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Novel Iraqi Fungal Isolates of Trichoderma reesei Registration

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Article Information	Abstract
Received: 04/04/2022	
Accepted: 09/05/2022	In Penguin city of Sulaymaniyah Province-Iraq is known for their
Keywords:	vegetarian richness and contained a wide variety of microorganisms
Identification, Novel Fungal	which have not been recognized yet. Seven new fungal isolates were
Isolates, NCBI, Penguin city,	identified as Trichoderma reesei fungi via phenotypic and molecular
Rhizospheric soil.	process i.e ITS rDNA gene and then registered in the gene bank NCBI
Corresponding Author	for the first time in Iraq.
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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 isolatation. *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±2°C for 5 days. All the fungal isolates were assigned as *Trichoderma reesei* by the taxonomic key of Samuels and Hebbar [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 (revere): 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 orginal 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* (Figure 1).

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].

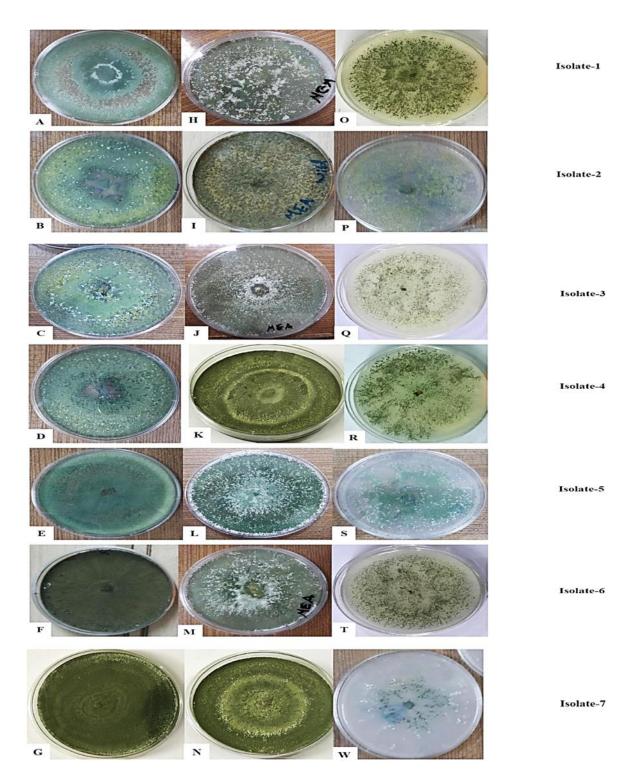


Fig. 1: *Trichoderma reesei* isolates after 5 days incubation period. A-G: PDA agar, H-N: MEA agar, O-W: SDA agar (15 migapixel).

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

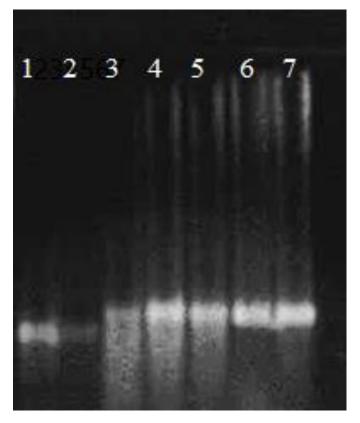


Fig. 2: 1% agarose gel electrophoresis of Trichoderma reesei genomic DNA.

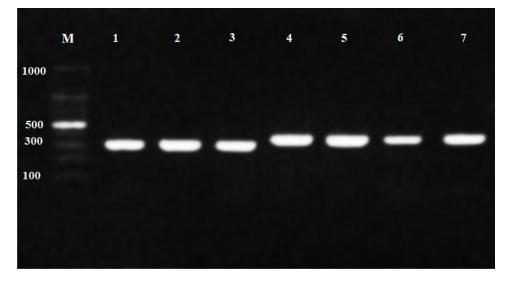


Fig. 3: 1.5% agarose gel electrophoresis of PCR product of *Trichoderma reesei* isolates by using ITS1-ITS4 primers. (1-7) bands of *Trichoderma reesei* PCR product, (M) DNA marker (1000 bp).

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 reseei* (AB1), *Trichoderma reseei* (Soil), *Trichoderma reseei* (AB2), *Trichoderma reseei* (AB1), *Trichoderma reseei* (Nb1), *Trichoderma reseei* (AB1), *Trichoderma reseei* (

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.

Isolate	Iraqi strain Codes		Accession	Sequences producin	g significant alignment	s
number	Registed in NCBI	Genus	Number	Description	Accession Number	Score similarity (%)
1	AB1	Trichoderma reesei	MF375204.1	Trichoderma reeseiF1-2018	MW789354.1	99%
2	Soil	Trichoderma reesei	MG597062.1	Trichoderma reeseiNTOU4438	MZ423065.1	99%
3	AB2	Trichoderma reesei	MF375205.1	Trichoderma reeseiSharify	KY031342.1	99%
4	AB1	Trichoderma reesei	MF375117.1	Trichoderma reeseiUFMGC1421	MW837788.1	98%
5	Nb1	Trichoderma reesei	MG822856.1	Trichoderma reeseiSikkim211810F	MZ596295.1	99%
6	AB1	Trichoderma reesei	MF375203.1	Trichoderma reeseiZ65-8	MZ543975.1	99%
7	C1C2	Trichoderma reesei	MG822870.1	Trichoderma reeseiS12	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.

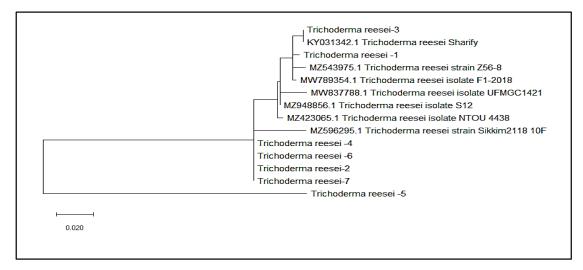


Fig. 4: The evolutionary history of *T. reesei* isolates.

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Fig. 5: Iraqi strain-1 registered as *T. reesei* AB1.

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

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Fig. 7: Iraqi strain-3 registered as *T. reesei* AB2.

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

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REFERENCE AUTHORS	1 (bases 1 to 196) hamdan,N.T.		Recent activity	
TITLE	Efficiency evaluation of mycodiesel production by some strains		Т	urn Off Clear
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REFERENCE	2 (bases 1 to 196)		ribosomal RNA gene and interna	
AUTHORS	hamdan,N.T. Direct Submission		Trichoderma reesei isolate Y.N.1 small subunit ribosomal RNA ger	
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Fig. 9: Iraqi strain-5 registered as *T. reesei* Nb1.

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

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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 rhizoshere 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.

References

- 1. Tisch, D., Pomraning, K. R., Collett, J. R., Freitag, M., Baker, S. E., Chen, C. L., *et al.*(2017). Omics Analyses of *Trichoderma reesei* CBS999.97 and OM6a indicate the relevance of female fertility to carbohydrate-active enzyme and transporter levels. *Environmental Microbiology*, 83:e01578-17.
- Hinterdobler, W., Li, G., Spiegel, K., Basyouni-Khamist, S., Gorfer, M. and Schmoll, M. (2021). *Trichoderma reesei* Isolated From Austrian Soil With High Potential for Biotechnological Application. *Frontiers in Microbiology*, (1), 10.
- 3. Bissett, J., Gams, W., Jaklitsch, W., and Samuels, G. J. (2015). Accepted *Trichoderma* names in the year 2015. *IMA Fungus*, 6, 263-295.
- Schoch, C. L.; Seifert K. A.; Huhndorf S.; Robert, V.; Spouge, J. L.; Levesque, C. A. and Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi, Proceedings of the National Academy of Sciences of the United State of America. *PNAS*, 109 (16), 6241-6246.
- 5. Haque, Z., Iabal, M.S., Ahmad A., Khan M. S and Prakash, J. (2020). Molecular Characterization of *Trichoderma* spp. Isolates by Internal Transcribed Spacer (ITS) Region Sequencing Technique and its Use as a Biocontrol Agent. *The Open Biotechnology Journal*, 14, 70-77.
- 6. Cornelia, M., Gradinger, K., Szakacs, G., Kubicek, Ch.P. (2002). Phylogeny and evolution of the genus Trichoderma :multigene approach. *Mycological Research*, 106, 757-767.
- 7. Samuels, G.J. and Hebbar, P. (2015). *Trichoderma* Identification and Agricultural Applications. The American Phytopathological Society, 1st Edn., St. Paul, Minnesto, USA, Pp196.
- 8. Iti, G.M; Niraji, T. and Shard, T. (2014). A simple and Rabid. DNA Extraction protocol for filamentous fungi efficient for molecular studies. *Indian Journal of Biotechnology*, 13,536-539.
- 9. White, T. J., Bruns, T., Lee, S. and Taylor, J.(1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR- Protocols and Applications A Laboratory Manual. Academic Press. pp. 315-322.
- 10. Tamura, K. and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10, 512-526.
- 11. Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547-1549.
- Arnau, J., Yaver, D., and Hjort, M. C. (2020). "Strategies and challenges for the development of industrial enzymes using fungal cell factories," in Grand challenges in Fungal Biotechnology, ed. H. Nevalainen (Cham: Springer Nature Switzerland AG), 179-210.
- 13. Sharma, K., Mishra, AK., Misra, RS. (2009). Morphological, biochemical and molecular characterization of *Trichoderma harzianum* isolates for their efficacy as biocontrol agents. *Journal of Phytopathology*,157(1), 51-56.

- 14. Shahid, M., Srivastava, M., Sharma, A. (2013). Morphological, molecular identification and SR marker analysis of a potential strain of *Trichoderma/Hypocrea* for production of a bioformulation. *Journal of Plant Pathology & Microbiology*, 4(10), 204-210.
- 15. Li, W. C., Huang, C. H., Chen, C. L., Chuang, Y. C., Tung, S. Y., and Wang, T. F. (2017). *Trichoderma reesei* complete genome sequence, repeat-induced point mutation, and partitioning of CAZyme gene clusters. *Journal of Biotechnology Biofuels* 10, 170.
- 16. Lücking, R., Aime, M. C., Robbertse, B., Miller, A. N., Ariyawansa, H. A., Aoki, T., *et al.* (2020). Unambiguous identification of fungi: where do we stand and how accurate and precise is fungal DNA barcoding. *IMA Fungus.* doi:10.1186/s43008-020-00033-2.
- 17. Beiggi, S., Piercey, M.(2007). Evolution of ITS Ribosomal RNA secondary structures in fungal and algal Symbionts of selected species of *Cladonia* sect.Cladonia (Cladoniaceae, Ascomycotina). *Journal of Molecular Evolution*, 64, 528-42. doi:10.1007/s00239-006-0115-x.
- Deng, W., Xi, D., Mao, H., Wanapat, M. (2008). The use of molecular techniques based on Ribosomal RNA and DNA for rumen microbial ecosystem studies. Review. *Molecular Biology Reports*, 35, 265-74. doi:10.1007/511033-007-9079-1.
- 19. Besse, P. (2014). Nuclear Ribosomal RNA Genes: ITS Region. In: Besse P, editor. Molecular plant taxonomy: methods and protocols, methods in molecular biology. New York: Springer Science. Business Media; v.1115. p.141-9.
- 20. Mendoza-Revilla, J. (2012). Aportes de la filogenética a la investigación médica. Rev Ded Hered. 23, 119-27.



تسجيل عزلات العراقية الجديدة لفطريات Trichoderma reesei

	نور طالب حمدان
	قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية
الخلاصة: تشتهر مدينة بنجوين في محافظة السليمانية /العراق بالثراء النباتي، اذ تحتوي على مجموعة	معلومات البحث:
متنوعة من الكائنات الحية الدقيقة التي لم يتم التعرف عليها بعد. تم عزل سبع عز لات فطرية	تأريخ الاستلام: 2022/04/04 تأريخ القبــول: 2022/05/09
جديدة وتم تحديدها على أنها Trichoderma reesei من خلال تشخيصها مظهريا وجزيئيا،	الكلمات المفتاحية:
وحيث سُجلت في بنك الجينات (المركز الوطني لمعلومات التقانة الحيوية) لإول مرة في	التشخيص، عز لات فطرية جديدة،
العراق.	مدينة بنجوين، تربة رايز وسفير.
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