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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 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℃ 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* (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].

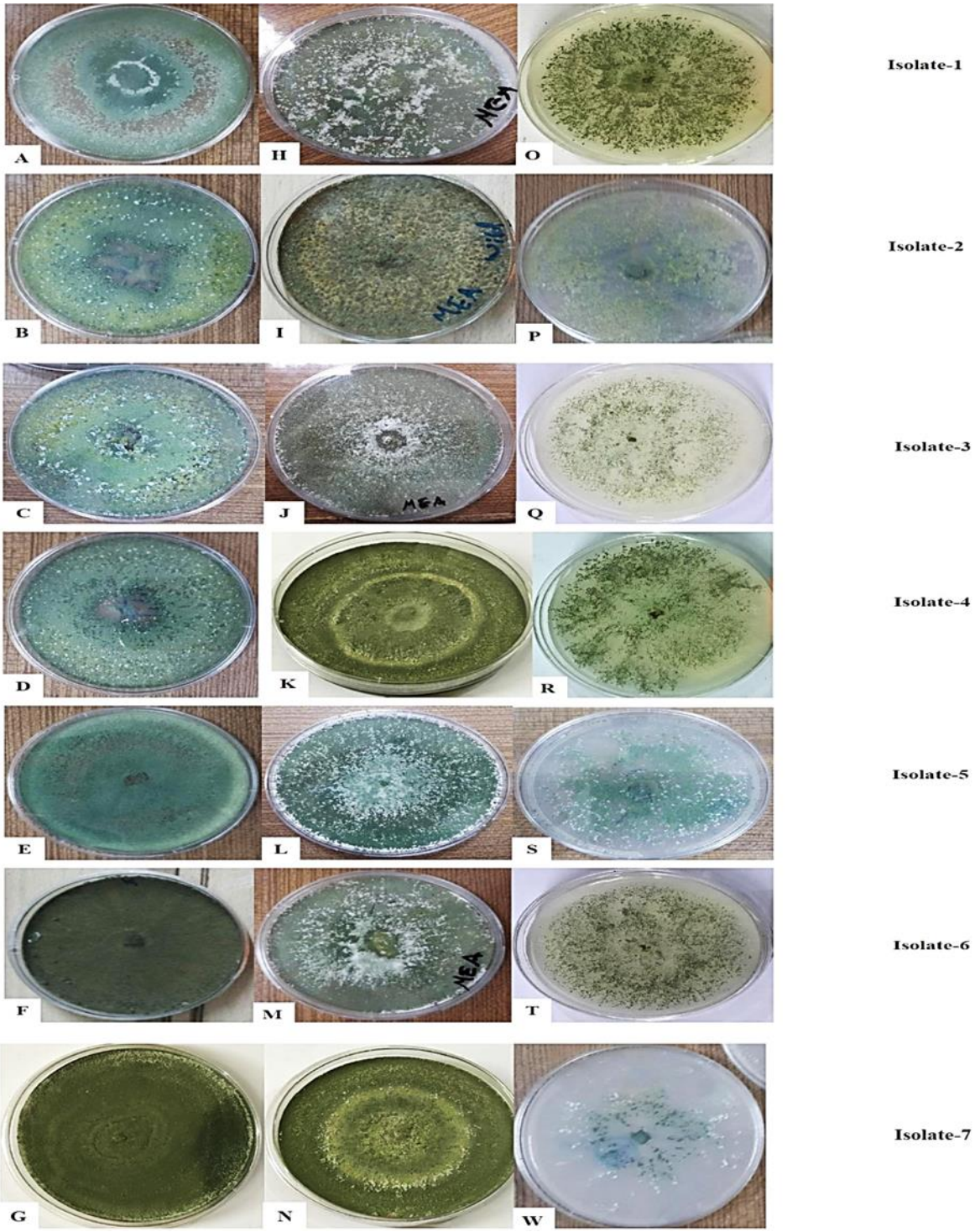

Fig. 1: *Trichoderma reesei* isolates after 5 days incubation period. A-G: PDA agar, H-N: MEA agar, O-W: SDA agar (15 migapixel).

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.

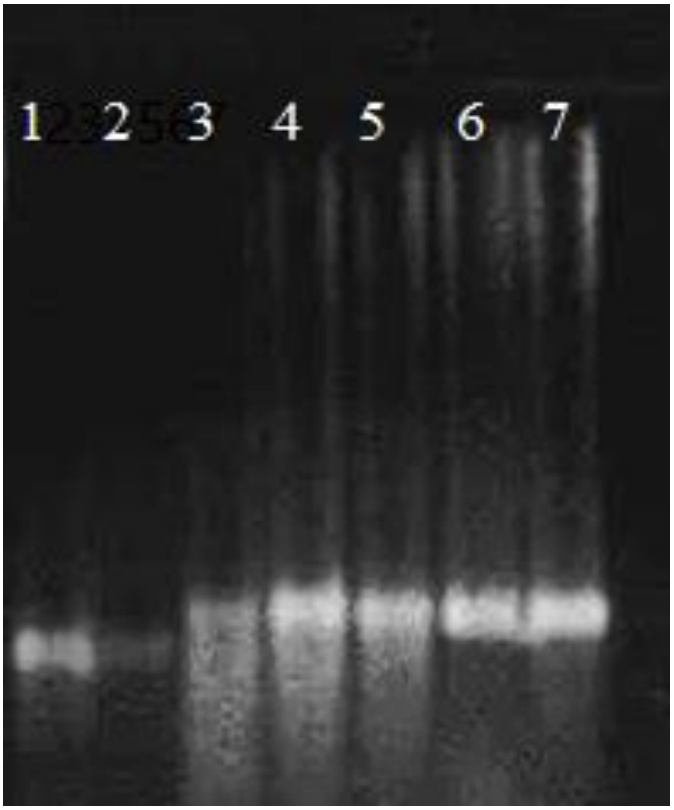

Fig. 2: 1% agarose gel electrophoresis of *Trichoderma reesei* genomic DNA.

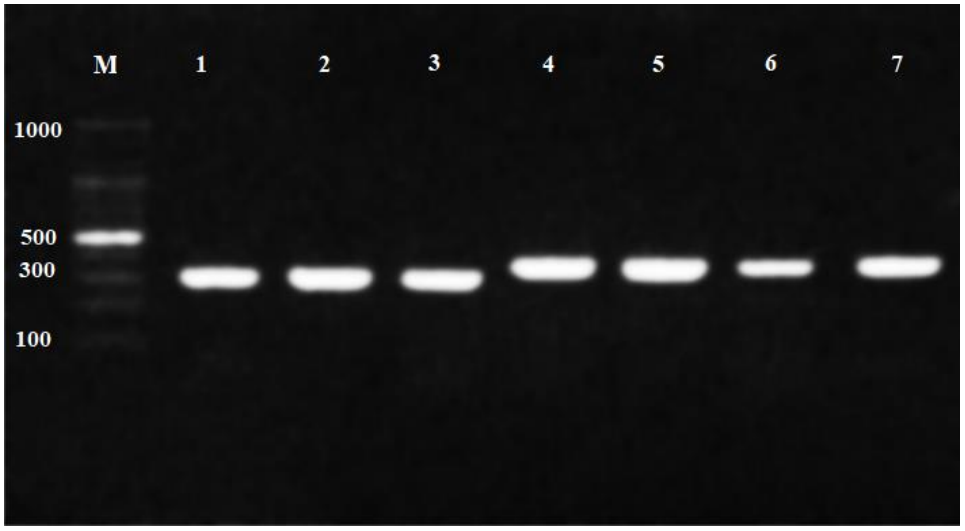

Fig. 3: 1.5% agarose gel electrophoresis of PCR product of *Trichoderma reesei* isolates by using ITS1-ITS4 primers. (1-7) bands of *Trichoderma reesei* PCR product, (M) DNA marker (1000 bp).

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* (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 Registed in NCBI	Genus	Accession Number	Sequences producing significant alignments		
				Description	Accession Number	Score similarity (%)
1	AB1	*Trichoderma reesei*	MF375204.1	*Trichoderma reesei*F1-2018	MW789354.1	99%
2	Soil	*Trichoderma reesei*	MG597062.1	*Trichoderma reesei*NTOU4438	MZ423065.1	99%
3	AB2	*Trichoderma reesei*	MF375205.1	*Trichoderma reesei*Sharify	KY031342.1	99%
4	AB1	*Trichoderma reesei*	MF375117.1	*Trichoderma reesei*UFMGC1421	MW837788.1	98%
5	Nb1	*Trichoderma reesei*	MG822856.1	*Trichoderma reesei*Sikkim211810F	MZ596295.1	99%
6	AB1	*Trichoderma reesei*	MF375203.1	*Trichoderma reesei*Z65-8	MZ543975.1	99%
7	C1C2	*Trichoderma reesei*	MG822870.1	*Trichoderma reesei*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.

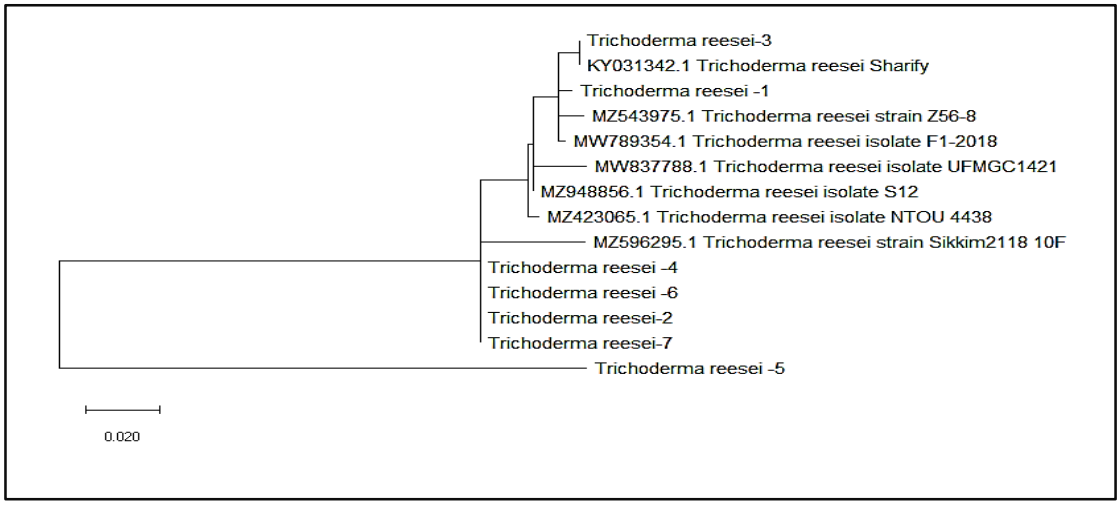

Fig. 4: The evolutionary history of *T. reesei* isolates**.**

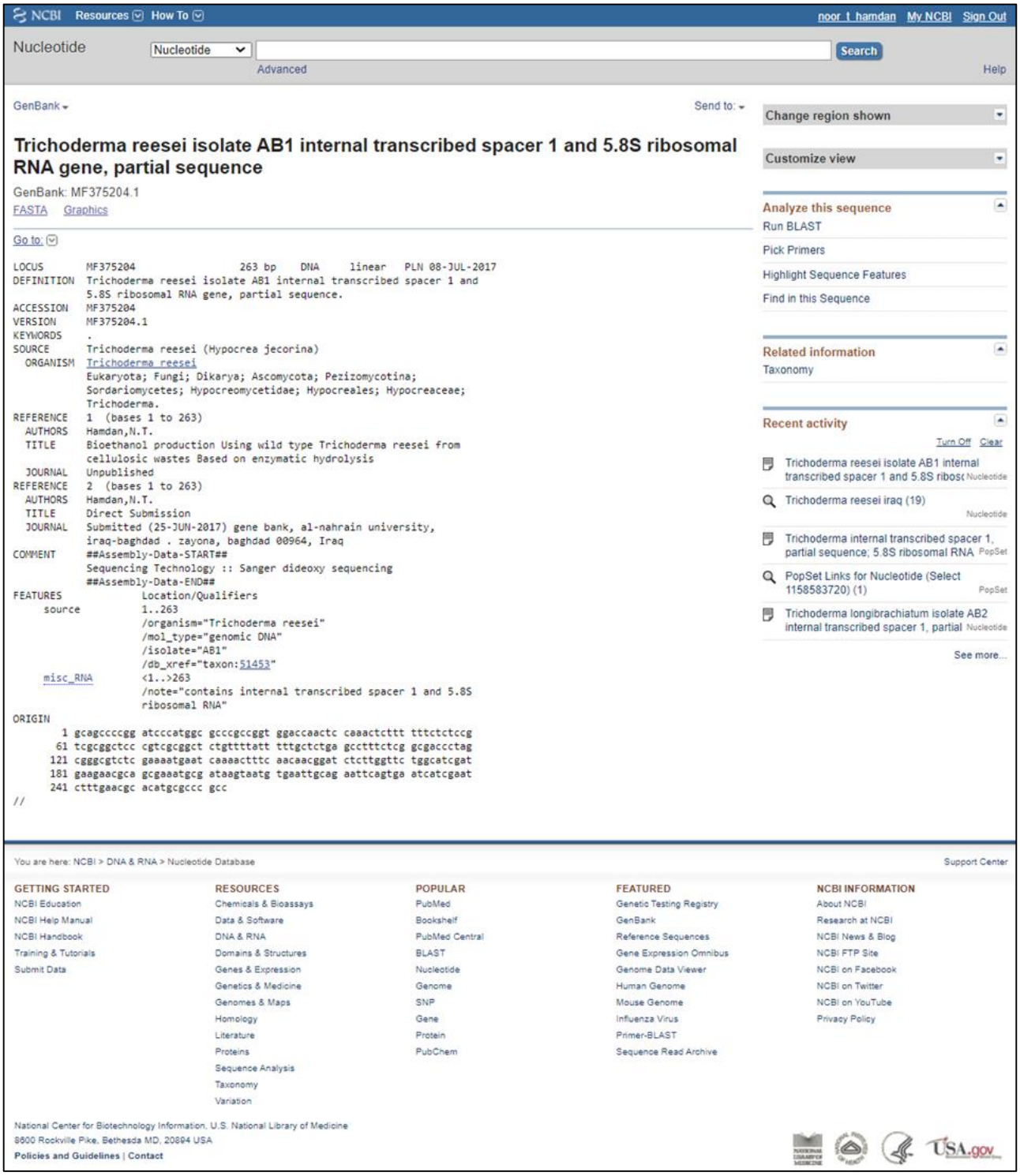

Fig. 5: Iraqi strain-1 registered as *T. reesei* AB1.

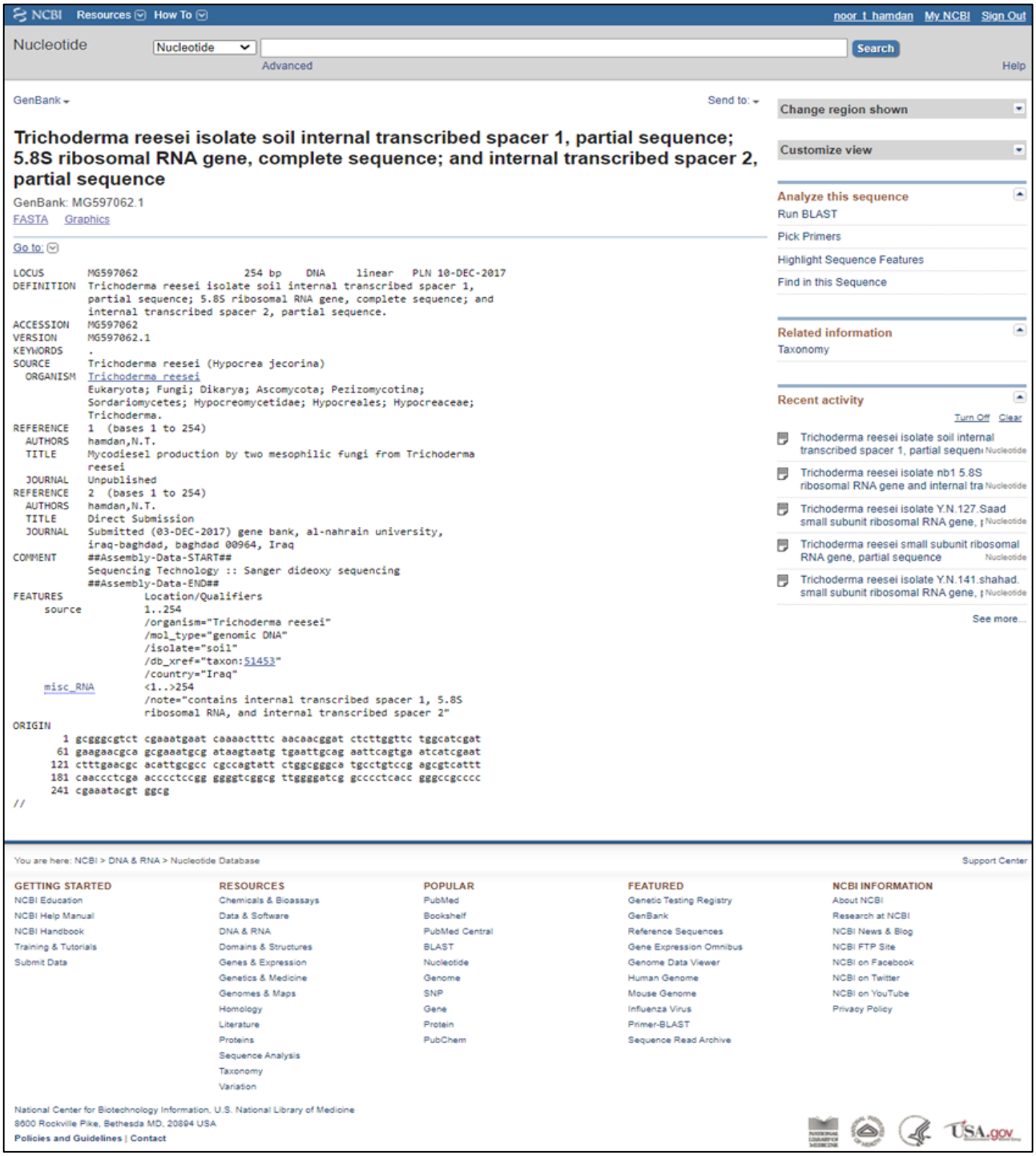

Fig. 6: Iraqi strain-2 registered as *T. reesei* Soil.

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

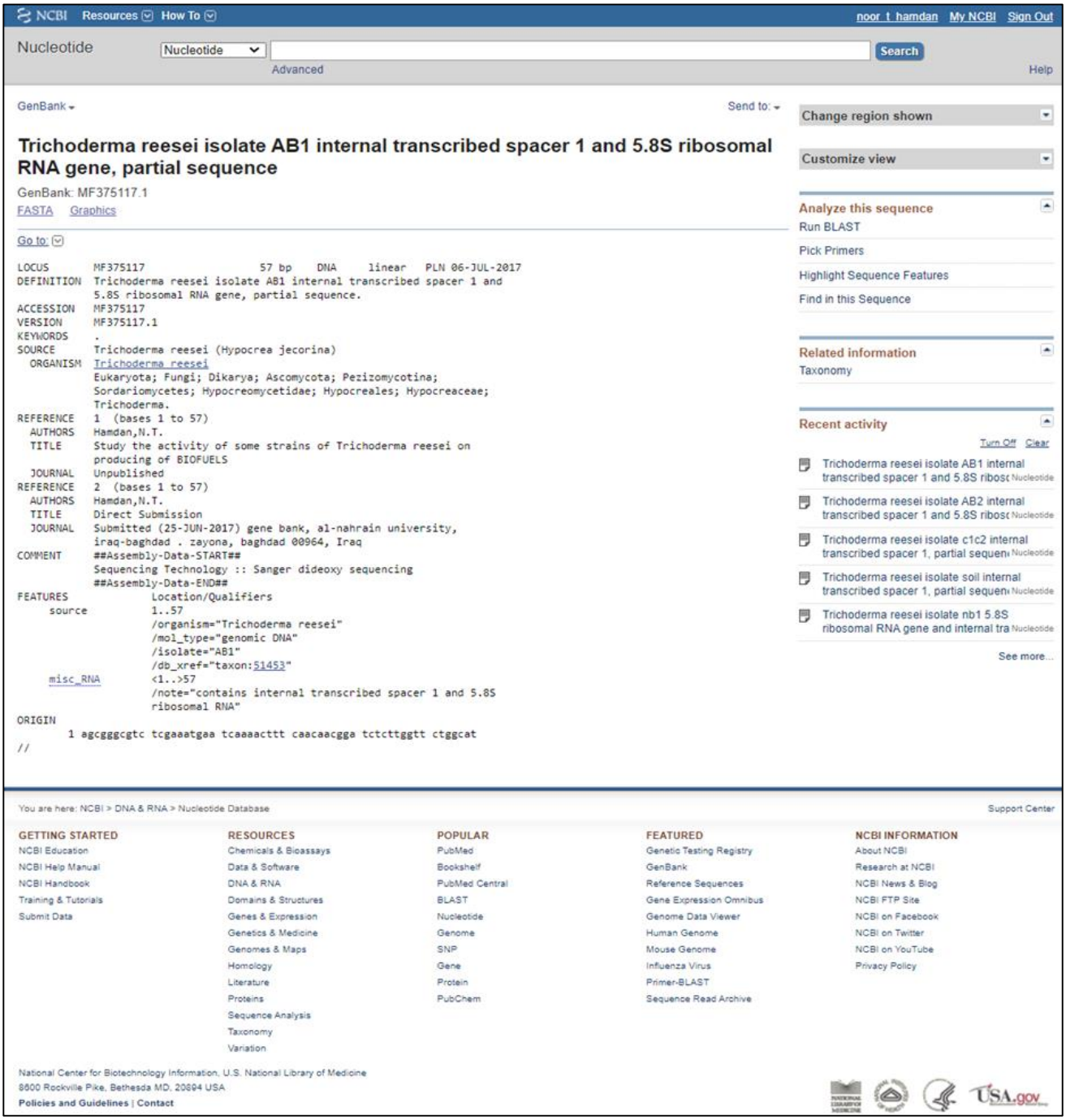

Fig. 8: Iraqi strain-4 registered as *T. reesei* AB1.

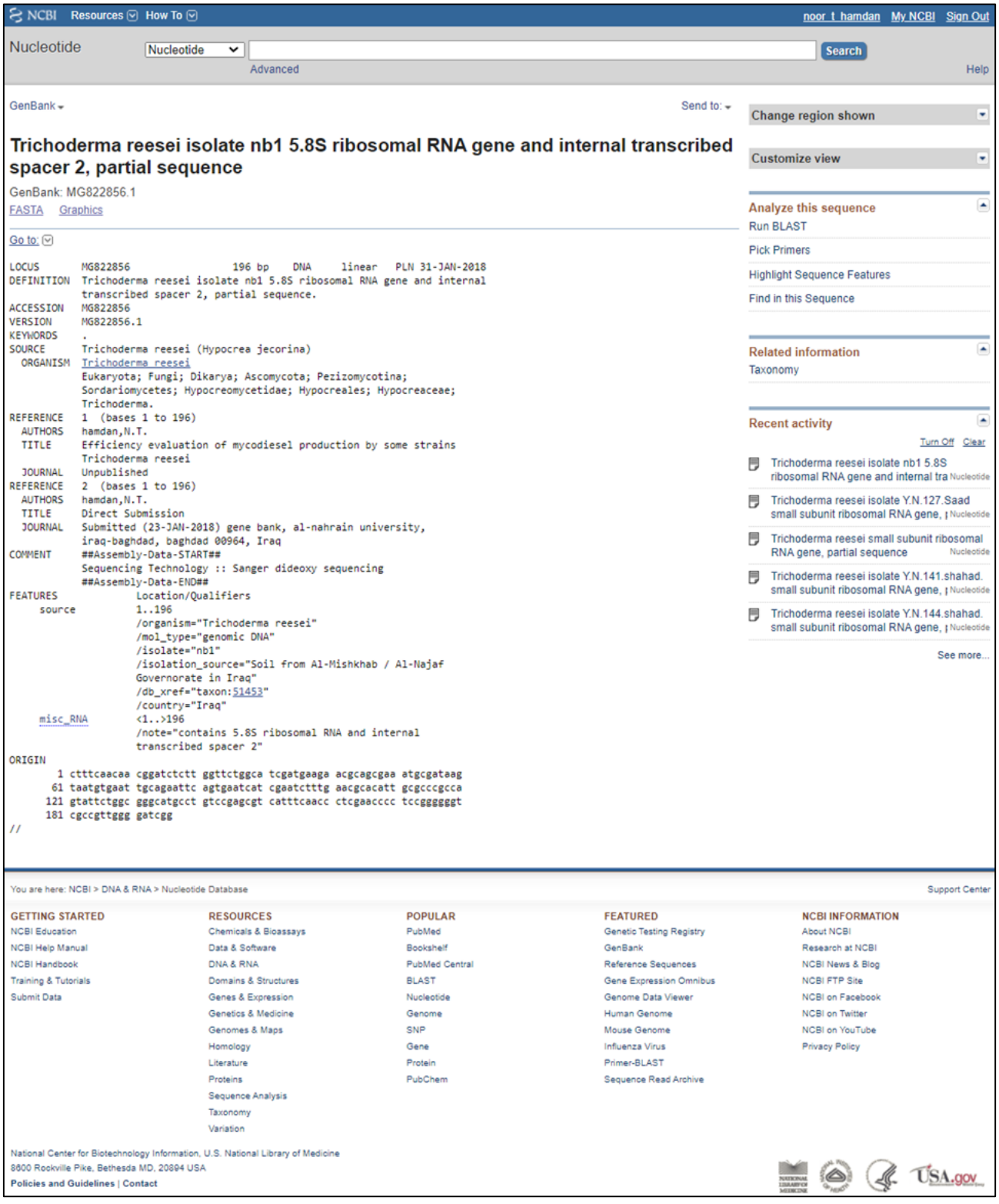

Fig. 9: Iraqi strain-5 registered as *T. reesei* Nb1.

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

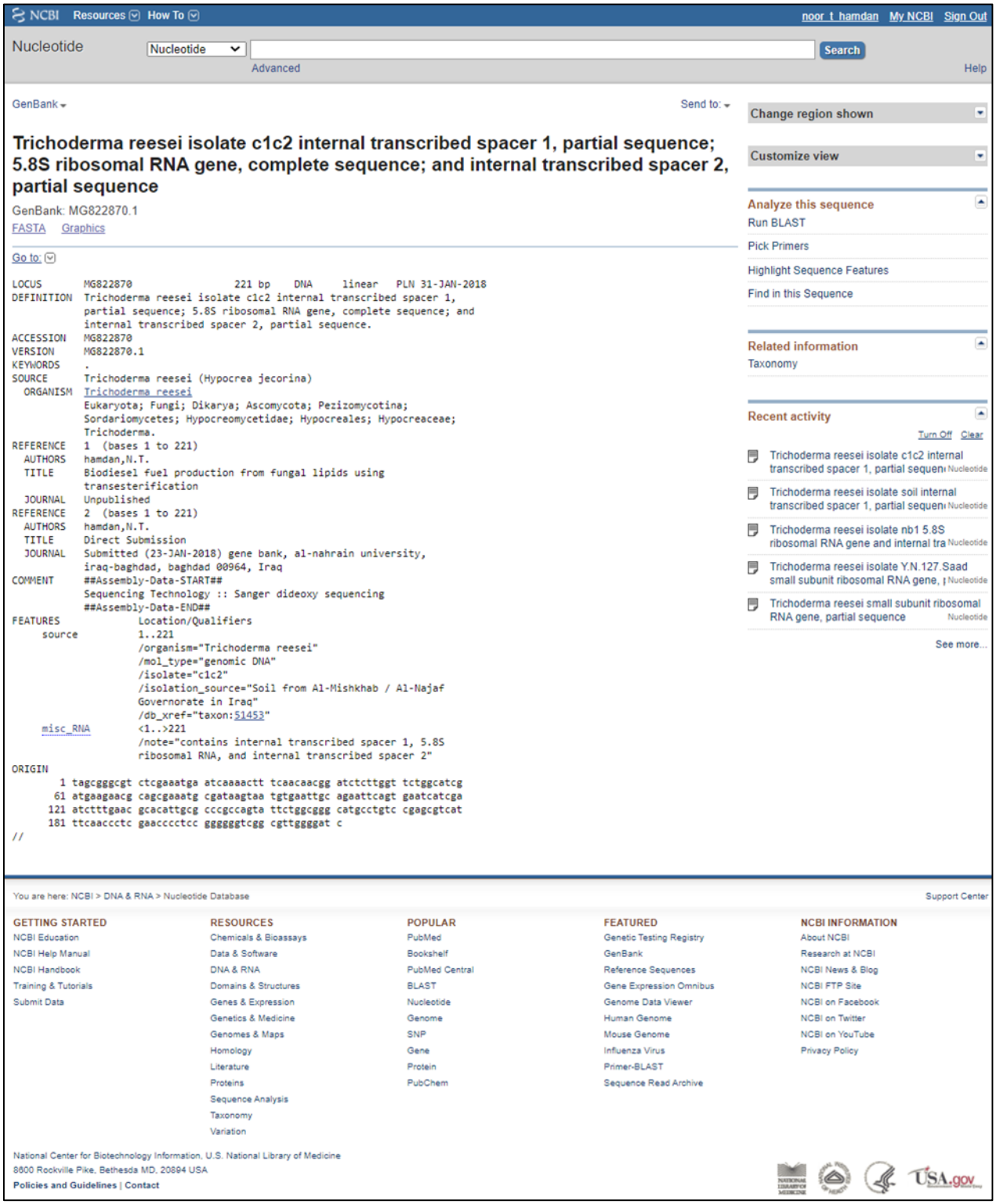

Fig. 11: Iraqi strain-7 registered as *T. reesei* C1C2

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.

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تسجيل عزلات العراقية الجديدة لفطريات *Trichoderma reesei*

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قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية

معلومات البحث:
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الكلمات المفتاحية:
التشخيص، عزلات فطرية جديدة، مدينة بنجوين، تربة رايزوسفير.

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 التقانة الحيوية) لإول مرة في العراق.

معلومات المؤلف

الايميل:
الموبايل: