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Original Article

Morphological and molecular identification of *Cladosporium sphaerospermum* isolates collected from tomato plant residues

Identificação morfológica e molecular de isolados de *Cladosporium sphaerospermum* coletados de resíduos de plantas de tomate

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Abstract

This study was conducted at the Agriculture College University of Karbala, Iraq to isolate and morphologically and molecularly diagnose thirteen *Cladosporium* isolates collected from tomato plant residues present in desert regions of Najaf and Karbala provinces, Iraq. We diagnosed the obtained isolates by PCR amplification using the ITS1 and ITS4 universal primer pair followed by sequencing. PCR amplification and analysis of nucleotide sequences using the BLAST program showed that all isolated fungi belong to *Cladosporium sphaerospermum*. Analysis of the nucleotide sequences of the identified *C. sphaerospermum* isolates 2, 6, 9, and 10 showed a genetic similarity reached 99%, 98%, 99%, and 99%, respectively, with those previously registered at the National Center for Biotechnology Information (NCBI). By comparing the nucleotide sequences of the identified *C. sphaerospermum* isolates with the sequences belong to the same fungi and available at NCBI, it was revealed that the identified *C. sphaerospermum* isolates 2, 6, 9, and 10 have a genetic variation with those previously recorded at the National Center for Biotechnology Information (NCBI); therefore, the identified sequences of *C. sphaerospermum* isolates have been registered in GenBank database (NCBI) under the accession numbers MN896004, MN896107, MN896963, and MN896971, respectively.

Keywords: *Cladosporium sphaerospermum*, PCR amplification, ITS1 and ITS4, fungi.

Resumo

Este estudo foi conduzido na Agriculture College University of Karbala, Iraque, para isolar e diagnosticar morfológica e molecularmente treze isolados de *Cladosporium* coletados de resíduos de plantas de tomate presentes nas regiões desérticas das províncias de Najaf e Karbala, no Iraque. Diagnosticamos os isolados obtidos por amplificação por PCR usando o par de *primers* universais ITS1 e ITS4 seguido de sequenciamento. A amplificação por PCR e a análise de sequências de nucleotídeos usando o programa BLAST mostraram que todos os fungos isolados pertencem a *Cladosporium sphaerospermum*. A análise das sequências de nucleotídeos dos isolados 2, 6, 9 e 10 de *C. sphaerospermum* identificados mostrou similaridade genética de 99%, 98%, 99% e 99%, respectivamente, com aqueles previamente registrados no *National Center for Biotechnology Informações* (NCBI). Ao comparar as sequências de nucleotídeos dos isolados de *C. sphaerospermum* identificados com as sequências pertencentes aos mesmos fungos e disponíveis no NCBI, foi revelado que os isolados 2, 6, 9 e 10 de *C. sphaerospermum* identificados têm variação genética com aqueles anteriormente registrados no *National Center for Biotechnology Information* (NCBI). Portanto, as sequências identificadas de isolados de *C. sphaerospermum* foram registradas no banco de dados GenBank (NCBI) sob os números de acesso MN896004, MN896107, MN896963 e MN896971, respectivamente.

Palavras-chave: *Cladosporium sphaerospermum*, amplificação por PCR, ITS1 e ITS4, fungos.

1. Introduction

Fungi exist in all environmental systems and have different effects between them as well as with the other organisms (Engelthaler & Litvintseva, 2020). *Cladosporium sphaerospermum* was first described by Albert Julius Otto Penzig in 1886 after isolating from the rotting leaves and branches of citrus (*Citrus aurantium*) (Bensch et al., 2010). *C. sphaerospermum* is a cosmopolitan fungus and present in many internal and external environments because of its airborne and rapid spread nature (Zalar et al., 2007; Bensch et al., 2010). *C. sphaerospermum* was isolated from very salty environments in Mediterranean and tropical as well as from temperate climates (Zalar et al., 2007).

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Within the buildings, this fungus can extensively grow on the interior surfaces of windows, bathrooms, and kitchens (Hocking et al., 1994; Adan et al., 2011).

C. sphaerospermum can be isolated from decaying leaves, stems of woody and herbaceous plants, vegetables, and fruits (Dugan et al., 2018). In India, Avasthi et al. (2016) reported that *C. sphaerospermum* is the causal agent of leaf spot disease on *Aloe vera* plants. Plant tissues can be attacked by *C. sphaerospermum* causing dead or senescent as well as it can cause spoilage of various post-harvested fruits and vegetables, e.g. citrus, strawberry, apple, and tomato fruits leading to a decrease in nutritional and marketing values of these fruits (Qiu-Xia et al., 2008).

There are very few reports implicating this species as a disease agent in humans (Hocking et al., 1994). *C. sphaerospermum* is a very rare cause of human sickness, but it can cause some different types of infections, including, sinus, skin, eye, and brain infections (Zalar et al., 2007; Maduri et al., 2015). It was reported that a female patient showed swelling on the dorsum of her hand and after testing with Lactophenol cotton blue, and Grocott's methenamine silver stain revealed the presence of hyphae compatible with the fungus *C. sphaerospermum* (Maduri et al., 2015). Another case reported where *C. sphaerospermum* caused cerebral phaeohyphomycosis but the patient was successfully treated and the symptoms were reduced (Lai et al., 2013). It was also mentioned that *C. sphaerospermum* can produce different allergenic compounds but has not been known to be able produce significant mycotoxins (Ng et al., 2012).

Morphological identification of fungi is still difficult and insufficient for identifying because some of the characteristics of the species are very similar to the characteristics in *Cladosporium* species (Dugan et al., 2008). Therefore, for accurate identification, various molecular methods have been successfully applied to diagnose many fungi such as *Fusarium* spp., *Rhizoctonia solani*, *Aspergillus* spp. (Salem et al., 2019; Abedalred et al., 2019). Among the molecular techniques used, the polymerase chain reaction (PCR) has been successfully applied to diagnose *Cladosporium* species using the internal transcribed spacer (ITS) that is the universal barcode of fungi and can be used for identification of *Cladosporium* spp. (Hensel et al., 2020; Han et al., 2020). ITS sequences are commonly used to construct the phylogenetic trees and identify fungi (Salem et al., 2019; Ao et al., 2020). Morphological identification and sequencing of ITS can be used to identify *Cladosporium* isolates to the species level. Therefore, the objective of this research was to diagnose some *Cladosporium* species isolated from tomato plant residues (roots and stems) using morphological and molecular characteristics.

2. Materials and Methods

2.1. Sampling and fungal isolation

Samples were collected from tomato residues (roots and stems) found in desert region farms in Najaf and Karbala provinces, Iraq and then brought to the laboratory of Plant Virology at Agriculture College in the Plant Protection Department, Karbala University for fungal isolation. Collected samples were washed by water, cut into small parts, sterilized with sodium hypochlorite solution (NaOCl 1%) for 2 min, and rinsed with distilled water. These pieces were dried using sterilized filter papers and transferred to the Petri dishes containing Potato Dextrose Agar medium (200 g potato, 20 g dextrose, 15 g agar, and 1 liter of water) supplemented with chloramphenicol antibiotic (200 mg/L). Inoculated Petri dishes were kept at 25 ± 2 °C for 3 days. Appeared fungal colonies were purified on PDA medium and initially diagnosed using the morphological characteristics. These isolates were also molecularly diagnosed by the PCR technology and determining the sequences generated from the PCR-amplified products according to the method given later.

Genomic DNA extraction, PCR amplification and sequencing of ITS region

From each isolate, about 100 mg of a 7-day-old colony were transferred using a sterile scalpel into an Eppendorf tube to extract DNA using a fungal/ bacteria DNA extraction kit (Zymo Research, Cat. No. D6005, USA) following the manufacturer's instructions. The purity ($A_{260/280}$) and concentration (A_{260}) of DNA extracted from each fungal isolate were determined using a UV spectrophotometer (Thermo Scientific, Germany). Thereafter, DNA was stored at -20°C until use.

The ITS (internal transcribed spacer) region of *C. sphaerospermum* isolates was amplified using the primer pair ITS1 (TCCGTTGGTGAACCAGCGG) and ITS4 (TCCTCCGCTTATGATA TGC) (White et al., 1990) and the PCR mixture shown in Table 1. PCR amplification carried out using the following conditions: initial denaturation for 1 min at 94 °C followed by 35 cycles each consists of final denaturation at 94 °C for 30 s, annealing temperature for 30s at 55 °C, initial extension for 1 min at 72 °C, and final extension for 5 min at 72 °C (White et al., 1990). PCR products were run on a 1% agarose gel for 150 min at 80V and 400 mA, ethidium bromide stained and visualized under UV illumination. Gel pictures were taken using gel documentation system, Vilber Lourmat, Taiwan.

For nucleotide sequencing, the PCR products were gel-purified by the FavorPrep PCR Purification Kit (Taiwan, Favorgen, Cat. No. FAGCK 001) and sent with the ITS1 and ITS4 primer pair to the Macrogen DNA sequencing service in South Korea. PCR products were sequenced in

Table 1. Components used for PCR amplification.

Component	Volume
1× PCR buffer	2 µL
template DNA (30 ng/µL)	3 µL
dNTPs (2 mM)	2 µL
Taq polymerase	1 unit
Forward primer (10 pmol)	1 µL
Reverse primer (10 pmol)	1 µL
Nuclease-free sterile distilled water	10 µL

both directions using the ITS1 and ITS4 primer pair. The nucleotide sequences were then aligned to compare with the sequences belonged to the *C. sphaerospermum* isolates deposited in NCBI using BLAST (Basic Local Alignment Search Tool) (Zhang et al., 2012). Multiple sequence alignments and construction of the phylogenetic trees were done using the neighbor-joining method using MEGA6 software (Tamura et al., 2013).

3. Results and discussion

3.1. Morphological and molecular identification of C. sphaerospermum isolates

C. sphaerospermum isolates were preliminary diagnosed based to the morphological characteristics. Colonies grown on PDA medium were dark olivaceous to greenish-black in color and velvety. The fungal conidiophores were closely septated with thick and darkened septa as well as branched. However, conidia were in branched-chain, small terminal conidia were apex rounded, brown to dark brown, globose to subglobose, base rounded to slightly attenuated each with a prominent scar at the end of the ramoconidia and conidium. The morphological characteristics of the isolates demonstrated that these fungal isolates belong to *C. sphaerospermum*. Bensch et al. (2018) reported that the main characteristics of *C. sphaerospermum* is the presence of numerous globose to subglobose terminal conidia, almost smooth to verruculose and conidiophores branched.

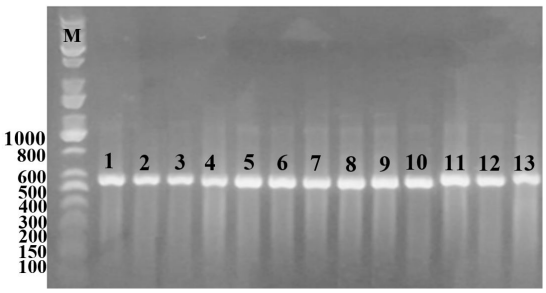

Figure 1. 1% Agarose gel electrophoresis of PCR products amplified using the primer pair ITS1 and ITS4 from the *C. sphaerospermum* isolates (1-13) obtained from tomato plant residues collected from different regions of Najaf and Karbala provinces. **M:** 1Kbp DNA Ladder (Promega, USA).

Avasthi et al. (2016) and Yew et al. (2016) also stated similar results regarding morphological characteristics of *C. sphaerospermum*.

The morphological identification of the *C. sphaerospermum* isolates were also confirmed by PCR amplification of DNA extracted from these isolates. PCR amplification showed the PCR products with sizes ranging between 550-600bp by the universal primer pair (ITS1-ITS4) (Figure 1).

The samples were sequenced in both directions and a BLAST search revealed that all the studied isolates belong to the fungus *C. sphaerospermum*. By comparing the partial sequence of ITS region of the *C. sphaerospermum* isolates (1-13) with the other *C. sphaerospermum* isolates previously available in GenBank, results showed that the similarity percentage of the nucleotide sequences ranged between 97%-100%. It was also noted that the nucleotide sequences of the ITS region (ITS1, 5.8S rDNA, and ITS4) amplified from the *C. sphaerospermum* isolates 5, 11, and 12, as well as 8 and 13, had a similarity of 100% (Table 2, Figure 2 and Figure 3). Comparison of the partial ITS region of the *C. sphaerospermum* isolates 1, 3, 4, 5, 7, 8, 11, 12, and 13 with formerly registered isolates in GenBank showed that the nearest nucleotide sequence similarity (100%) with *C. sphaerospermum* already isolated and identified in Japan (AB572902), Brazil (MK913353), China (MK356737), China (MK281560), China (MK356729), Iran (KY046241), China (MK281560), China (MK281560), and Iran (KY046241), respectively.

Analysis of the *C. sphaerospermum* isolate 2 showed distinct differences in some nucleotide positions with the *C. sphaerospermum* isolates previously identified and registered in GenBank (Figure 2). This isolate (2) had a 98%-99% nucleotide sequence similarity with the other isolates available at NCBI (Figure 3).

A comparison of the nucleotide sequence obtained from this isolate (2) with those recorded in GenBank revealed that the highest similarity was 99% with the *C. sphaerospermum* isolates previously diagnosed in China (MK952405 and KU350727). Whereas, minimum nucleotide sequence similarity (98%) for this *C. sphaerospermum* isolate was observed with the isolates already identified in China (MG787259), Japan (LC317549, LC317550, LC375369, and LC317551), and Germany (MH482916) (Figure 4). The constructed phylogenetic tree showed that the *C. sphaerospermum* isolate 2 appeared in a separate clade

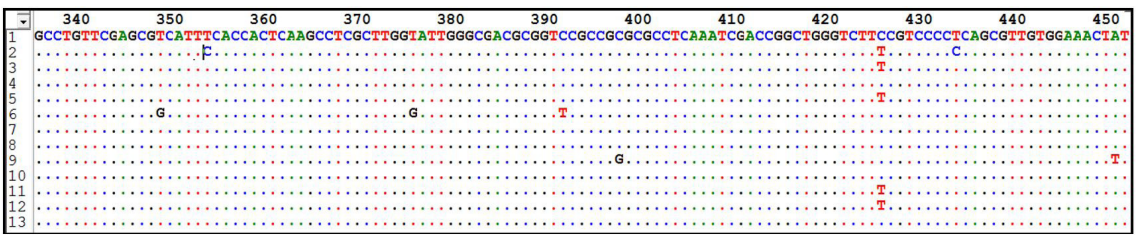

Figure 2. The similarity and difference in the nucleotide sequences of the *C. sphaerospermum* isolates (1-13) identified in the present study. Similar nucleotides are stated in dots. Numbers given on the right side of the figure represent the nucleotide sequences obtained from the *C. sphaerospermum* isolates.

from *C. sphaerospermum* isolates previously identified and recorded in GenBank (Figure 5).

By comparing the nucleotide sequence obtained from the *C. sphaerospermum* isolate 6 with the other isolates registered in NCBI indicated that there was a clear similarity ranges between 95%-98% (Figure 6). The nearest sequence similarity was 98% with the *C. sphaerospermum* isolates already isolated in China (GQ901954), India (MF380926), and Iran (Iran). In contrast, the lowest homology percentage of 95% was noticed with the other isolates of *C. sphaerospermum* identified in China, Australia, the USA, as well as New Zealand. To determine the genetic relationship among these, the sequence of the *C. sphaerospermum* isolate (6) with the sequences of those *C. sphaerospermum* isolates available in GenBank were used for constructing a phylogenetic tree. The phylogenetic tree showed the appearance of this isolate 6 in a separate clade apart from the *C. sphaerospermum* isolates deposited in GenBank (Figure 7).

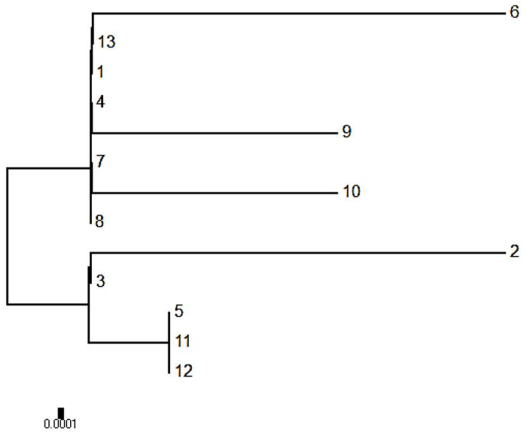

Figure 3. A phylogenetic tree constructed by the neighbor-joining method depending on a comparison of the nucleotide sequences obtained from the *C. sphaerospermum* isolates (1-13).

As shown in Figure 8 and Figure 9, the *C. sphaerospermum* isolate 9 investigated in this study had clear differences in the nucleotide sequences ranged from 97%-99% with the *C. sphaerospermum* isolates previously registered in NCBI. *C. sphaerospermum* 9 revealed that the highest nucleotide sequence similarity was 99% with the *C. sphaerospermum* isolates already identified in Bangladesh (MH393182), Iran (KY046241), France (MH393182), and Australia (EF568045). Minimum similarity (97%) for this *C. sphaerospermum* isolate 9 was found with *C. sphaerospermum* isolates

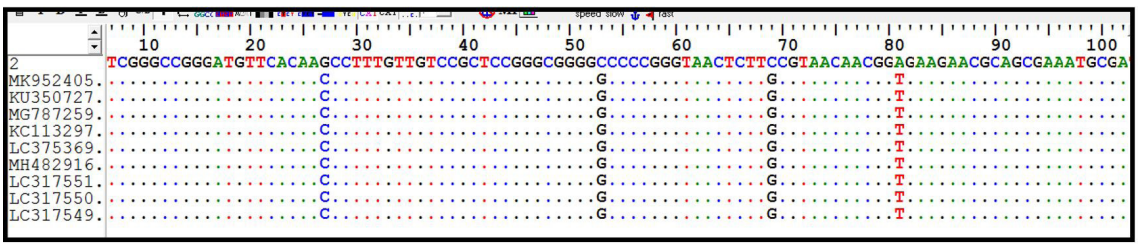

Figure 4. The similarity and difference of nucleotide sequences in the *C. sphaerospermum* isolate 2, identified in this study, with those *C. sphaerospermum* isolates previously registered in NCBI. Identical nucleotides are given in dots.

Table 2. Similarity rates among the *C. sphaerospermum* isolates identified in this study.

													Isolate no.
												-	**1**
											-	98	**2**
										-	99	99	**3**
									-	99	98	100	**4**
								-	99	100	98	99	**5**
							-	98	99	98	97	97	**6**
						-	99	99	100	99	98	100	**7**
					-	100	99	99	100	99	98	100	**8**
				-	99	99	98	98	98	99	99	97	**9**
			-	98	99	99	98	98	99	99	97	99	**10**
		-	98	98	99	99	98	100	99	100	98	99	**11**
	-	100	98	98	99	99	98	100	99	100	98	99	**12**
-	99	99	99	99	100	100	99	100	99	98	98	100	**13**
13	**12**	**11**	**10**	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	

registered in Australia (KU059910), Chin (KP269061), and Netherlands (MF473024).

A BLAST search was also done on the identified sequence of the *C. sphaerospermum* isolate 10 and analysis of nucleotide sequence alignment was carried out with the other sequences deposited in GenBank. Results showed that the sequence obtained from *C. sphaerospermum* isolated in the present study had a high degree of nucleotide identity (99%) with *C. sphaerospermum* isolates formerly isolated from Malaysia (JX966565 and JX966567), India (MF46882), Australia (EF568045) and China (KX885028 and KT852577). Whereas, the lower identity (98%) was revealed with *C. sphaerospermum* isolates previously identified in Japan (LC317550 and LC317551) (Figure 10). As illustrated in Figure 11, construction of the phylogenetic tree showed clustering of *C. sphaerospermum* 10 with the other *C. sphaerospermum* isolates already identified in Australia (EF568045), India (MF46882), and Malaysia (JX966565 and JX966567).

The new *C. sphaerospermum* isolates identified in this study may be more devastating for some economic plants. In this study, PCR was successfully used to identify different isolates of *C. sphaerospermum* because of its high sensitivity and accuracy in the identification of many organisms, including fungi such as *Fusarium oxysporium*, *Alternaria alternata*, *F. solani*, *Trichoderma* spp., *R. solani*, and *Aspergillus* spp. (Al-Abedy et al., 2018; Khan et al., 2018).

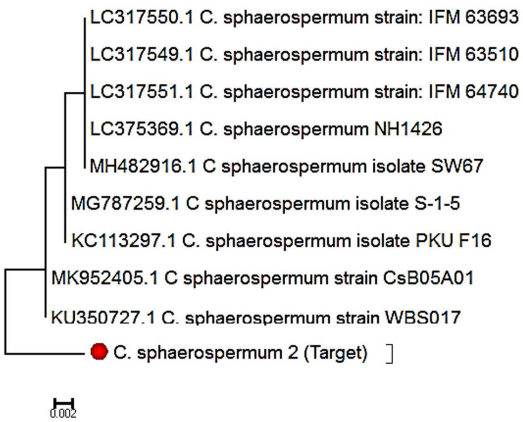

Figure 5. A phylogenetic tree was generated using the neighbor-joining method which shows the genetic relationship between *C. sphaerospermum* 2 (as indicated in red circle) and the other *C. sphaerospermum* isolates deposited in GenBank (NCBI)

Fungal identification has increased dramatically with the introduction of some molecular tools such as PCR. PCR and sequencing have widely invested as a quick and precise technique to identify many pathogenic and non-pathogenic plant fungi, bacteria, and viruses. Molecular markers have a role in eliminating the limitations in identification based on morphological characteristics (Henry et al., 2000; AL-Abedy et al., 2020). Despite this, morphological characters are being used in sorting fungal isolates into smaller groups but it requires time, efforts and expertise to be a taxonomist for accurate identification, particularly up to species level (Zhang et al., 2012; Huang et al., 2006; Abdullah et al., 2019). Morphological characteristic also depends on many other factors, e.g. moisture, pH, light, and composition of the growth medium that can change the color, shapes, and sizes of spores as well as the fungal colonies growing pattern (Zhang et al., 2012; Huang et al., 2006).

In Malaysia, studies on *Fusarium* spp. are often based on some morphological characters that could lead to incorrect identification (Hsuan et al., 2011). Previous studies revealed some limitations related to the use of morphological characters for the identification of some fungi, e.g. species in the *G. fujikuroi* species complex as some species, i.e., *F. sacchari*, *F. subglutinans, F. verticillioides, F. proliferatum, F. fujikuroi*, and *F. andiyazi*, have very close morphological characters (Hsuan et al., 2011). It was also noticed through the re-diagnosis by using the PCR technique that there was a mistake in the morphological identification of several fungi identified in previous studies like *Fusarium* spp., e.g., *F. verticillioides,* and *Fusarium subglutinans* (Hsuan et al., 2011).

Differences in the ITS (Internal Transcribed Spacer) regions of the rDNA (ribosomal DNA), repeat units are present in copies in fungi that can be found and selected by PCR (Al-Fadhal et al., 2018). PCR amplification of the ITS region has shown a high efficiency in identifying several fungi, e.g. *Pythium* spp., *R. solani,* and *F. verticillioides* (Hsuan et al., 2011; Al-Fadhal et al., 2018; AL-Abedy et al., 2018). It is concluded that the newly identified *C. sphaerospermum* isolates 2, 6, 9, and 10 were genetically different from the other *C. sphaerospermum* isolates and not previously recorded in NCBI; therefore, they were registered

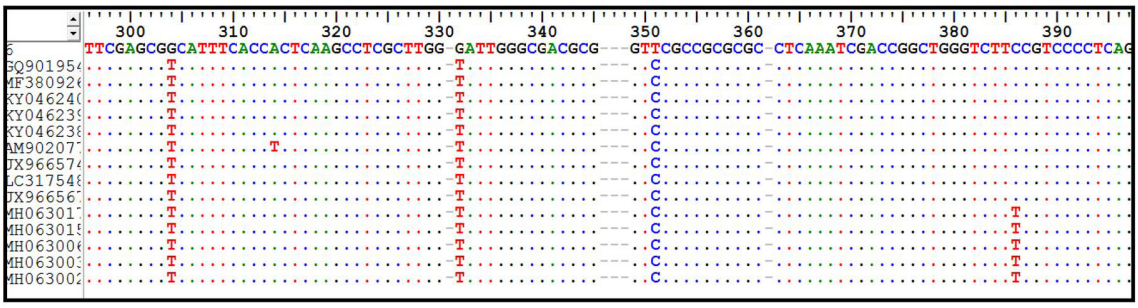

Figure 6. Nucleotide sequence alignment of the ITS region of the *C. sphaerospermum* isolate 6 identified in the current study and the other isolates already recorded in NCBI.

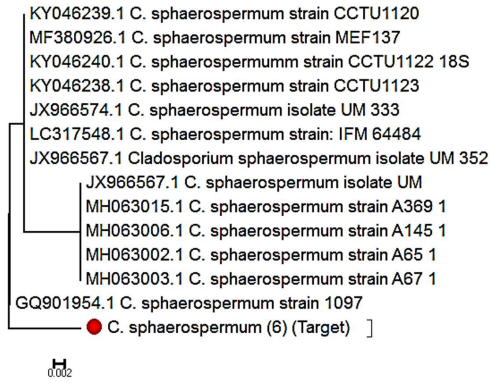

Figure 7. The Neighbor-Joining tree analysis shows the genetic relationship of the *C. sphaerospermum* isolate 6, investigated in this study, and the other isolates already recorded in NCBI.

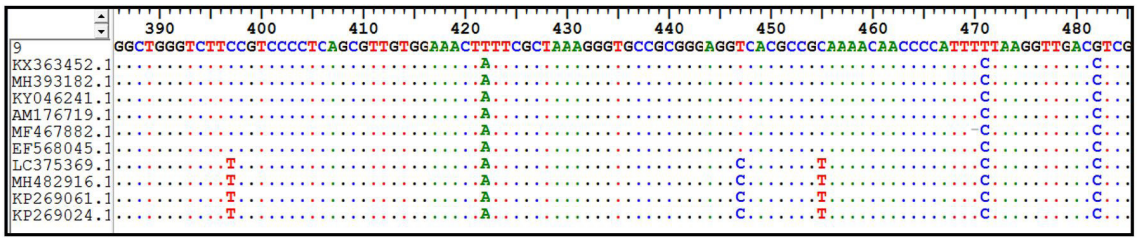

Figure 8. Nucleotide sequence alignment of the ITS (ITS1, 5.8S rDNA and ITS4) region of the *C. sphaerospermum* isolate 9 and the other isolates already recorded in NCBI.

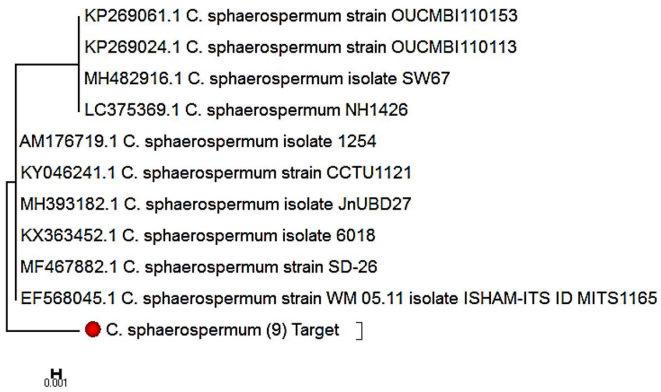

Figure 9. A phylogenetic tree shows the genetic relationship between the *C. sphaerospermum* isolate 9 investigated in this study as indicated by red dote (●), and the *C. sphaerospermum* isolates available in NCBI.

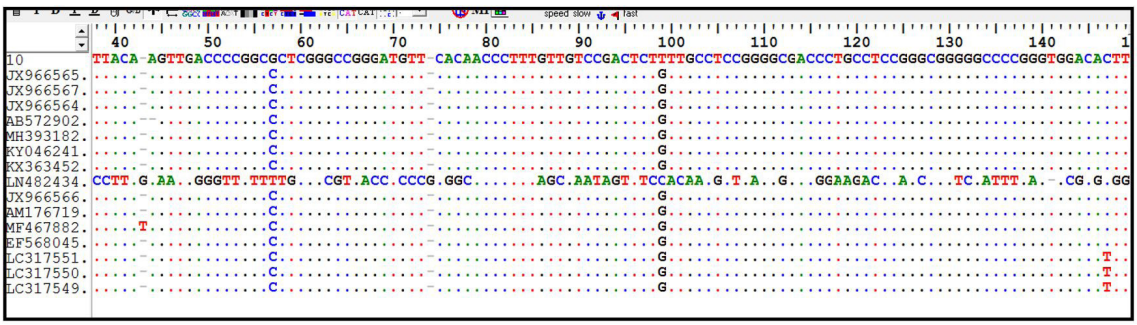

Figure 10. Nucleotide sequence alignment of the ITS (ITS1, 5.8S rDNA and ITS4) region of the *C. sphaerospermum* isolate 10 and the other isolates already recorded in NCBI.

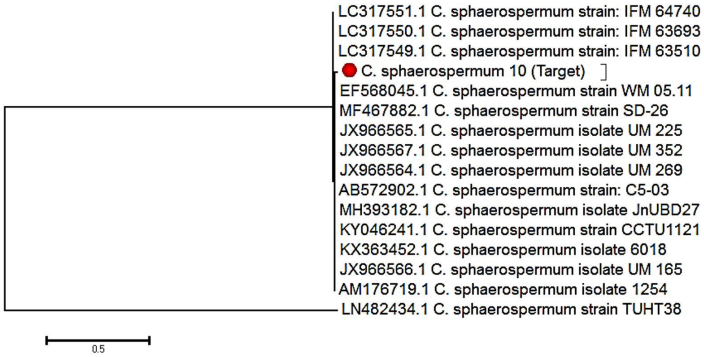

Figure 11. A phylogenetic tree constructed using the neighbor-joining method depending on the comparison of the obtained nucleotide sequence of *C. sphaerospermum* isolate 10 as indicated by red dote (●) with those of *C. sphaerospermum* isolates deposited in NCBI.

in GenBank under the accession numbers: MN896004, MN896107, MN896963, and MN896971, respectively.

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References

ABDULLAH, A.A., DEWAN, M.M., and AL-ABEDY, A.N. (2019). Genetic variation of some isolates of *Cladosporium sphaerospermum* isolated from different environments. *IOP Conference Series: Earth and Environmental Science*, Vol. 388, No. 1, p. 012016. http://dx.doi.org/10.1088/1755-1315/388/1/012016.

ABEDALRED, E.M., ISMAIL, W.M., ABDULMOOHSIN, R.G. and AL-KARHI, M.A. (2019). First molecular identification of Fusarium *fujikuroi* causing pollen rot of palm trees (*Phoenix dactylifera* L.) in Iraq and evaluation efficacy of some nanoparticles against it. *IOP Conference Series: Earth and Environmental Science*, vol. 388, No. 1, p. 012007. http://dx.doi.org/10.1088/1755-1315/388/1/012007.

ADAN, O.C., HUININK, H.P. and BEKKER, M. (2011). Water relations of fungi in indoor environments. In: O.C.G. ADAN and R.A. SAMSON. *Fundamentals of mold growth in indoor environments and strategies for healthy living*. Wageningen: Wageningen Academic Publishers. http://dx.doi.org/10.3920/978-90-8686-722-6_2.

AL-ABEDY, A.N., AL-FADHAL, F.A., KAREM, M.H., AL–MASOUDI, Z. and AL-MAMOORI, S.A., 2018. Genetic variability of different isolates of *Rhizoctonia solani* Kühn isolated from Iranian imported potato tubers (*Solanum tuberosum* L.). *International Journal of Agricultural and Statistics Sciences*, vol. 14, no. 2, pp. 587-598.

AL-ABEDY, A.N., AL-JANABI, R.G., AL-TMEME, Z.A., SALIM, A.T., and ASHFAQ, M., 2020. Molecular characterization of novel isolates of *Rhizoctonia solani, Trichoderma atroviride* and *Fusarium* spp. Isolated from different plants and cutting woods in Iraq. *Pakistan Journal of Botany*, vol. 52, no. 3, pp. 1-10.

AL-FADHAL, F.A., AL-ABEDY, A.N. and AL-JANABI, M.M., 2018. Molecular identification of novel isolates of *Rhizoctonia solani* Kühn and *Fusarium* spp.(Matsushima) isolated from petunia plants (*Petunia hybrida* L.). *Plant Archives*, vol. 18, no. 1, pp. 703-711.

AO, T., DEB, C.R. and RAO, S.R., 2020. Molecular strategies for identification and characterization of some wild edible mushrooms of Nagaland, India. *Molecular Biology Reports*, vol. 47, no. 1, pp. 621-630. http://dx.doi.org/10.1007/s11033-019-05170-2. PMid:31754929.

AVASTHI, S., GAUTAM, A. and BHADAURIA, R., 2016. First report of Cladosporium sphaerospermum causing leaf spot disease of Aloe vera in India. *Journal of Crop Protection*, vol. 5, no. 4, pp. 649-654.

BENSCH, K., GROENEWALD, J.Z., DIJKSTERHUIS, J., STARINK-WILLEMSE, M., ANDERSEN, B., SUMMERELL, B.A., SHIN, H.D., DUGAN, F.M., SCHROERS, H.J., BRAUN, U. and CROUS, P.W., 2010. Species and ecological diversity within the Cladosporium cladosporioides complex (Davidiellaceae, Capnodiales). *Studies in Mycology*, vol. 67, pp. 1-94. http://dx.doi.org/10.3114/sim.2010.67.01. PMid:20877444.

DUGAN, F.M., BRAUN, U., GROENEWALD, J.Z. and CROUS, P.W., 2008. Morphological plasticity in *Cladosporium sphaerospermum*. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, vol. 21, no. 9, pp. 9-16. http://dx.doi.org/10.3767/003158508X334389. PMid:20396574.

ENGELTHALER, D.M. and LITVINTSEVA, A.P. (2020). Genomic epidemiology and forensics of fungal pathogens. In: B. BUDOWLE, S. SCHUTZER and S. MORSE. *Microbial forensics*. London: Academic Press, pp. 141-154. http://dx.doi.org/10.1016/B978-0-12-815379-6.00010-6.

HAN, X., DAI, Y., HU, M., XIAO, R., ZHANG, S., LI, J., WANG, Z., HU, X. and GU, Z., 2020. Investigation of multiple pathogens in black-spotted *Dendrobium officinale* based on culture-independent and dependent methods. *Journal of Biobased Materials and Bioenergy*, vol. 14, no. 2, pp. 249-257. http://dx.doi.org/10.1166/jbmb.2020.1959.

HENRY, T., IWEN, P.C. and HINRICHS, S.H., 2000. Identification of Aspergillus species using internal transcribed spacer regions 1 and 2. *Journal of Clinical Microbiology*, vol. 38, no. 4, pp. 1510-1515. http://dx.doi.org/10.1128/JCM.38.4.1510-1515.2000. PMid:10747135.

HENSEL, M., MEASON-SMITH, C., PLUMLEE, Q.D., MYERS, A.N., COLEMAN, M.C., LAWHON, S., RODRIGUES HOFFMANN, A. and RECH, R.R., 2020. Retrospective analysis of Aetiological agents associated with Pulmonary Mycosis secondary to Enteric Salmonellosis in Six Horses by Panfungal Polymerase Chain Reaction. *Journal of Comparative Pathology*, vol. 174, pp. 1-7. http://dx.doi.org/10.1016/j.jcpa.2019.10.002. PMid:31955794.

HOCKING, A.D., MISCAMBLE, B.F. and PITT, J.I., 1994. Water relations of *Alternaria alternata, Cladosporium cladosporioides, Cladosporium sphaerospermum, Curvularia lunata* and *Curvularia pallescens. Mycological Research*, vol. 98, no. 1, pp. 91-94. http://dx.doi.org/10.1016/S0953-7562(09)80344-4.

HSUAN, H.M., SALLEH, B. and ZAKARIA, L., 2011. Molecular identification of Fusarium species in *Gibberella fujikuroi* species complex from rice, sugarcane and maize from Peninsular Malaysia. *International Journal of Molecular Sciences*, vol. 12, no. 10, pp. 6722-6732. http://dx.doi.org/10.3390/ijms12106722. PMid:22072914.

HUANG, A., LI, J.W., SHEN, Z.Q., WANG, X.W. and JIN, M., 2006. High-throughput identification of clinical pathogenic fungi by hybridization to an oligonucleotide microarray. *Journal of Clinical Microbiology*, vol. 44, no. 9, pp. 3299-3305. http://dx.doi.org/10.1128/JCM.00417-06. PMid:16954264.

KHAN, M., WANG, R., LI, B., LIU, P., WENG, Q. and CHEN, Q., 2018. Comparative evaluation of the LAMP assay and PCR-based assays for the rapid detection of *Alternaria solani. Frontiers in Microbiology*, vol. 9, no. 2, pp. 1-11. http://dx.doi.org/10.3389/fmicb.2018.02089. PMid:30233554.

LAI, C.C., LIN, W.R., CHEN, C.Y., CHANG, K., LU, P.L., CHEN, Y.H. and LIN, C.Y., 2013. Acute meningitis caused by *Cladosporium sphaerospermum. The American Journal of the Medical Sciences*, vol. 346, no. 6, pp. 523-525. http://dx.doi.org/10.1097/MAJ.0b013e3182a59b5f. PMid:24263083.

MADURI, A., PATNAYAK, R., VERMA, A., MUDGETI, N., KALAWAT, U. and ASHA, T., 2015. Subcutaneous infection by *Cladosporium sphaerospermum*-A rare case report. *Indian Journal of Pathology & Microbiology*, vol. 58, no. 3, pp. 406-407. http://dx.doi.org/10.4103/0377-4929.162936. PMid:26275282.

NG, K. P., YEW, S. M., CHAN, C. L., SOO-HOO, T. S., NA, S. L., HASSAN, H, NGEOW, Y.F., HOH, C.-C., LEE, K.-W. and YEE, W.-Y., 2012. Sequencing of Cladosporium sphaerospermum, a dematiaceous fungus isolated from blood culture.. *Eukaryotic Cell*, vol. 11, no. 5, pp. 705-706. http://dx.doi.org/10.1128/EC.00081-12. PMid:22544899.

QIU-XIA, C., CHANG-XING, L., WEN-MING, H., JIANG-QIANG, S., WEN, L. and SHUN-FANG, L., 2008. Subcutaneous phaeohyphomycosis caused by *Cladosporium sphaerospermum. Mycoses*, vol. 51, no. 1, pp. 79-80. PMid:18076601.

SALEM, M.Z., BEHIRY, S.I. and EL-HEFNY, M., 2019. Inhibition of *Fusarium culmorum, Penicillium chrysogenum* and *Rhizoctonia solani* by n-hexane extracts of three plant species as a wood-treated oil fungicide. *Journal of Applied Microbiology*, vol. 126, no. 6, pp. 1683-1699. http://dx.doi.org/10.1111/jam.14256. PMid:30887609.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. and KUMAR, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, vol. 30, no. 12, pp. 2725-2729. http://dx.doi.org/10.1093/molbev/mst197. PMid:24132122.

WHITE, T.J., BRUNS, T.S., LEE, S.B. and TAYLOR, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M.A. INNIS, D.H. GELFAND, J.J. SNINSKY and T.J. WHITE, ed. *PCR protocols: a guide to methods and applications*. New York, NY: Academic Press, Inc., pp. 315-322.

YEW, S.M., CHAN, C.L., NGEOW, Y.F., TOH, Y.F., NA, S.L., LEE, K.W., HOH, C.C., YEE, W.Y., NG, K.P. and KUAN, C.S., 2016. Insight into different environmental niches adaptation and allergenicity from the *Cladosporium sphaerospermum* genome, a common human allergy-eliciting Dothideomycetes. *Scientific Reports*, vol. 6, no. 1, pp. 27008. http://dx.doi.org/10.1038/srep27008. PMid:27243961.

ZALAR, P.D., DE HOOG, G.S., SCHROERS, H.J., CROUS, P.W., GROENEWALD, J.Z. and GUNDE-CIMERMAN, N., 2007. Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. *Studies in Mycology*, vol. 58, pp. 157-183. http://dx.doi.org/10.3114/sim.2007.58.06. PMid:18490999.

ZHANG, S., ZHAO, X., WANG, Y., LI, J., CHEN, X., WANG, A. and LI, J., 2012. Molecular detection of *Fusarium oxysporum* in the infected cucumber plants and soil. *Pakistan Journal of Botany*, vol. 44, no. 4, pp. 1445-1451.