *Lycium barbarum*, a plant native to Uzbekistan known for producing highly active medicinal compounds. This plant was found to be rich in endophytic bacteria, with a total of 18 isolates obtained: 3 from leaves, 3 from fruits, 5 from stems, and 7 from roots. The isolated bacteria were studied for their ability to control phytopathogens, promote plant growth through the synthesis of phytohormones such as indole-3-acetic acid (IAA), cytokinins, and gibberellic acids, and enhance growth by facilitating phosphate solubilization, nutrient cycling, and the secretion of bioactive metabolites. According to our findings, this is the first report characterizing and studying the growth-promoting properties of *Lycium barbarum* endophytic bacteria in Uzbekistan, aiming to enhance the growth performance of this plant.

### 3.2 Production of Phytohormones by Active Isolates.

Active endophytic bacterial isolates were cultured in Nutrient Broth (NB) media and analyzed for their production of gibberellic acid (GA) and indole-3-acetic acid (IAA). The study revealed a significant impact of phytohormone production on bacterial growth and activity. Bacteria were cultured in liquid nutrient medium for up to 5 days to determine the highest concentrations of GA and IAA during the growth dynamics of the isolates. It was observed that the isolates exhibited higher concentrations of cellulose and sucrose as carbohydrate sources compared to the control (**Fig 1**).

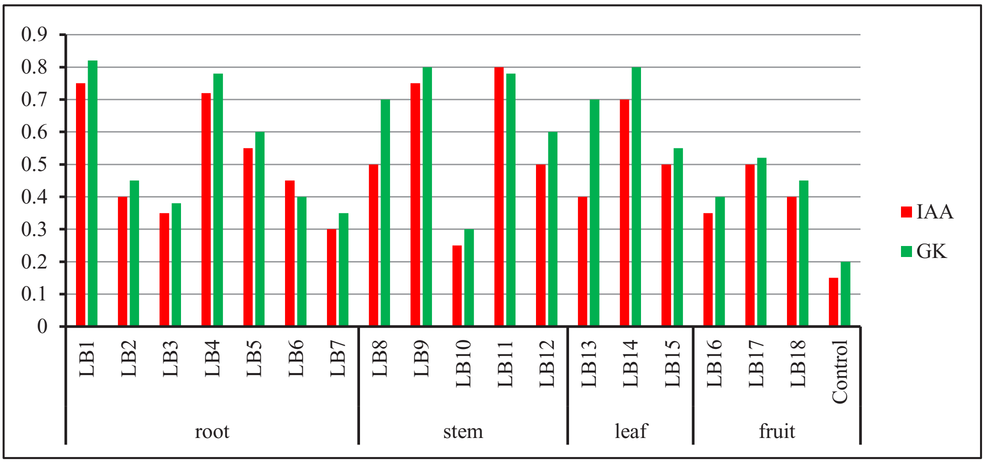


**Fig.1.** Amount of IAA GA synthesis of isolated isolates

The peak synthesis of plant hormones occurred on the fifth day of cultivation. Among the isolates, strain LB1 demonstrated the highest levels of phytohormone production, with 0.76 mg/ml of IAA and 0.82 mg/ml of GA. By comparison, the control produced 0.12 mg/ml of IAA and 0.17 mg/ml of GA on the fifth day. However, beyond the fifth day, the production of IAA began to decline.

### 3.3 Study of Phosphate Mobilization Properties of Endophytic Bacteria.

Phosphorus is a critical nutrient required for plant growth, development, and productivity. Modern agricultural practices heavily rely on synthetic phosphorus fertilizers; however, the efficiency of phosphorus uptake by plants is significantly influenced by microbial communities, particularly bacteria. These bacteria play a pivotal role in converting insoluble phosphates into soluble forms, making them readily available to plants. Phosphorus-solubilizing bacteria constitute only 0.1–0.5% of the microbial population in soil, with endophytic bacteria being key players in enhancing phosphate absorption. Therefore, this study aimed to investigate the phosphate solubilization properties of isolated endophytic bacteria under in vitro conditions.

The results revealed that 40% of the 18 isolated endophytic bacterial strains demonstrated the ability to solubilize insoluble phosphorus. Specifically, eight isolates—LB1, LB2, LB4, LB5, LB8, LB9, LB11, and LB12—exhibited solubilization activity, forming activity zones of varying diameters: 21 mm, 19 mm, 18 mm, 15 mm, 18 mm, 20 mm, 15 mm, and 22 mm, respectively. These isolates showed the highest phosphate solubilization potential, indicating their significant role in phosphorus mobilization (**Fig. 2**).

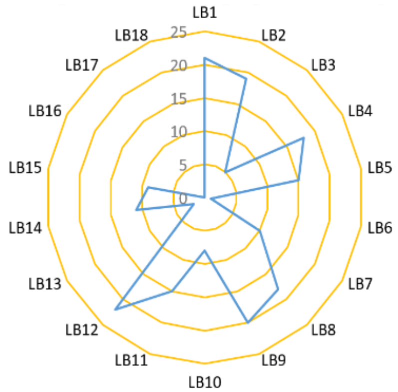


**Fig. 2.** Phosphate mobilization properties of endophytic bacteria

### 3.3 Activity of Isolated Isolates Against Pathogenic Fungi.

Endophytes are known to produce a variety of antifungal metabolites, including steroids, quinones, alkaloids, terpenoids, isocoumarin derivatives, flavonoids, phenols, phenolic acids, and peptides. These compounds play a crucial role in inhibiting the growth of phytopathogenic fungi. To further investigate the antifungal properties of halotolerant endophytes, experiments were conducted as part of this research. The endophytic fungi were first cultivated in liquid nutrient medium for 10 days. After this period, the biomass was separated from the culture liquid through centrifugation. Subsequently, the antifungal activity of the endophyte extracts and culture fluids was tested against common plant pathogens, including *Fusarium oxysporum*, *Fusarium solani*, *Fusarium moniliforme*, and *Verticillium dahliae*. The results of these experiments are illustrated in **Figure 3**.

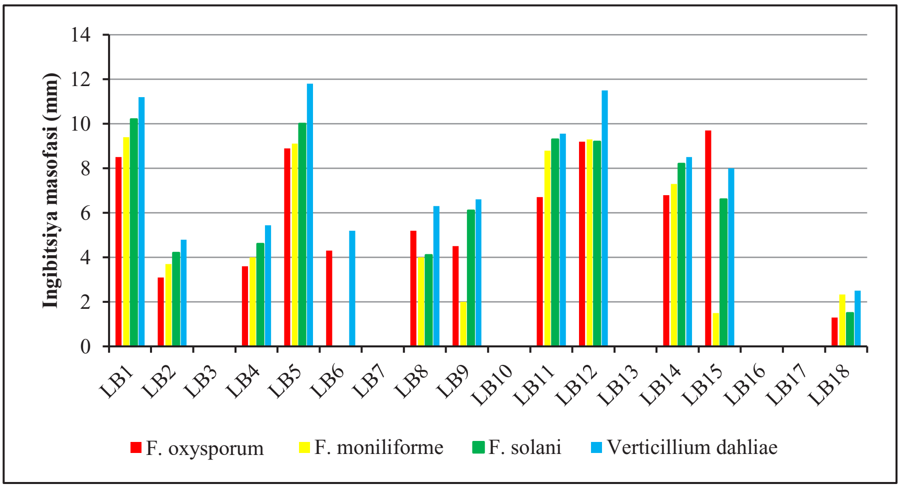


**Fig. 3.** Antifungal properties of endophytic bacteria

The results presented in **Figure 3** demonstrate that approximately 50% of the tested endophytes exhibited varying levels of antifungal activity against plant pathogens. These endophytes showed significant antagonistic activity, particularly against *Fusarium oxysporum* and *Fusarium moniliforme*, where the inhibition zone of the growth ring ranged from 5 mm to 15 mm. Moreover, the highest inhibition was observed against *Verticillium dahliae*, with growth ring suppression measured between 10 mm and 20 mm using the agar block method.

## 4 Conclusion

In our study, nine endophytic bacterial isolates were successfully isolated from the *Lycium barbarum* plant. These isolates demonstrated various plant growth-promoting abilities, including phosphate solubilization, and the production of indole-3-acetic acid (IAA) and gibberellic acid (GA). Among them, eight isolates (LB1, LB2, LB4, LB5, LB8, LB9, LB11, and LB12) exhibited superior activity in phosphate solubilization and IAA production compared to the others. Additionally, all fungal isolates showed varying levels of antagonistic activity against pathogenic fungal strains such as *Fusarium solani*, *Fusarium moniliforme*, and *Fusarium oxysporum*. Notably, isolates LB1, LB2, LB4, LB5, LB8, LB11, LB12, LB14, and LB15 demonstrated strong antifungal activity against several pathogenic fungi. These findings highlight the potential of these endophytic bacterial isolates in the development of eco-friendly biofertilizers, offering promising applications in sustainable agriculture.

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