

Novel Iraqi Fungal Isolates of *Trichoderma reesei* Registration

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Abstract

In Penguin city of Sulaymaniyah Province-Iraq is known for their vegetarian richness and contained a wide variety of microorganisms which have not been recognized yet. Seven new fungal isolates were identified as *Trichoderma reesei* fungi via phenotypic and molecular process i.e ITS rDNA gene and then registered in the gene bank NCBI for the first time in Iraq.

Introduction

Trichoderma reesei fungus can be found in soils, decomposing wood, and crop residues. Their ability to persist in variety of diverse areas that contributed to their physiological variety, great reproduction rate, and competitive capabilities. Furthermore, the optimum temperature for the growth of *T. reesei* ranges between (20-28) °C while the growth pH for *T. reesei* is between 3-9 and the optimum pH is between 4.5 [1]. *T. reesei* is a well-studied genus, owing to its very well application in the manufacture of bioenergy-related and cell wall disintegrating enzymes, as well as as antimicrobials toward plant diseases [2]. *T. reesei* teleomorph previously referred as *Hypocrea jecorina*, which is commonly published in species documents [3].

DNA Barcoding approach which provides limited and defined DNA zone with specific trend and regarded as one of the highest efficient and fast approach to classifying unknown endophytes [4]. For example, Internal Transcribed Spacer (ITS) region was widely employed since it considers greatest sequenced zone for endophytic classification of species. The ITS region was highly variable of non-coding in plenty of phylogenetic elements for allowing species level sequences isolation. *Trichoderma reesei* is a filamentous fungus that is one of the most ubiquitous genera worldwide due to the using in an industrial scale to produce enzymes of biotechnological interest [5]. Through this study, seven unrecorded fungal species in the Penguin city were discovered, identified and registered from these cities.

Materials and Methods

Phenotypic Characterization

The seven *Trichoderma reesei* isolates were isolated from rhizosphere of rice straw field which located at Penguin city of Sulaymaniyah Province-Iraq. The isolation processes were involved via serial dilution and sub-culturing strategies [6]. The Phenotypic characterization was identified by culturing on PDA, SNA and MEA agars at $25\pm 2^{\circ}\text{C}$ for 5 days. All the fungal isolates were assigned as *Trichoderma reesei* by the taxonomic key of Samuels and Hebbbar [7]. Freshly cultured pure colony was then employed for DNA extraction.

Molecular characterization

DNA extraction

The dominance fungi (7 isolates) were collected from the plate and transferred to a new PDA medium plate to obtain single colony for DNA extraction. pure culture was used to extract DNA by using Genetic DNA isolation kit (Doctor protein INC, Korea), following by manufactures instructions [8]. The absorbance of diluted DNA solution at 260 and 280 nm was measured using Nanodrop to estimate DNA concentrations (25-100 ng/rxn) and purity (1.6-1.8) (Thermos Scientific, Germany). The purity of the DNA was tested by electrophoresis on a 1% agarose gel dyed with ethidium bromide. The solutions were kept at -20°C until they were needed.

Amplification method of genomic DNA

Two universal primers, ITS1 (forward): 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 (reverse): 5'-TCCTCCGCTTATTGATATGC-3', have been employed to amplify the fungi's ITS region [9]. For PCR experiments, we used DNA Polymerase (Doctor protein INC, Korea) according to the manufacturer guidelines. The following were the PCR amplification circumstances: Denaturation was started at 95°C for 5 minutes, then 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, followed by a final extension at 72°C for 7 minutes. Electrophoresis then used to assess the integrity and quantity for PCR product at 1.5 percent agarose gel. At the Macrogen sequencing laboratory, gene sequences have been verified from both strands of PCR amplification products (Macrogen Inc., Seoul, Korea). The gene sequence analyses have been performed using sequences available in GeneBank of the National Center for Biotechnology Information (NCBI) for identification at species via BLAST tool.

Evolutionary studies via Maximum Likelihood technique

The Tamura-Nei modelling and the Maximum Likelihood technique were used to determine the evolutionary history [10]. It presented the tree with the largest log likelihood (-1943.49). The original tree(s) of metaheuristic have been dynamically utilizing by the Neighbor-Join and BioNJ algorithm to a matrix of pairwise lengths computed via the Maximum Composite Likelihood (MCL) technique, and thereafter picking the topological for the optimal log likelihood ratio. Numbers of 14 nucleotide sequences were analyzed in this study. The 1st+2nd+3rd+Noncoding codon locations were included. In the end, the dataset had 988 locations. Evolutionary studies have been determined at MEGA X [11].

Results and Discussion

Identification of fungal strains using phenotypic and molecular characteristics

Firstly, the ITS region sequences of the seven *Trichoderma reesei*, that had been phenotypic diagnosed in Biology Department/ Science college/ Mustansiriyah University, Iraq, previously published via Samuels and Hebbar [7] were confirmed. The isolates diagnosed via the morphological and microscopic descriptions. These isolates were readily distinguished using phenotypic characteristics such as colony color (dark green and cottony whitish green colonies), growth pattern, form and size of conidiophore, phialides and conidia microscopically viewed. The phenotypic discovered isolates have named as *Trichoderma reesei* (Figure1).

T. reesei fungi are significant commercially since they provide commercial enzymes and antibiotics, as well as serving as biostimulants[12]. Because of their great degree of resemblance, morphological characterizations at species proved problematic [13, 14]. Furthermore, since of their sensitivity to environmental influences, classification related to host selectivity beside phenotypic variations isn't trustworthy; hence, molecular approaches have subsequently been devised for detailed characterization.

As a result, these differences should have no effect on molecular patterns in genomic DNA, which can be helpful for species identification and resolving ambiguous situations [15, 16].

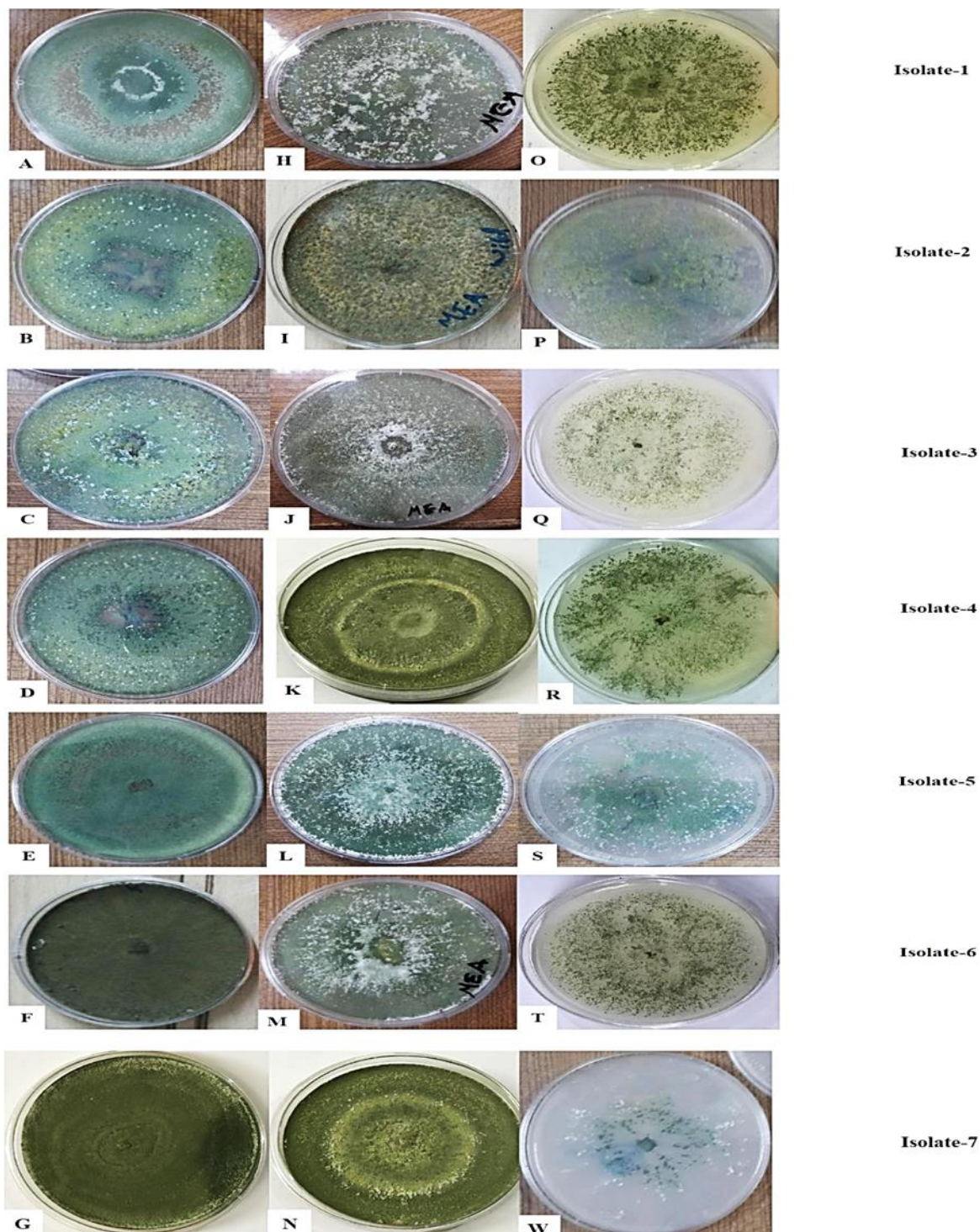


Fig. 1: *Trichoderma reesei* isolates after 5 days incubation period. A-G: PDA agar, H-N: MEA agar, O-W: SDA agar (15 megapixel).

The PCR amplification products of ITS region size were around 300 bp to 350bp on 1.5% agarose gel. Fig. 3 shows that the fungal isolates of isolates 1,2,3 gave 300bp, and other isolates 4,5,6,7, gave 350bp amplified bands respectively.

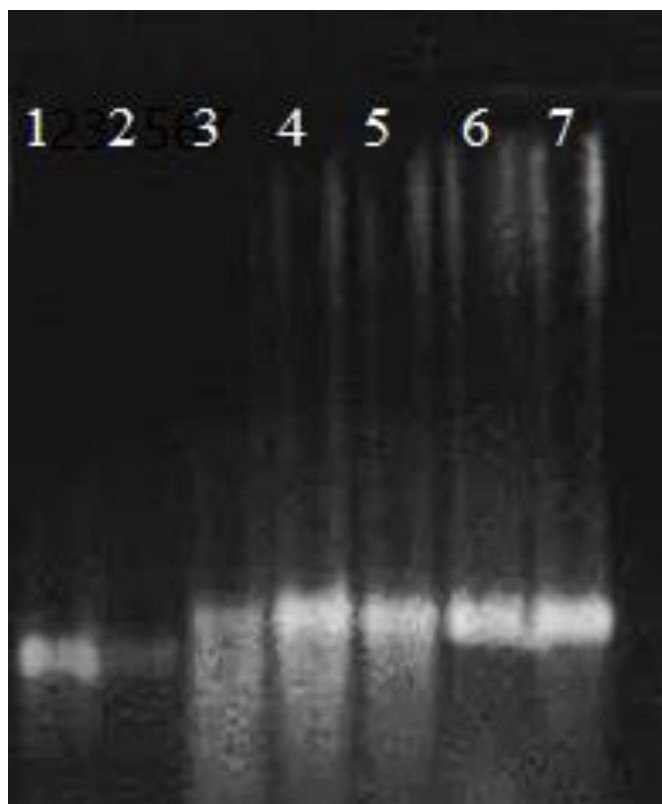


Fig. 2: 1% agarose gel electrophoresis of *Trichoderma reesei* genomic DNA.

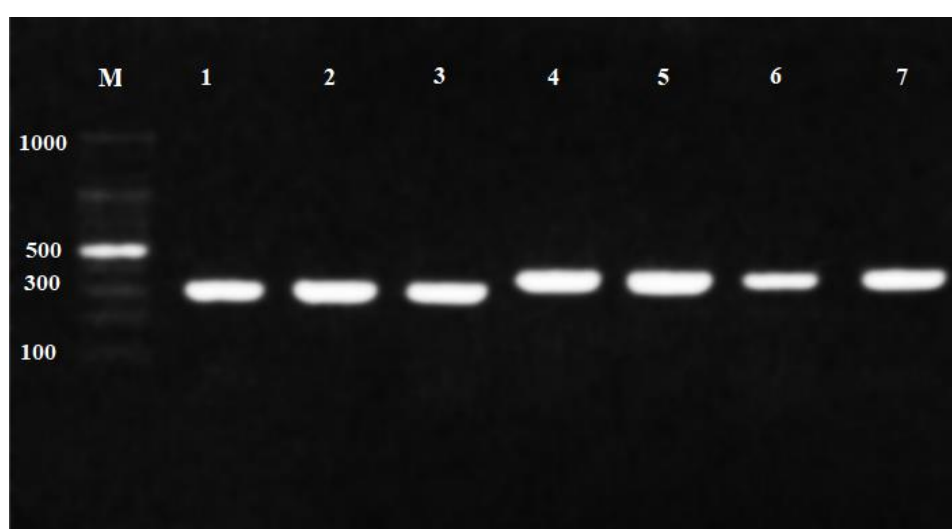


Fig. 3: 1.5% agarose gel electrophoresis of PCR product of *Trichoderma reesei* isolates by using ITS1-ITS4 primers. (1-7) bands of *Trichoderma reesei* PCR product, (M) DNA marker (1000 bp).

After that, The BLAST data were determined through searching the GenBank dataset with partial nucleotide sequences. Furthermore, the percentage similarity was revealed by Blast analysis. Sequence for the identified fungi including *Trichoderma reesei* (AB1), *Trichoderma reesei* (Soil), *Trichoderma reesei*(AB2), *Trichoderma reesei*(AB1), *Trichoderma reesei* (Nb1), *Trichoderma reesei* (AB1), *Trichoderma reesei* (C1C2) were then published to the NCBI GenBank website and deposited with the accession number [MF375204.1, MG597062.1, MF375205.1, MF375117.1, MG822856.1, MF375203.1, MG822870.1, respectively] table (1).

Therefore in sense, The sequence was effective for determining phylogenetic and evolutionary relations which incorporated highest preserved 5.8s rRNA gene beside it enveloped via two high variability regions that differed among species [17, 18, 19].

All of the nucleotide sequences from the ITS region found in this investigation matched 99-98 percent of the previous sequences of *T. reesei* in gene bank accession number *T. reesei* F12018 (MW789354.1), *T. reesei* NTOU4438 (MZ423065.1), *T. reesei* Sharify (KY031342.1), *T. reesei* UFMGC1421 (MW837788.1), *T. reesei* Sikkim211810F (MZ596295.1), *T. reesei* Z65-8 (MZ543975.1), *T. reesei* S12 (MZ948856.1) respectively.

Table 1: Major alignments between *T. reesei* sequences.

Isolate number	Iraqi strain Codes Registered in NCBI	Genus	Accession Number	Sequences producing significant alignments		
				Description	Accession Number	Score similarity (%)
1	AB1	<i>Trichoderma reesei</i>	MF375204.1	<i>Trichoderma reesei</i> F1-2018	MW789354.1	99%
2	Soil	<i>Trichoderma reesei</i>	MG597062.1	<i>Trichoderma reesei</i> NTOU4438	MZ423065.1	99%
3	AB2	<i>Trichoderma reesei</i>	MF375205.1	<i>Trichoderma reesei</i> Sharify	KY031342.1	99%
4	AB1	<i>Trichoderma reesei</i>	MF375117.1	<i>Trichoderma reesei</i> UFMGC1421	MW837788.1	98%
5	Nb1	<i>Trichoderma reesei</i>	MG822856.1	<i>Trichoderma reesei</i> Sikkim211810F	MZ596295.1	99%
6	AB1	<i>Trichoderma reesei</i>	MF375203.1	<i>Trichoderma reesei</i> Z65-8	MZ543975.1	99%
7	C1C2	<i>Trichoderma reesei</i>	MG822870.1	<i>Trichoderma reesei</i> S12	MZ948856.1	99%

On the other hand, the evolutionary tree was derived from seven strains published in the NCBI gene bank and as represented in figure (4) with high match ratio of 98%-99%. The fungal isolates 1, 2, 3, 4, 5, 6, 7 of *T. reesei* were shown a close relationship with accession numbers MF375204.1, MG597062.1, MF375205.1, MF375117.1, MG822856.1, MF375203.1, MG822870.1 respectively. According to Mendoza-Revilla [20] two taxa are most linked to each other if they share more common ancestors. Finally, these fungal isolates were then recorded in NCBI as AB1, Soil, AB2, AB1, Nb1, AB1, C1C2 For the first time in Iraq as shown in figure (5,6,7,8,9,10,11). To our facts, this paper is the first in Iraq.

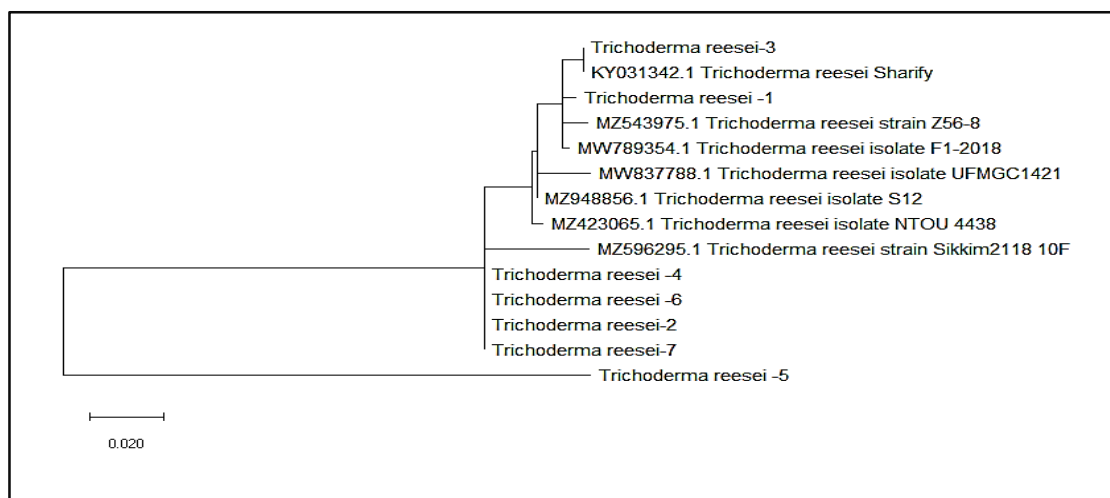


Fig. 4: The evolutionary history of *T. reesei* isolates.

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Trichoderma reesei isolate AB1 internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence

GenBank: MF375204.1
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 VERSION MF375204.1
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 SOURCE Trichoderma reesei (Hypocrea jecorina)
 ORGANISM [Trichoderma reesei](#)
 Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
 Sordariomycetes; Hypocreomycetidae; Hypocreales; Hypocreaceae;
 Trichoderma.
 REFERENCE 1 (bases 1 to 263)
 AUTHORS Hamdan, N.T.
 TITLE Bioethanol production Using wild type Trichoderma reesei from cellulosic wastes Based on enzymatic hydrolysis
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 263)
 AUTHORS Hamdan, N.T.
 TITLE Direct Submission
 JOURNAL Submitted (25-JUN-2017) gene bank, al-nahrain university, iraq-baghdad . zayona, baghdad 00964, Iraq
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 Trichoderma reesei iraq (19) Nucleotide
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 Trichoderma longibrachiatum isolate AB2 internal transcribed spacer 1, partial Nucleotide
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Fig. 5: Iraqi strain-1 registered as *T. reesei* AB1.

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Trichoderma reesei isolate soil internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MG597062.1

[FASTA](#) [Graphics](#)

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ACCESSION MG597062

VERSION MG597062.1

KEYWORDS .

SOURCE Trichoderma reesei (Hypocrea jecorina)

ORGANISM [Trichoderma reesei](#)

Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreomycetidae; Hypocreales; Hypocreaceae; Trichoderma.

REFERENCE 1 (bases 1 to 254)

AUTHORS hamdan,N.T.

TITLE Mycodiesel production by two mesophilic fungi from Trichoderma reesei

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 254)

AUTHORS hamdan,N.T.

TITLE Direct Submission

JOURNAL Submitted (03-DEC-2017) gene bank, al-nahrain university, iraq-baghdad, baghdad 00964, Iraq

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Trichoderma reesei isolate soil internal transcribed spacer 1, partial sequence Nucleotide

Trichoderma reesei isolate nb1 5.8S ribosomal RNA gene and internal tra Nucleotide

Trichoderma reesei isolate Y.N.127.Saad small subunit ribosomal RNA gene, p Nucleotide

Trichoderma reesei small subunit ribosomal RNA gene, partial sequence Nucleotide

Trichoderma reesei isolate Y.N.141.shahad small subunit ribosomal RNA gene, p Nucleotide

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Fig. 6: Iraqi strain-2 registered as *T. reesei* Soil.

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Trichoderma reesei isolate AB2 internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence

GenBank: MF375205.1
[FASTA](#) [Graphics](#)

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 VERSION MF375205.1
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 ORGANISM [Trichoderma reesei](#)
 Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
 Sordariomycetes; Hypocreomycetidae; Hypocreales; Hypocreaceae;
 Trichoderma.
 REFERENCE 1 (bases 1 to 215)
 AUTHORS Hamdan, N.T.
 TITLE Production of cellulase enzyme and Application in the production of Bio-ethanol by Trichoderma reesei
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 215)
 AUTHORS Hamdan, N.T.
 TITLE Direct Submission
 JOURNAL Submitted (25-JUN-2017) gene bank, al-nahrain university, Iraq-baghdad . zayona, baghdad 00964, Iraq
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Fig. 7: Iraqi strain-3 registered as *T. reesei* AB2.

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Trichoderma reesei isolate AB1 internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence

GenBank: MF375117.1
[FASTA](#) [Graphics](#)

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 Trichoderma.
 REFERENCE 1 (bases 1 to 57)
 AUTHORS Hamdan, N.T.
 TITLE Study the activity of some strains of Trichoderma reesei on producing of BIOFUELS
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 57)
 AUTHORS Hamdan, N.T.
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- Trichoderma reesei isolate AB1 internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence Nucleotide
- Trichoderma reesei isolate AB2 internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence Nucleotide
- Trichoderma reesei isolate c1c2 internal transcribed spacer 1, partial sequence Nucleotide
- Trichoderma reesei isolate soil internal transcribed spacer 1, partial sequence Nucleotide
- Trichoderma reesei isolate nb1 5.8S ribosomal RNA gene and internal transcribed spacer 1, partial sequence Nucleotide

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Fig. 8: Iraqi strain-4 registered as *T. reesei* AB1.

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Trichoderma reesei isolate nb1 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence

GenBank: MG822856.1
[FASTA](#) [Graphics](#)

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 Sordariomycetes; Hypocreomycetidae; Hypocreales; Hypocreaceae;
 Trichoderma.

REFERENCE 1 (bases 1 to 196)
 AUTHORS hamdan, N.T.
 TITLE Efficiency evaluation of mycodiesel production by some strains
 Trichoderma reesei
 JOURNAL Unpublished

REFERENCE 2 (bases 1 to 196)
 AUTHORS hamdan, N.T.
 TITLE Direct Submission
 JOURNAL Submitted (23-JAN-2018) gene bank, al-nahrain university,
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	Genetics & Medicine	Genome	Human Genome	NCBI on Twitter
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	Homology	Gene	Influenza Virus	Privacy Policy
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	Sequence Analysis			
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	Variation			

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Fig. 9: Iraqi strain-5 registered as *T. reesei* Nb1.

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Trichoderma reesei isolate AB1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MF375203.1
[FASTA](#) [Graphics](#)

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LOCUS MF375203 218 bp DNA linear PLN 08-JUL-2017
 DEFINITION Trichoderma reesei isolate AB1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.
 ACCESSION MF375203
 VERSION MF375203.1
 KEYWORDS .
 SOURCE Trichoderma reesei (Hypocrea jecorina)
 ORGANISM Trichoderma reesei
 Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreomycetidae; Hypocreales; Hypocreaceae; Trichoderma.
 REFERENCE 1 (bases 1 to 218)
 AUTHORS Hamdan, N.T.
 TITLE Bioethanol production using co-culture of Trichoderma reesei and saccharomyces cerevisiae from steam pretreated wheat straw in solid state fermentation
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 218)
 AUTHORS Hamdan, N.T.
 TITLE Direct Submission
 JOURNAL Submitted (25-JUN-2017) gene bank, al-nahrain university, iraq-baghdad . zayona, baghdad 00964, Iraq
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 Trichoderma reesei isolate AB1 internal transcribed spacer 1, partial sequence Nucleotide
 Trichoderma reesei isolate AB1 internal transcribed spacer 1 and 5.8S ribosomal RNA gene Nucleotide
 Trichoderma reesei isolate AB2 internal transcribed spacer 1 and 5.8S ribosomal RNA gene Nucleotide
 Trichoderma reesei isolate c1c2 internal transcribed spacer 1, partial sequence Nucleotide
 Trichoderma reesei isolate soil internal transcribed spacer 1, partial sequence Nucleotide
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	Homology	Gene	Influenza Virus	Privacy Policy
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	Variation			

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Fig. 10: Iraqi strain-6 registered as *T. reesei* AB1.

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Trichoderma reesei isolate c1c2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MG822870.1
[FASTA](#) [Graphics](#)

Go to: (v)

LOCUS MG822870 221 bp DNA linear PLN 31-JAN-2018
 DEFINITION Trichoderma reesei isolate c1c2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.
 ACCESSION MG822870
 VERSION MG822870.1
 KEYWORDS
 SOURCE Trichoderma reesei (Hypocrea jecorina)
 ORGANISM [Trichoderma reesei](#)
 Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreomycetidae; Hypocreales; Hypocreaceae; Trichoderma.
 REFERENCE 1 (bases 1 to 221)
 AUTHORS hamdan, N.T.
 TITLE Biodiesel fuel production from fungal lipids using transesterification
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 221)
 AUTHORS hamdan, N.T.
 TITLE Direct Submission
 JOURNAL Submitted (23-JAN-2018) gene bank, al-nahrain university, iraq-baghdad, baghdad 00964, Iraq
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 Sequencing Technology :: Sanger dideoxy sequencing
 ##Assembly-Data-END##
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 /db_xref="taxon:51453"
 misc_RNA
 <1..>221
 /note="contains internal transcribed spacer 1, 5.8S ribosomal RNA, and internal transcribed spacer 2"
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 121 atctttgaac gcacattgac ccgccagta ttctggcggg catgcctgac cgagcgtcat
 181 ttcaaccctc gaacccctcc gggggctcgg cgttggggat c
 //

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- Trichoderma reesei isolate c1c2 internal transcribed spacer 1, partial sequence Nucleotide
- Trichoderma reesei isolate soil internal transcribed spacer 1, partial sequence Nucleotide
- Trichoderma reesei isolate nb1 5.8S ribosomal RNA gene and internal tra Nucleotide
- Trichoderma reesei isolate Y.N.127.Saad small subunit ribosomal RNA gene, i Nucleotide
- Trichoderma reesei small subunit ribosomal RNA gene, partial sequence Nucleotide

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Fig. 11: Iraqi strain-7 registered as *T. reesei* C1C2

Conclusion

Seven Iraqi strains of the fungus *T. reesei* were morphologically and molecularly identified in isolates from the rhizosphere soil of rice straw field in the Penguin city of Sulaymaniyah Province-Iraq. The fungal strains are currently used for biotechnological interest i.e in producing industrial enzymes. To our facts, this paper is the first in Iraq.

Acknowledgment

The author would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq) Baghdad Iraq for its support in the present work.

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تسجيل عزلات العراقية الجديدة لفطريات *Trichoderma reesei*

نور طالب حمدان

قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية

معلومات البحث:

تاريخ الاستلام: 2022/04/04

تاريخ القبول: 2022/05/09

الكلمات المفتاحية:

/التشخيص، عزلات فطرية جديدة،
مدينة بنجوين، تربة رابوزسفير.

معلومات المؤلف

الايمل:

الموبايل:

الخلاصة:

تشتهر مدينة بنجوين في محافظة السليمانية /العراق بالثراء النباتي، اذ تحتوي على مجموعة متنوعة من الكائنات الحية الدقيقة التي لم يتم التعرف عليها بعد. تم عزل سبع عزلات فطرية جديدة وتم تحديدها على أنها *Trichoderma reesei* من خلال تشخيصها مظهريا وجزيئيا، وحيث سجلت في بنك الجينات (المركز الوطني لمعلومات التقنية الحيوية) لأول مرة في العراق.