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Molecular study of endophytic *Aspergillus terreus* isolated from *Glycyrrhiza glabra* plant

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Abstract

Plants contain many types of endophytic fungi, and these have several applications in farming and biofuel production. Vegetables, trees, fodder, fruits, cereal grains, and wild plants, including *Glycyrrhiza glabra,* harbor them. The present study was conducted to isolate and molecularly characterize *Aspergillus terreus* from *Glycyrrhiza glabra* plant roots obtained from Al-Jadiria Company gardens in Baghdad, Iraq. Twenty healthy *Glycyrrhiza glabra* plant samples were collected from various locations in Al-Jadiria Company gardens in Baghdad, Iraq. Endophytic fungi were isolated from the root samples. Morphological and microscopic examination were used to identify the fungal isolates. The identification of the fungal isolates was confirmed using molecular analysis. Polymerase chain reaction (PCR) was used to amplify the internal transcribed spacer (ITS) region of the *rRNA* gene. The PCR product was purified and sequenced. The nucleotide sequence was subjected to bioinformatics analysis. The results revealed that 70% (14\20) of the *Glycyrrhiza glabra* plant root samples tested positive for *Aspergillus* species according to the morphological and microscopic examination. A fragment size of 650 bp was obtained by amplifying the ITS region of the rRNA gene. The bioinformatics analysis confirmed that the endophytic fungus was *Aspergillus terreus*. After amplification of fungi's ribosomal RNA, primers (ITS) and phylogenetic structuring tree analysis were carried out by sequences and confirmation of microorganism homogeneous data utilizing a database (NCBI). The results indicated that when compared to the wild type of the ITS gene from the gene bank, *Aspergillus terreu* isolate IAA1 small subunit ribosomal RNA gene displayed 100% identity.

Keywords: Endophytic mycota**,** *Glycyrrhiza glabra*, *Aspergillus terreus*, Molecular study, ITS region.

دراسة جزيئية للرشاشيات الارضية المعزولة كنابوت داخلي من نبات عرق السوس

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الخلاصة

تحتوي النباتات على العديد من أنواع الفطريات الداخلية، ولها العديد من التطبيقات في الزراعة وإنتاج الوقود الحيوي. تؤويها الخضروات والأشجار والأعلاف والفواكه والحبوب والنباتات البرية، ومن بينها *Glycyrrhiza glabra*. أجريت الدراسة الحالية لعزل وتوصيف *Aspergillus terreus* من جذور نبات

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Glycyrrhiza glabra التي تم الحصول عليها من حدائق مجمع الجادرية في بغداد، العراق. تم جمع عشرين عينة صحية من نبات *Glycyrrhiza glabra* من مواقع مختلفة في حدائق مجمع الجادرية في بغداد، العراق. تم عزل الفطريات الداخلية من عينات الجذور. تم إجراء الفحص الخارجي والمجهري للتعرف على العزلات الفطرية. تم التأكد من هوية العزلات الفطرية باستخدام التحليل الجزيئي. حيث تم استخراج الحمض النووي من العزلة الفطرية وتحديد التركيز من خلال استخدام تفاعل البلمرة المتسلسل (PCR) لتضخيم منطقة فراغ النسخ الداخلي (ITS) لجين الرنا الرايبوسومي (rRNA). وتنقية منتج PCR وتسلسله. ان تسلسل النوكليوتيدات تم تعرضه لتحليل المعلوماتية الحيوية. حيث أظهرت النتائج أن 14 (70٪) من أصل 20 عينة من جذور نبات *Glycyrrhiza glabra* عرق السوس كانت إيجابية لأنواع الرشاشيات وفقا للفحص الخارجي والمجهري. وكان تركيز الحمض النووي المعزول 19 نانوغرام / ميكرولتر. حيث تم الحصول على حجم جزء قدره 650 نقطة أساس لتضخيم منطقة ITS في جين الرنا الرايبوسومي. أكد تحليل المعلوماتية الحيوية أن الفطر الداخلي هو *Aspergillus terreus*. بعد تضخيم الحمض النووي الريبي الريبوسومي للفطريات، تم إجراء تحليل شجرة التركيب الفطري (ITS) وتحليل التركيب الشجري النشوئي عن طريق التسلسل وتأكيد بيانات التماثل بين الكائنات الحية الدقيقة باستخدام قاعدة بيانات (NCBI) حيث أشارت النتائج إلى أنه عند مقارنتها بالنوع البري لجين ITS من بنك الجينات، فإن *Aspergillus terreu* المعزول IAA1 من جين الريبوسوم RNA ذو الوحدة الفرعية الصغيرة أظهر توافقًا بنسبة 100٪.

1. Introduction

Plants have a widespread species of endophytic fungi. They exist endo- and exophyte for at low partition of their sustenance without displaying apparent syndromes. Almost every plant contains endophytes inside it [1] [2]. It is difficult to choose the right plant to explore endophytes for bioactive substances [3] [4]. As a result, pharmaceutical plants, which have long been used as a supplementary therapy, are a valuable source of endophytic bioprospecting [1]. Endophytes have various applications in several approaches, such as agriculture and biofuel production [5]. Endophytic fungal strains have been identified from trees, fodder, vegetables, fruits, cereal grains, and wild plants, such as *Glycyrrhiza glabra* [6]. *Glycyrrhiza glabra* plant is a perennial herbaceous legume indigenous to Western Asia, North Africa, and Southern Europe [7]. Its extracts have been utilized in traditional medicine and herbalism [8] [9]. *Glycyrrhiza glabra* is effective in treating allergies, bronchitis, cough, arthritis, adrenal insufficiency, and duodenal or stomach ulcers [10] [11]. The major active compounds of *Glycyrrhiza glabra* roots involve glycyrrhizin, flavonoids, glycosides and glycyrrhizic acid [12] [13]. These active compositions from *Glycyrrhiza glabra* roots may be joined to a specific microbiological factor [14]. Plentiful endophytic fungi from medicinally significant plants are thought to be able to produce secondary metabolites identical to or comparable to those produced by the plant [15]. Endophytic fungi meaningfully enhance the strength of the host plant in plant societies [16] and could boost plant prosperity and development [17].

Many species of fungi were isolated from *Glycyrrhiza glabra* roots, such as *Paraboereia putaminum*, *Chaetomium* spp, *Acrocalymma vagum* and *Aspergillus* spp [16] [18].A rapprochement of sequence similarity and phylogenetic analysis of the internal transcribed spacer (ITS) region of the *rRNA* gene enables the molecular identification of these fungi. *Aspergillus terreus* is a filamentous fungus belonging to the Ascomycetes family, found globally in the soil [19] [20]. It has been demonstrated to be an abundant source of secondary metabolites with intriguing biological activity [21] [22]. *Aspergillus terrus* has several active materials of processing and medicinal importance, and these compounds include antibacterials, butanolide (anti-inflammatory agent), and aurasperone H (anticancer agent) [23] [24] [25]. *Aspergillus terreus* is a prevalent fungus found in the chemical and

pharmaceutical industries, and it is recognized for producing lovastatin and itaconic acid [3] [26] [27].

Since the fungus is isolated from a medicinal plant, it is believed that it contains the same active substances as the medicinal plant, So the current study aimed to separate *Aspergillus terreu*s from *Glycyrrhiza glabra* plant roots that were collected from Baghdad, Iraq's Al-Jadiria Company gardens and molecular method for identification.

2. Materials and Methods

2.1 Sources of Chemical Materials

The absolute ethanol was obtained from ROMIL Pure Chemistry, UK. ABIO Pure TM total DNA kit was bought from ABIO, USA. Agarose, ethidium bromide solution (10 mg/ml), 100 bp DNA ladder marker, nuclease-free water, Go tag green master mix, TAE buffer (40X), and Quant dsDNA System were provided by Promega (USA).

2.2 Plant Sample Collection

Twenty healthy *Glycyrrhiza glabra* plant samples were collected on 20 - 29 September 2022 from different locations in Al-Jadiria Company gardens in Baghdad, Iraq. This area is characterized by its flatness, with a height of 34 m, and its agricultural area. It is located near the banks of the Tigris River. The longitude and latitude of this area is 33.1630" N - 33.1637" N, 44.2245" E (figure 1). The temperature of this area in the collection period was 18-38°C with humidity of 30%, and annual rainfall was 340-512 mm annually. The plant samples (Figure 2) were identified and authenticated in the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq. The plant samples were placed in sterile plastic bags and refrigerated at 4°C with 35-40% humidity till use (The storage period in the refrigerator should not exceed 2-3 days to avoid spoilage) [6].

Figure 1: The longitude and latitude of Al-Jadiria Company gardens in Baghdad, Iraq.

Figure 2: *Glycyrrhiza glabra* plant collected from Al-Jadiria Company gardens in Baghdad, Iraq.

2.3 Isolation of Endophytic Fungus

Collected *Glycyrrhiza glabra* root samples were washed with tap water for 10 min. The plant samples were then dried [28] and chopped into 1×1 cm by a sterilized scalpel. These root samples were immersed in 75% ethanol for 1 minute, followed by distilled water for 1-5 minutes, then 4% NaOCl for 1 minute, and finally distilled water for 1-5 minutes. The dried plant samples were sterilized with filter paper and inoculated in potato dextrose agar (PDA; HIMEDIA, USA) containing antibiotics (0.2 g/L chloramphenicol). Four pieces of root samples were inoculated in each plate). After that, the culture was incubated at 28±2C° for 3-21 days [4] [28]. The endophytic fungal strain was identified by morphological (color, textile, and appearance in the medium) and microscopic examination. Furthermore, the most frequent fungi were chosen for this study, and the identity of the fungal isolate was confirmed by the polymerase chain reaction (PCR) technique [18].

2.4 Macroscopic and microscopic examination

Several preparations from various fungal growth regions were examined under a microscope by staining a clean slide with lactophenol cotton blue to reveal conidia and mycelium. To remove air bubbles and aid with staining, the slide was gently heated under a spirit lamp (extra stain was wiped off using tissue paper).

The following factors were employed to identify the growth:

1. Features of the colony: color, consistency, geography.
2. The colony reverses (color, major pigment).
3- Microscopic morphology (shape, conidia arrangement, and hyphal structures).

2.5 Molecular identification of fungal isolation based on the ITS region

After the morphological and microscopic examination, a PCR-based technique was used to identify the fungal isolation. The molecular procedure included the extraction of the genomic DNA from the fungal isolate, PCR amplification, sequencing, and bioinformatics analysis.

2.5.1 DNA extraction from the endophytic fungus

Using the ABIO pure DNA extraction kit, genomic DNA was isolated from the growing fungal isolate following the manufacturer's instructions (Tiny pieces of the mycelia mat were extracted into 1.5 ml tubes and the culture medium for each of the 14 *A. terreus* isolates were frozen for one hour, followed by 5-minutes vortexing). A loop of filamentous hypha was suspended in 200 µL of buffer CL for pelleting the cells. To digest and lyse the cells, 20 µl of cell pellets were mixed with 200 µL of buffer CL, and 20 mg/ml of Proteinase K enzyme. The suspension was then vigorously agitated with a vortex mixer and then incubated at 50ºC for 60 minutes. An aliquot of 200 µL of buffer BL was added to the sample, and the tube was vigorously agitated with a vortex mixer before incubation at 70°C for 60 minutes. A pulse vortex machine was applied to fully mix up the sample after adding 200 µL of absolute ethanol. The supernatant was carefully removed and placed in the mini column. The mini column was then centrifuged for 1 min at 8,000 rpm, after which the tube for collection was replaced with a new one. Following the addition of 600 µL of BW buffer to the mini column, it was centrifuged for 1 min at 8,000 rpm. The buffer was drained off, and 700 µL solution TW was added. The mini column was then centrifuged at 8,000 rpm for 1 minute. The flow-through from the mini-column was discarded. High-speed (>13,000 x g) centrifugation was applied for 1 min to remove residual buffer. After that, the mini column was inserted into a new tube (1.5 ml). An aliquot of 100 µL of TAE buffer was added and incubated at room temperature for 1 minute before centrifugation at 5,000 rpm for 5 min [29].

2.5.2 Quantification of DNA Extract

A quantum fluorimeter was used to quantify the extracted DNA. An aliquot of 1 µL of DNA was mixed with 200 µL of the quantfluor dye. The preparation was incubated for 5 minutes at room temperature, and the concentration of the DNA was determined.

2.5.3 Amplification of ITS regions by polymerase chain reaction

The oligonucleotide primers used for amplification were provided by Macrogen, Korea. The forward primer's sequence (ITS1) was 5`-TCCGTAGGTGAACCTGCGG-3`, and the reverse primer's sequence (ITS4) was 5`-TCCTCCGCTTATTGATATGC-3`, with an annealing temperature of 55°C with an expected PCR product size of 650 bp [30] [31]. The lyophilized primers (ITS1 and ITS4) were prepared by dissolving in 300 µL nuclease-free water to make a final concentration of 100 pmol/µL (stock solution) as indicated by the manufacturing company. Then, 90 µL of nuclease-free water was combined with 10 µL of primer stock solution (stored at -20 C°) to make a working solution of 10 pmol/µL of these primers [32]. To set up the 25 µL PCR reaction, 12.5 µL of the master mix (2X), 1 µL of the forward primer (10 µM), 1 µL of the reverse primer (10 µM), 3 µL of DNA template (10 ng/µl), and 7.5 µL of nuclease-free water were combined in a PCR tube. The tube was vortexed briefly and spun in a microcentrifuge. The tube was loaded with a thermal cyclist. Table 1 displays the amplification conditions [30].

Table 1: Polymerase chain reaction amplification conditions

Step	°C	min: sec	Cycle
Initial denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	55	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

2.5.4 Agarose gel electrophoresis of polymerase chain reaction-amplified product

After the PCR amplification, agarose gel electrophoresis was performed to detect the amplified product. In setting up the gel electrophoresis apparatus, 1.5% agarose gel containing 1 µL of ethidium bromide (10 mg/ml) was prepared. It was stirred to mix thoroughly and heated in a microwave oven for 1 min. The agarose solution was allowed to cool between 50 and 60ºC [31]. After sealing the gel tray's edges with cellophane tape on both sides, the agarose solution was poured into the gel tray containing a plastic comb and allowed to solidify for 30 min at room temperature. After carefully removing the tape, the gel tray was placed inside the gel tank containing 1 X TBE-electrophoresis buffer and covered. The PCR product was loaded into the well of the agarose gel alongside a 100 bp DNA ladder marker [32]. The electrophoretic tank was connected to the power source, and the electrophoresis was run at 100 V for 1 hour. At the end of the electrophoresis, the Gel Documentation System was used to view the PCR product in the gel.

2.5.5 DNA sequencing of polymerase chain reaction-amplified product

To subject the PCR products to the sequencing procedure, the PCR product was purified to remove the left-over reaction components. The ABI3730XL DNA sequencer (Macrogen Corporation, Korea) was utilized for the sequencing procedure. The nucleotide sequence was analyzed with bioinformatics software as described by Rassin, *et al*.[33].

3. Results and Dissuasion

3.1 Identification of Glycyrrhiza glabra

The *Glycyrrhiza glabra* was identified and authenticated in the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq, by experts and professors of plant taxonomy. It was classified depending on the external features as it is characterized by annual stoloniferous roots, downy stems, pinnate leaves, and a maximum height of two meters; liquorice is a hardy perennial. The roots are long, cylindrical (round), straight segments of wrinkly, fibrous wood that extend horizontally below the surface. The inside of liquorice roots is yellow, whereas the outside is brown. It produces loose spikes of pale blue to violet pea-like blooms in late summer, which are followed by oblong pods that hold two to four kidney-shaped seeds each.

3.2 Isolation of endophytic fungus from plant samples

Of the 20 *Glycyrrhiza glabra* plant root samples, 14 were positive for *Aspergillus terreus* when cultured in a PDA medium. The fungal isolation was identified by morphological (growth features) and microscopic examination depending on [34]. The color of colonies is usually cinnamon-buff to sand-brown, suede-like, with a yellow to deep dirty brown reverse. Conidial heads are biseriate, compact, and columnar, with a diameter of up to 500 x 30–50 µm. Phialides and mettulae have the same length. Hyaline conidiophore stipes and walls that are smooth. Conidia are smooth-walled, globose to ellipsoidal, 1.5–2.5 µm in diameter, hyaline to slightly yellow, as shown in Figure 3.

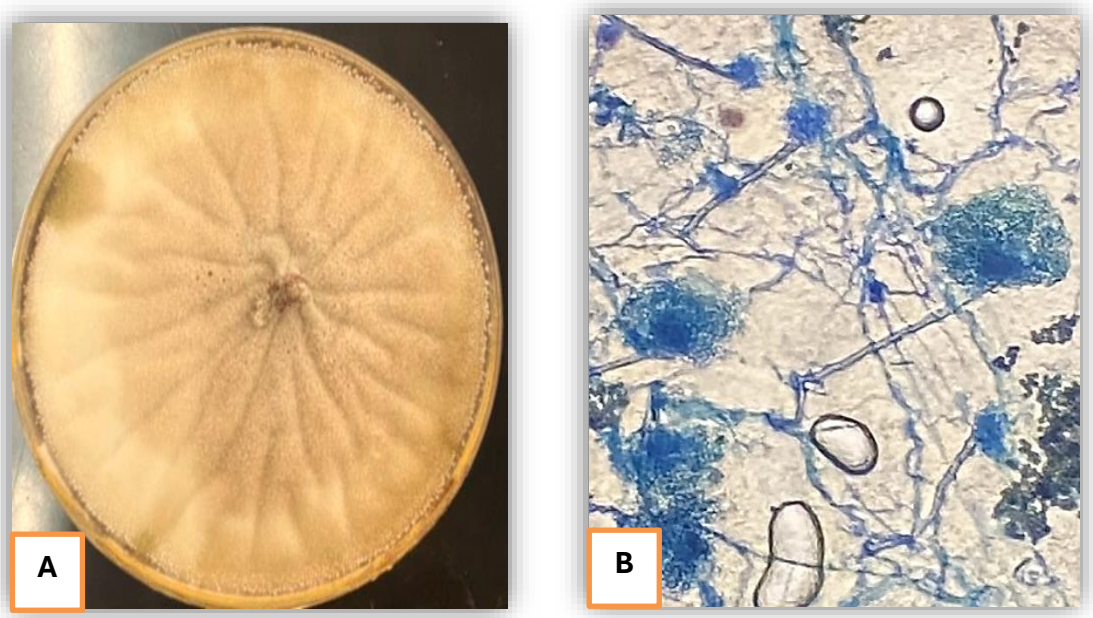

Figure 3: Characterization of *Aspergillus terreus* isolate. (A) Colony growth on potato dextrose agar medium after 3-5 days incubate. (B) Microscopic examination (40X).

3.3 Confirmation of Aspergillus terreus isolate using molecular analysis

In addition to the morphological and microscopic characterization of the fungal isolate, the identity of the isolate was confirmed using molecular techniques. Figure (4) shows the amplification of the target DNA segment. The results showed that the PCR product was positive for the ITS region with a PCR product size of 650 bp. The PCR product was sequenced, and the nucleotide sequences, as shown in Figure 5, were subjected to bioinformatics analysis. The result of the basic local alignment search tool (BLAST) is shown in Figure 6. *Aspergillus terreus* was the most often isolated fungus that has been selected for molecular identification by PCR employing primers (ITS1 and ITS 4) and phylogenetic structuring tree. Once the ribosomal RNA of the fungus was amplified, an analysis was carried out utilizing sequences, and the homogenous data was confirmed using the NCBI database.

3.4 Sequencing and alignment of NCBI

The PCR product of the *Aspergillus terreus* sample was sent for sequencing analysis. The sample was processed by Macrogen Corporation-Korea Company using an automated DNA sequence, the ABI3730XL Applied Biosystem equipment. Blast examined the sequence analysis result in the National Center for Biotechnology Information (NCBI) online database. *Aspergillus terreus* isolate showed 100% compatibility with score (1086) and 99% compatibility with score (1085), as shown in Table 6.

3.5 Local Iraqi isolate submission to NCBI

Aspergillus terreus isolate IAA1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence. GenBank: PQ394972.1 were registered after the correspondence of the National Centre Biotechnology Information (NCBI) obtained an accession number and became a reference to Iraq and the Middle East. It is available for download at https://www.ncbi.nlm.nih.gov/nuccore/PQ394972

3.6 The structure of phylogenetic trees

A phylogenetic tree diagram that reflected the true evolutionary paths and displayed neighbor-joining species produced by the Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0, as depicted in Figure (7).

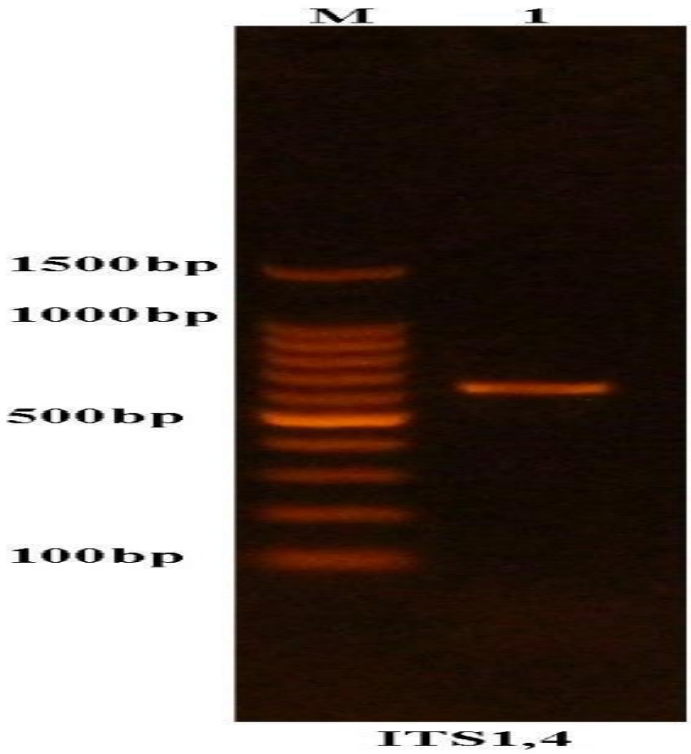

Figure 4: Agarose gel electrophoresis of polymerase chain reaction amplification of the internal transcribed spacer (ITS) region of the *rRNA* gene from *Aspergillus terreus*.

GCCTGCGGAAGGATCATTACCGAGTGCGGGTCTTTATGGCCCAACCTCCCACCCG
TGACTATTGTACCTTGTTGCTTCGGCGGGCCCGCCAGCGTTGCTGGCCGCCGGGG
GGCGACTCGCCCCCGGGCCCGTGCCCGCCGGAGACCCCAACATGAACCCTGTTCT
GAAAGCTTGCAGTCTGAGTGTGATTCTTTGCAATCAGTTAAAACTTTCAACAATG
GATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGT
GAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGG
TATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGT
GTGTTGGGCCCTCGTCCCCCGGCTCCCGGGGGACGGGCCCGAAAGGCAGCGGCG
GCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCTTCCGCTCCGTAGGCCCG
GCCGGCGCCCGCCGACGCATTTATTTGCAACTTGTTTTTTTCCAGGTTGACCTCGG
ATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATA

Figure 5: The nucleotide sequence of the internal transcribed spacer (ITS) region of the rRNA gene from *Aspergillus terreus*.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Aspergillus terreus isolate MD32_7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans	1086	1086	100%	0.0	100.00%	JQ697508.1
Aspergillus terreus isolate 2011F5 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inter	1085	1085	99%	0.0	100.00%	MT558839.1
Aspergillus terreus clone SF_31 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and interna	1085	1085	99%	0.0	100.00%	MT529307.1
Aspergillus terreus strain DTO 403-C9 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and	1085	1085	99%	0.0	100.00%	MT316343.1
Aspergillus terreus strain ORG-1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and intern	1085	1085	99%	0.0	100.00%	MT138424.1
Aspergillus terreus strain NF1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequ	1085	1085	99%	0.0	100.00%	MT077160.1
Aspergillus terreus strain SO11 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal	1085	1085	99%	0.0	100.00%	MT077141.1
Aspergillus terreus isolate VSGF2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inter	1085	1085	99%	0.0	100.00%	MN818690.1
Aspergillus terreus isolate 4100062L51-1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, a	1085	1085	99%	0.0	100.00%	MN559622.1
Aspergillus terreus strain EUR1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and interna	1085	1085	99%	0.0	100.00%	MF590162.1
Aspergillus terreus isolate CER2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and intern	1085	1085	99%	0.0	100.00%	MN173141.1
Aspergillus sp. isolate A3 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans	1085	1085	99%	0.0	100.00%	MN114540.1
Uncultured fungus clone SONO-6 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inter	1085	1085	99%	0.0	100.00%	MK189062.1
Uncultured fungus clone NEPA-8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete se	1085	1085	99%	0.0	100.00%	MK189050.1
Aspergillus terreus strain 8 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tran	1085	1085	99%	0.0	100.00%	MK541019.1
Aspergillus sp. isolate NIAB-FB15 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inter	1085	1085	99%	0.0	100.00%	MH741436.1

Figure 6: Basic local alignment search tool (BLAST) result of the internal transcribed spacer (ITS) region of the rRNA gene from *Aspergillus terreus*.

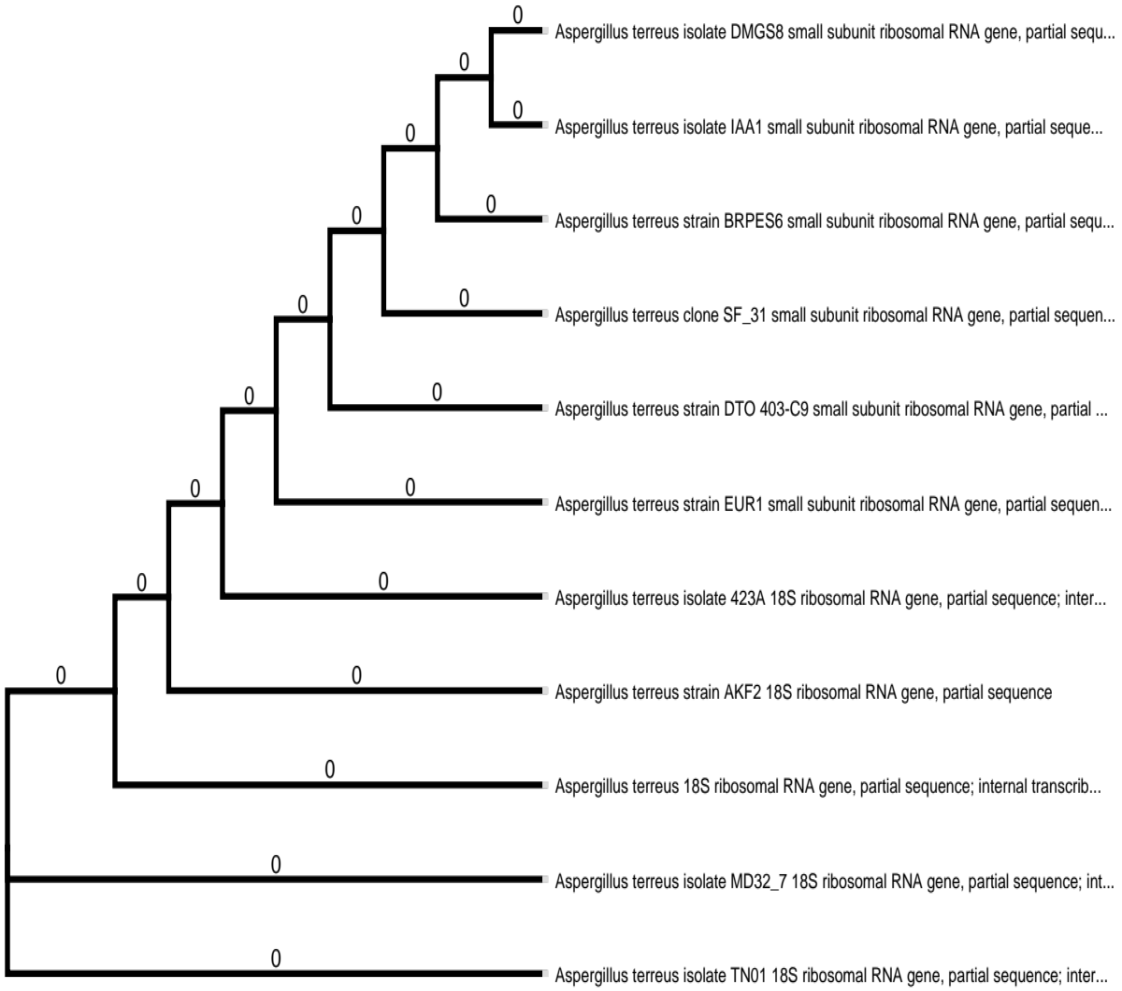

Figure 7: Phylogenetic tree of *Aspergillus terreus* IAA1 isolated from *Glycyrrhiza glabra* plant roots obtained from Al-Jadiria Company gardens in Baghdad, Iraq as endophytic fungus.

Endophytic fungi are symbiotic with their hosts, invading intercellular and intracellular parts of healthy plant tissues. These microorganisms produce antibiotics, enzymes, and other bioactive compounds, which allow them to survive in competitive environments with other microbes [35] [36]. Endophytic filamentous fungi are among the most innovative forms of producers of secondary metabolites, and they play essential biological roles in human life. They could be the source of substances used in medical, agricultural, and ecological sectors [37] [38]. Therefore, this study focused on the endophytic fungus in the *Glycyrrhiza glabra* plant, which has various benefits for humans. *Glycyrrhiza* species is a 4000-year-old medicinal plant that was used in several applications. It is part of the Fabaceae family. Glycyrrhizin (glycyrrhizic acid or glycyrrhetinic acid) is the major active component of *Glycyrrhiza* species. It has 50 times as much sugar as sucrose, with 6 to 14% concentrations. *Glycyrrhiza* species produce a variety of compounds, including coumarins, triterpenoids, flavonoids, isoflavonoids, kumatakenin, tannins, phytosterols, chalcones, and licorice [39] [40].

Three endophytes, *Aspergillus terreus, Bionectria* species, and *Stagonosporopsis cucurbitacearum,* also significantly enhanced the growth of shoot and rhizome. This endophytic mycota might produce endophyte-based solutions for agricultural applications and increase the production of *Glycyrrhiza glabra* [41]. *Aspergillus terreus* was isolated from

the *Glycyrrhiza glabra* plant obtained from Al-Jaderia Company garden, in Baghdad, Iraq. The fungus has been studied molecularly in many parts of the world, except in Iraq, especially in Baghdad. To the best of our knowledge, this present study is the first in Baghdad. Three fungi with various kinds of glycyrrhizin (GL) transformative potential were found in samples collected from soil from a *Glycyrrhiza glabra* farming zone in China. The three fungi, *Aspergillus terreus* Li-20, *Aspergillus ustus* Li-62, and *Penicillium purpurogenum* Li-3, were identified based on morphological characteristics and *18S rDNA* gene sequence analysis [42]. Molecular identification studies showed that the isolated fungal strain AM2 is 98% related to *Aspergillus terreus* and has been placed into the gene bank under the accession number MW444551.1. Using BLAST algorithms, fungal strains had 98% similarity to ITS sequences of *rRNA* genes from neighbouring isolates on the evolutionary tree [43] [44] [45]. The findings of Imran and Al Rubaiy demonstrate a great deal of variation in each isolate's phenotypic characteristics. The PCR results from the genotyping validated the diagnosis by primer pairs (ATE1 and ATE2), indicating that they were all unquestionably *A. terreus*. By highlighting the colonies' yellow hue, they effectively dispelled any misconception that would have caused people to mistake these isolates for different species. This result is consistent with the outcomes of Imran and Al Rubaiy [46] and. The idea of gene-environment correlation accounts for variations in phenotypic characteristics [47] [48] [49]. The study of Zhang *et al.* out of the 140 isolates *of A. terreus* that had their Cmd gene sequenced, 138 (GenBank accession nos. KM386696–KM386816 and KM458096–KM458112) demonstrated 99% homology (query coverage ranging from 98–100%) with isolates of *A. terreus* in GenBank (accession nos. KJ146014, JF927632, EU147582) [50].

Conclusion

The present study successfully isolated *Aspergillus terreus* from the *Glycyrrhiza glabra* plant roots, and molecular identification of the isolate was achieved by DNA sequencing of the PCR-amplified product of the ITS (ITS1 and ITS4) region of the *rRNA* gene. The future direction of this study is to explore the isolation of bioactive compounds from the endophytic *Aspergillus terreus* for agricultural and industrial applications.

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