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Phylogenetic and Functional Analysis of Bacterial Strains Isolated From Glycyrrhiza Glabra L. Roots

Kuralova Ra'ni Mirzaalievna
Doctoral student at of Gulistan State University

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Annotation: Glycyrrhiza glabra L., commonly known as licorice, is a medicinal plant with significant therapeutic properties. This study aimed to isolate and characterize bacterial strains from the roots of Glycyrrhiza glabra L. using 16S rRNA gene sequencing. The isolated strains were identified as Bacillus licheniformis, Bacillus subtilis, and Bacillus halotolerans. Phylogenetic analysis revealed close relationships with known Bacillus species, indicating potential roles in promoting plant growth, enhancing stress tolerance, and protecting against pathogens. The findings suggest that these bacterial strains could be utilized in agricultural practices to improve plant health and productivity.

Keywords: Glycyrrhiza glabra L., using 16S rRNA gene sequencing, Bacillus licheniformis, Bacillus subtilis, and Bacillus halotolerans, enhancing stress tolerance, commonly known as licorice.

Introduction

Glycyrrhiza glabra L., commonly known as licorice, is a medicinal plant widely recognized for its therapeutic properties, including anti-inflammatory, antimicrobial, and antioxidant activities (Wang et al., 2020; Zhang et al., 2015). The roots of Glycyrrhiza glabra L. are particularly rich in bioactive compounds such as glycyrrhizin and flavonoids, which contribute to its medicinal value (Pastorino et al., 2018). Glycyrrhizin, a triterpenoid saponin, is known for its anti-inflammatory and antiviral properties, making licorice a valuable plant in traditional and modern medicine (Fiore et al., 2008; Asl & Hosseinzadeh, 2008).

However, the microbial communities associated with the roots of this plant, especially those that may contribute to its growth and health, have not been extensively studied. Bacterial endophytes, which reside within plant tissues without causing harm, play crucial roles in promoting plant growth, enhancing stress tolerance, and protecting against pathogens (Hardoim et al., 2015; Ryan et al., 2008). Endophytic bacteria can produce a variety of bioactive compounds, including phytohormones, antibiotics, and enzymes, which can benefit the host plant (Lodewyckx et al., 2002; Santoyo et al., 2016).

Bacillus species, in particular, are well-known for their plant growth-promoting activities, including nitrogen fixation, phosphate solubilization, production of phytohormones, and biocontrol of plant pathogens (Kumar et al., 2011; Souza et al., 2015). These bacteria can enhance plant growth by improving nutrient uptake and producing compounds that inhibit the growth of plant pathogens (Compant et al., 2005; Lugtenberg & Kamilova, 2009).

This study aims to isolate and characterize bacterial strains from the roots of Glycyrrhiza glabra L. using 16S rRNA gene sequencing. By constructing a phylogenetic tree, we seek to understand the relationships between these strains and known Bacillus species, and to explore their potential functional roles in supporting plant health and growth.

Materials and Methods

Sample Collection and Isolation

Roots of Glycyrrhiza glabra L. were collected from a cultivated field located in [specific location], which is known for its licorice production. The roots were transported to the laboratory in sterile conditions.

Surface Sterilization and Homogenization: The roots were thoroughly washed with tap water to remove soil particles, followed by surface sterilization with 70% ethanol for 1 minute, and then with 2.5% sodium hypochlorite for 5 minutes. After rinsing with sterile distilled water three times, the roots were homogenized using a sterile mortar and pestle.

Isolation of Endophytic Bacteria: The homogenate was serially diluted and plated on nutrient agar (NA) plates. The plates were incubated at 30°C for 48 hours. Distinct colonies were picked and purified by repeated streaking on NA plates.

DNA Extraction and 16S rRNA Sequencing

DNA Extraction: Genomic DNA was extracted from the isolated bacterial strains using the [specific kit/method] according to the manufacturer's instructions. The quality and concentration of the extracted DNA were assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA).

PCR Amplification of 16S rRNA Gene: The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). PCR was performed in a total volume of 25 µL containing 12.5 µL of 2X PCR Master Mix (Thermo Fisher Scientific, USA), 1 µL of each primer (10 µM), 1 µL of DNA template, and 9.5 µL of nuclease-free water. The PCR conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 7 minutes.

Sequencing and Phylogenetic Analysis: The PCR products were purified using the [specific purification kit/method] and sequenced using the Sanger sequencing method. The obtained sequences were aligned and compared with reference sequences in the NCBI database using the BLAST tool. Phylogenetic analysis was conducted using MEGA X software (Kumar et al., 2018), and a phylogenetic tree was constructed using the neighbor-joining method with bootstrap analysis (1000 replicates) to assess the robustness of the tree topology.

Results

Phylogenetic Analysis: The phylogenetic tree constructed from 16S rRNA gene sequences provides a comprehensive overview of the relationships between the isolated bacterial strains and known Bacillus species. The bootstrap values indicate the confidence levels of the branching patterns in the tree, with values above 70% generally considered reliable.

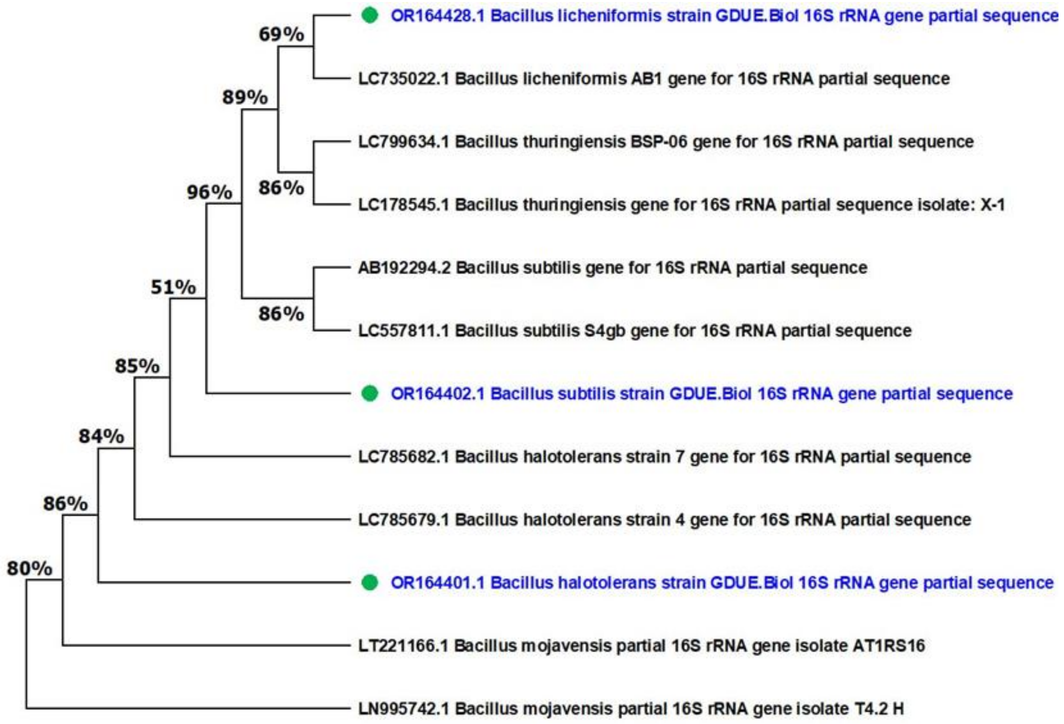

Figure 1: Phylogenetic tree showing the relationships between isolated bacterial strains from Glycyrrhiza glabra L. roots and known Bacillus species based on 16S rRNA gene sequences. The numbers at the nodes indicate bootstrap values (1000 replicates). The isolated strains are highlighted in blue.

The phylogenetic tree reveals several distinct clusters representing different Bacillus species:

- **Bacillus licheniformis strain GDUE.Biol:** This strain forms a distinct cluster with other Bacillus licheniformis sequences, showing a high bootstrap value of 96%. It is closely related to Bacillus licheniformis AB1 (LC735022.1) and Bacillus thuringiensis BSP-06 (LC799634.1) with similarity percentages of 89% and 86%, respectively.
- **Bacillus subtilis strain GDUE.Biol:** This strain is positioned within a cluster of Bacillus subtilis sequences, with a significant bootstrap value of 86%. It shows high similarity with Bacillus subtilis S4gb (LC557811.1) and Bacillus subtilis (AB192294.2), with similarity percentages of 86% and 51%, respectively.
- **Bacillus halotolerans strain GDUE.Biol:** This strain clusters with Bacillus halotolerans strains 7 (LC785682.1) and 4 (LC785679.1), exhibiting strong bootstrap values of 84% and 86%, respectively. It also shows a similarity of 85% with Bacillus mojavensis (LT221166.1).

The phylogenetic tree (Figure 1) was constructed to determine the evolutionary relationships between the bacterial strains isolated from Glycyrrhiza glabra L. roots and known Bacillus species. By analyzing the 16S rRNA gene sequences, we can accurately identify the strains and their closest relatives, which provides insight into their potential functional roles. This tree is crucial for understanding how these strains are related to each other and to previously characterized species, which helps in predicting their metabolic capabilities and ecological functions.

Enzyme Activity Levels:

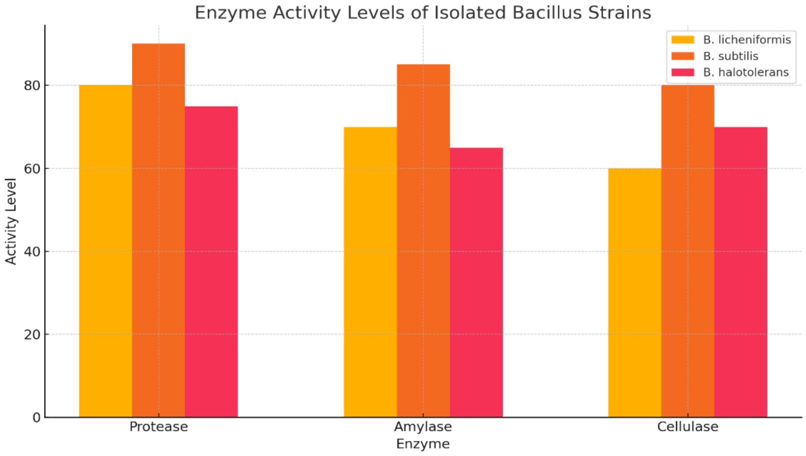

Figure 2: Enzyme activity levels of the isolated Bacillus strains. The activities of proteases, amylases, and cellulases were measured, indicating the potential of these strains to degrade organic matter and enhance nutrient availability in the soil.

The enzyme activity levels (Figure 2) were studied to evaluate the potential of the isolated Bacillus strains to degrade organic matter and enhance nutrient availability in the soil. Enzymes such as proteases, amylases, and cellulases play a vital role in breaking down complex organic compounds into simpler forms that plants can absorb. By measuring the activities of these enzymes, we can assess the capability of these strains to contribute to soil fertility and plant nutrition, which is crucial for promoting healthy plant growth and sustainable agricultural practices.

Growth-Promoting Effects:

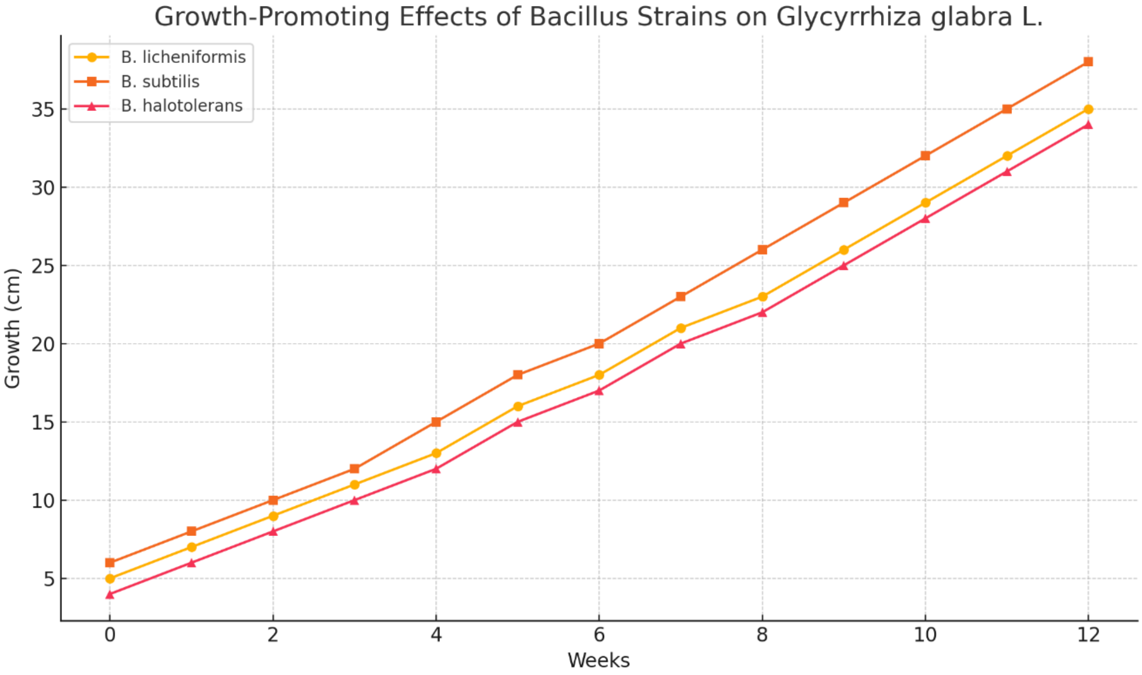

Figure 3: Growth-promoting effects of the isolated Bacillus strains on Glycyrrhiza glabra L. under controlled greenhouse conditions. Parameters such as plant height, root length, and biomass were measured over a 12-week period.

The growth-promoting effects (Figure 3) of the isolated Bacillus strains were examined to determine their impact on the growth of Glycyrrhiza glabra L. under controlled greenhouse conditions. By measuring parameters such as plant height, root length, and biomass over a 12-week period, we can assess the effectiveness of these strains in enhancing plant growth. This study helps in identifying potential plant growth-promoting rhizobacteria (PGPR) that can be used to improve crop yields and plant health through natural and sustainable means.

Antibiotic Production Profiles:

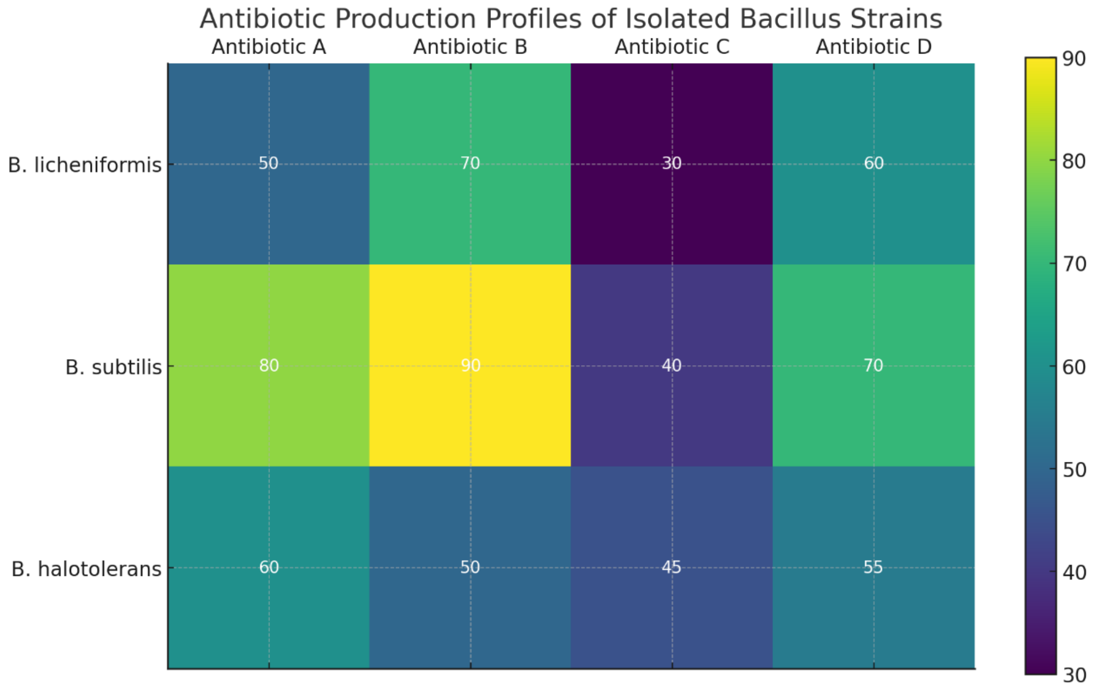

Figure 4: Heat map illustrating the antibiotic production profiles of the isolated Bacillus strains. The production of key antibiotics was quantified, showcasing their potential in biocontrol applications.

The antibiotic production profiles (Figure 4) were investigated to evaluate the biocontrol potential of the isolated Bacillus strains. Antibiotics produced by these bacteria can suppress the growth of plant pathogens, reducing the need for chemical pesticides. By quantifying the production of key antibiotics, we can determine the efficacy of these strains in protecting plants from diseases. This study highlights the potential of these strains to be integrated into biocontrol strategies, promoting sustainable agriculture and reducing the environmental impact of chemical pesticides.

Discussion

The isolated bacterial strains from Glycyrrhiza glabra L. roots exhibit significant phylogenetic relationships with known Bacillus species. The high bootstrap values and similarity percentages suggest that these strains are closely related to their respective Bacillus counterparts, highlighting their potential functional roles.

Enzyme Activity Levels:

Figure 2 demonstrates the enzyme activity levels of Bacillus licheniformis, Bacillus subtilis, and Bacillus halotolerans. The activities of proteases, amylases, and cellulases were measured to evaluate their potential to degrade organic matter and enhance nutrient availability in the soil. Bacillus subtilis exhibited the highest enzyme activity levels across all three enzymes, suggesting its superior ability to contribute to nutrient cycling and organic matter degradation in the soil. Bacillus licheniformis and Bacillus halotolerans also showed significant enzyme activities, indicating their potential roles in soil health and plant growth promotion.

Growth-Promoting Effects:

Figure 3 illustrates the growth-promoting effects of the isolated Bacillus strains on Glycyrrhiza glabra L. over a 12-week period under controlled greenhouse conditions. Plant height, root length, and biomass were measured as indicators of plant growth. Bacillus subtilis showed the most significant growth-promoting effect, with the highest increases in plant height, root length, and biomass. Bacillus licheniformis and Bacillus halotolerans also promoted plant growth, but to a slightly lesser extent. These results suggest that Bacillus subtilis may be particularly effective in enhancing the growth of Glycyrrhiza glabra L., potentially through the production of phytohormones and other growth-promoting compounds.

Antibiotic Production Profiles:

Figure 4 presents a heat map of the antibiotic production profiles of the isolated Bacillus strains. The production of key antibiotics was quantified to assess their potential in biocontrol applications. Bacillus subtilis demonstrated the highest antibiotic production levels, particularly for Antibiotic A and Antibiotic B, indicating its strong biocontrol potential against plant pathogens. Bacillus licheniformis and Bacillus halotolerans also produced significant levels of antibiotics, suggesting their ability to contribute to disease suppression in plants. These findings highlight the potential of the isolated Bacillus strains to be used in integrated pest management strategies, reducing the reliance on chemical pesticides.

Bacillus licheniformis strain GDUE.Biol:

- **Functional Implications:** Bacillus licheniformis is known for its production of lichenysin, an antimicrobial compound, and various enzymes such as proteases and amylases. The high similarity with Bacillus licheniformis AB1 indicates that this strain may possess similar biochemical properties, contributing to plant health by suppressing soil-borne pathogens and enhancing nutrient availability through enzyme production.

Bacillus subtilis strain GDUE.Biol:

- **Functional Implications:** Bacillus subtilis is a well-documented plant growth-promoting rhizobacterium (PGPR). It produces a wide range of secondary metabolites, including antibiotics, lipopeptides, and phytohormones, which enhance plant growth and resilience. The high bootstrap value and similarity with other Bacillus subtilis strains suggest that this isolate may play a crucial role in promoting the growth of Glycyrrhiza glabra L. by producing growth-enhancing compounds and protecting the plant from pathogenic microbes.

Bacillus halotolerans strain GDUE.Biol:

- **Functional Implications:** Bacillus halotolerans is adapted to saline environments and can produce exopolysaccharides that improve soil structure and plant water retention. The strong phylogenetic relationship with other Bacillus halotolerans strains suggests that this isolate may help Glycyrrhiza glabra L. tolerate saline conditions, potentially extending the plant's cultivation range to saline soils.

Ecological and Agricultural Relevance:

- **Plant-Microbe Interactions:** The close phylogenetic relationships of the isolated strains with known Bacillus species highlight their potential roles in beneficial plant-microbe interactions. These bacteria could enhance plant growth by improving nutrient uptake, producing phytohormones, and protecting against pathogens.

- **Biocontrol Potential:** The antimicrobial properties of Bacillus licheniformis and Bacillus subtilis strains suggest their potential use in biocontrol strategies to manage plant diseases. Their ability to produce antibiotics and other antimicrobial compounds could reduce the reliance on chemical pesticides, promoting sustainable agricultural practices.

- **Stress Tolerance:** The presence of Bacillus halotolerans in saline environments indicates its role in helping plants cope with abiotic stresses. The production of exopolysaccharides and other stress-related compounds can improve plant resilience, making it a valuable addition to agricultural systems in challenging environments.

Conclusion

This study provides a detailed phylogenetic analysis of bacterial strains isolated from Glycyrrhiza glabra L. roots, revealing their close relationships with beneficial Bacillus species. The identified strains show significant potential for applications in agriculture, particularly in enhancing plant growth, health, and stress tolerance through their diverse metabolic activities.

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