SHORT REPORT

Molecular identification of *Hypomyces chrysospermus* mycoparasitic fungus isolated from mushrooms in a local market of Iraq

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Abstract

In this study, mycoparasitic fungus was identified by using the rDNA internal transcribed spacer barcode marker, i.e. ITS rDNA gene. Amplicons were sequenced and identified by NCBI – BLAST (Basic local alignment search tool). The BLAST results revealed that NOOR strain matched 100% with accession numbers [MH854754.1].

The phylogenetic tree shows close relationship between NOOR strain and *H. chrysospermus* [MH854754-1], *H. chrysospermus* [MZ389105-1], *H. chrysospermus* [MK605327-1], *H. chrysospermus* [MT595227-1], H. [EU816370-1], *H. chrysospermus* [MG685885-1].

The Iraqi isolate was recorded as NOOR strain in the National Centre for Biotechnology Information for the first time in Iraq.

Keywords: *Hypomyces chrysospermus,* Phylogeny, DNA, Ribosomal, Biotechnology

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Introduction

Hypomyces are considered one of the biggest genus of ascomycetes including only fungicolous fungi. There are around 53 species in this genus that are recognised globally.[1-3]. *H. chrysospermus* parasitises the fruiting bodies of Agaricales, Boletales, Helotiales, Pezizales, and Polyporales causing it to rot and die.[4, 5] *H. chrysospermus* has a thin white coating that turns golden and then pimpled, giving it a greenish-brown colour. In the yellow stage, spores are oval-shaped and smooth, measuring 5-12 µm in diameter, whereas the white stage spores are hairy, spherical, or thicker-walled, measuring 10-25 µm in diameter.[6, 7] As previously stated, *H. chrysospermus* is not palatable and may be harmful.[8]

Furthermore, standard approaches to identify *H. chrysospermus* via morphological and cultural tools are vulnerable to mistakes and inadequate recording of isolates at the stock culture because isolates are not "adequately differentiated". The phylogenomic characteristics in combination with standard approaches are a more effective strategy for verifying isolates and discovering new strains.[9-11]

Therefore, the isolation of *H. chrysospermus* mycoparasitic fungus from a mushroom from local market in Iraq was of interest. Furthermore, *H. chrysospermus* has been discovered for the first time in Iraq.

Materials and Methods

This research was approved by Human Research Ethics Committee, Mustansiriyah University, College of Medicine (No: 0003; date: 01 /02/ 2021).

The fungal culture utilised was obtained as a parasitic fungus from a mushroom available in an Iraqi market in Spring (01/03/2021-30/05/2021) at Mustansiriyah University, College of Science, Department of Biology. The fruiting bodies were chopped into 01cm pieces, soaked for 10 minutes in a two percent sodium hypochlorite solution, and then rinsed twice in deionised water. The fungus was placed on plates with Rose Bengal medium and cultured for seven days at 28°C. A pure culture of the isolate was cultivated on Rose Bengal medium at 4°C and then transferred to a new medium to create actively proliferating mycelia for investigation. With the aid of appropriate publications, the fungus was identified using standard culture and microscopic tools.[12, 13]

Molecular approaches have focussed on amplification and sequencing of the Internal Transcribed Spacer (ITS) region that was used to identify the isolated fungus, as stated by Wagner who introduced a modified TAB technique[14] (Cetyl Trimethylammonium Bromide) for extracting DNA. For ITS amplification, two universal primers were used: ITS1 forward 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 reverse 5'-TCCTCCGCTTATTGATATGC-3'.[15] The PCR procedures were 95°C for five minutes, accompanied by 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for one minute, with a final extension at 72°C for seven minutes.

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The amplified products were prepared according to the manufacturer's recommendations through QIAquick PC Purification Kit (Qiagen, USA), and afterwards sequenced in both directions at the Macrogen sequencing facility (Macrogen Inc., Seoul, Korea). For taxa classification[16], the genomic sequence data have been analysed with sequences available in the National Centre for Biotechnology Information (NCBI) online database (GenBank) via BLAST tool. Phylogenetic analysis was conducted in MEGA6.[16]

Results and Discussion

Through standard methodologies, it was proven that the Iraqi fungal isolate corresponded to the *Hypomyces chrysospermus*. A white colony appears first, followed by a colony that is golden to yellow-greenish in colour. These fungal spores were 10-15 µm long with rigid walls as well as oval in form (Figure 1). Our results were compatible with previous studies which observed that the size of spores *H. chrysospermus* was 10-16 µm and the walled spores were thick.[17]

The PCR amplification products of ITS region size were around 650bp on 1.5% agarose gel. Figure (2 A) shows that the fungal isolates of *H. chrysospermus* gave 650bp amplified bands. The BLAST results were obtained by searching with partial nucleotide sequences at the GenBank database. Furthermore, the percentage similarity was revealed by BLAST analysis. Sequence for the identified species including *H. chrysospermus* was then

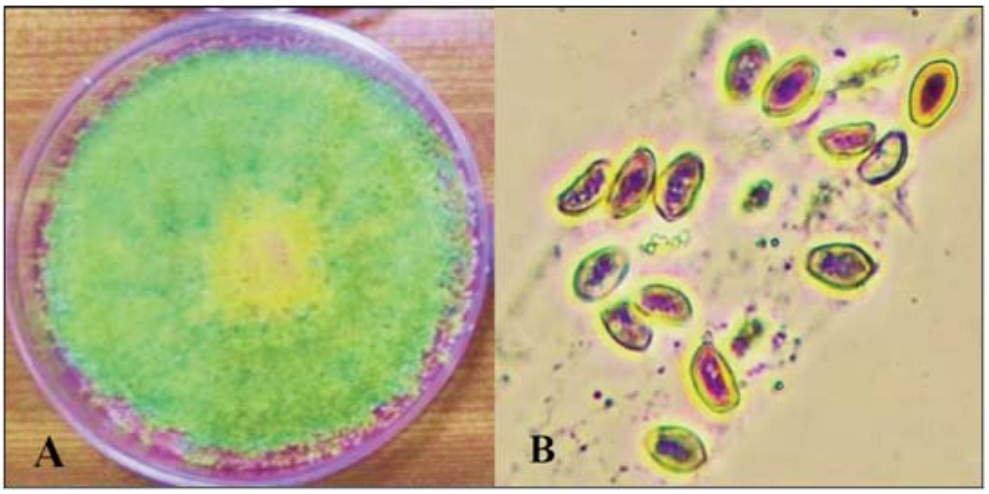

Figure-1: H. *chrysospermus*. (A): Fungal colony on Rose Bengal agar, (B): Spores.

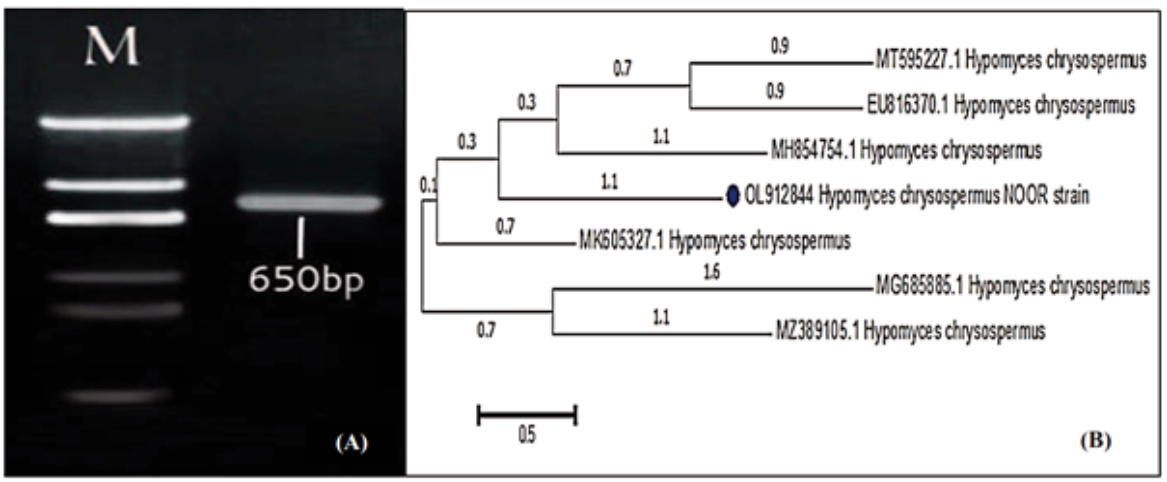

Figure-2: (A): PCR amplified products of ITS regions of H. *chrysospermus* M=DNA marker (100 bp), (B): The phylogenetic tree of novel isolate H. *chrysospermus* NOOR strain.

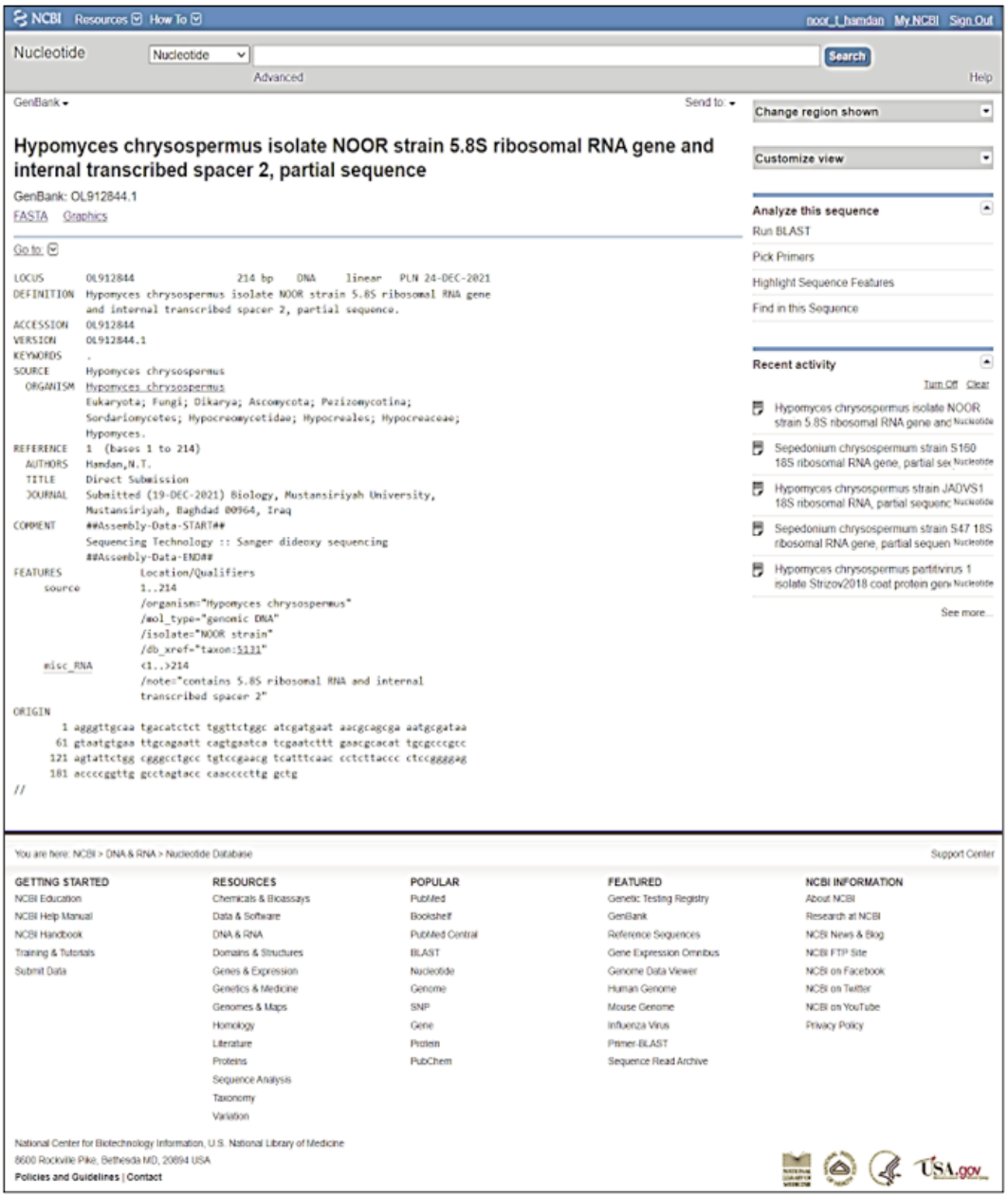

Figure-3: Novel Iraqi strain registered as Hypomyces chrysospermus NOOR strain in NCBI (https://www.ncbi.nlm.nih.gov/nuccore/OL912844).

submitted at the NCBI GenBank under the accession number [(MH854754.1% similarity100]. Similar results were obtained by Kim[18] using the ITS region of *H. chrysospermus* for amplification and was identified as *H. chrysospermus* based on a blast analysis in the NCBI Genbank database [AY344796].

On the other hand, the evolutionary tree was derived from six strains published in the NCBI Genbank and as represented in Figure (2 B) with high match ratio of 99%-100%. Therefore, the sequence was effective for determining phylogenetic and evolutionary relations which incorporated highest preserved 5.85 rRNA gene; besides that, it enveloped via two high variability regions that differed among species.[19-21] These results are in agreement with that recorded by Kim M, Ahn C and Kim et al[18] as well as Sun et al, 2019,[22] which showed the phylogenetic tree of *H. chrysospermus* OZ2 and *H. chrysospermus* CBS, respectively. Furthermore, the Iraqi isolate was recorded as NOOR strain in the National Centre for Biotechnology Information for the first time in Iraq (Figure 3).

Conclusion

The study's findings reveal that the novel Iraqi parasitic fungus of *H. chrysospermus* NOOR strain is of interest because it may seriously influence the mushroom industries and affect consumers' health. This study is considered original research as its results were recorded for the first time in Iraq; therefore, further studies are required to eliminate the parasitic fungi via biocontrol.

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Conflict of Interest: None.

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