

Molecular characterization of *Trichophyton indotineae* and Its Virulence-Associated Genes (Subtilisin and Fungalsin) in Human Isolates from Basrah, Iraq

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Abstract

Background/Aims

The dermatophytes genome encodes a large number of proteolytic enzymes like proteases which may be associated with persistence of dermatophytosis. This study was designed to assess the presence of the serine protease-encoding subtilisin (SUB1-7) and metalloprotease-encoding fungalsin (MEP3 and MEP4) genes and to evaluate the phylogenetic relationships between Iraqi *Trichophyton indotineae* isolates and global strains.

Subjects and Methods

A total of 150 clinical samples were collected and screened between February 2024 and September 2025. Twenty-five isolates were molecularly confirmed as *T. indotineae*. These isolates were obtained from patients suffering from recurrent and treatment-resistant dermatophytosis. Virulence-associated genes (SUB1–SUB7, MEP3 and MEP4) were detected by PCR using previously published and newly designed primers. Phylogenetic analysis was performed using the Neighbor-Joining method implemented in MEGA (Version 11) software.

Results

PCR results demonstrated successful amplification of most target genes, with all isolates showing 100% positivity for SUB1, SUB2, SUB3, SUB4, SUB6, SUB7, MEP3, and MEP4, whereas SUB5 was detected in 60% of isolates. Sequence alignment and phylogenetic analysis indicated a high degree of genetic similarity between Iraqi and global isolates, characterized by limited nucleotide variation, short branch lengths, and low genetic distances, suggesting low genetic divergence and may be consistent with a relatively recent introduction and possible regional spread of *T. indotineae*.

Conclusion

The widespread detection of SUB and MEP genes supports their potential contribution to the virulence of *T. indotineae*. The observed genetic similarity between Iraqi and global isolates suggests recent dissemination of this emerging species. Further studies incorporating gene expression analysis, protease activity assays, and larger epidemiological investigations are need to clarify the functional role of these virulence-associated genes and the transmission patterns of *T. indotineae*.

Keywords: dermatophytes, *Trichophyton, indotineae*, subtilisin, fungalysin.

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Introduction

Keratinophilic fungi called dermatophytes exclusively infect the stratum corneum, human hair, and nails. Dermatophytes create virulence factors to infect host tissues and cause disease [1]. Proteases are classified as enzymes that can break down proteins into peptides and amino acids [2]. Different species of dermatophytes have several proteases that showed keratinolytic, elastinolytic and/or collagenolytic activities [3]. Secretory proteases are divided into exoproteases and endoproteases. Endoproteases secreted by dermatophytes are divided into two groups: subtilisins (serine proteases) and fungalysins (metalloproteases). Five MEP (genes for metalloproteases) and seven SUB (genes for serine proteases) genes were detected [4]. Although dermatophytosis is treatable, an obvious increase in the number of patients with recurrent dermatophytosis has been observed during the last few years. Compared with the classical presentation of the infection, recurrent dermatophytosis is characterized by persistent symptoms, widespread, more inflammatory, running a recurrent course, and reduced responsiveness to conventional treatments [5]. Dermatophytosis poses a significant global public health challenge. The epidemiology of these infections differs depending on the specific population and the geographic location. The extensive availability and accessibility of antimicrobials has led to an increased risk of developing resistant strains through inappropriate treatment. Of particular global concern is the emergence of

Trichophyton indotineae [6]. *T. indotineae* is an emerging novel species causing severe outbreaks, and is already spreading globally through travel and physical contact [7]. It can now become chronic and recurrent, requiring prolonged treatment due to increasing antifungal resistance [8]. To the best of The emergence of *T. indotineae* has significantly transformed the treatment landscape for dermatophytosis. Once easily managed with short-term antifungal therapy, mycotic infection knowledge, despite the increasing incidence of recurrent dermatophytosis and the emergence of antifungal resistant dermatophytes, molecular data on virulence-associated genes in *T. indotineae* remain limited, particularly in Iraq. Therefore, the aim of the present study was to detect the presence of subtilisin and fungalysin genes in clinical *T. indotineae* isolates obtained from human cases and to investigate the phylogenetic relationships between Iraqi isolates and global reference strains using conventional polymerase chain reaction (PCR) and ITS sequence analysis.

2. Patients and Methods

2.1. Source of isolates and their identification

Twenty-five clinical isolates of *T. indotineae*, obtained from patients with recurrent and treatment-resistant dermatophytosis were included in this study. The isolates were collected from hair and skin lesions of patients attending the outpatient clinic of Basrah Teaching

Hospital, AlSadr Teaching Hospital, and other private clinics between Feb. 2024 and Sep. 2025. Skin scales were scraped near the edges of the skin lesions using a blunt scalpel. Damaged hair root were collected using sterile forceps. The specimens were cultured on Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM) supplemented with Chloramphenicol and Cycloheximide. After 1 to 2 weeks of incubation at 25 ± 2 °C, microscopic examination was performed by using Lactophenol Cotton Blue (LCB) staining. Species identification was confirmed by sequencing the amplified DNA fragment at the National Instrumentation Center for Environmental Management(NICEM)(http://nicem.snu.ac.kr/main/?en_skin=index.html). Sequence alignment and analysis were conducted using the Basic Local Alignment Search Tool (BLAST) available at the National Center Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>)

2.2. primers design

According to Singh et al [10] and Tang et al [11] whole genome sequencing of the epidemic isolates, revealed that differentiating *T. indotineae* from the *T. mentagrophytes*/ *T. interdigitale* complex is difficult, as this complex includes several clones that may differ in anonymous genetic markers while displaying similar evolutionary behavior. Moreover, *T. mentagrophytes* is considered a common ancestor of *T. indotineae*, since *T. indotineae* is a new name for *T. mentagrophytes* genotype VIII [12]. Based on this close genetic relationship, the present study used previously published

placed on a grease-free glass slide, treated with 2-3 drop of a mixture of 10% Potassium hydroxide (KOH) with 40% Dimethyl sulphoxide (DMSO), covered with a cover slip, and examined after kept for 5-8 minutes [9]. All primers that were originally designed to detect virulence genes in *T. mentagrophytes*, in order to assess the presence of the same genes in *T. indotineae*. Primers targeting subtilisin (SUB1-7) and fungalsin (MEP4) genes were adopted from a previous publication [13,14]. While primers targeting fungalsin (MEP3) genes were designed by the authors of this study with sequence data retrieved from the National Center for Biotechnology Information (NCBI), Oligo primer analysis software was used to generate primers from conserved areas of homologous genes in other dermatophytes. Primer specificity was evaluated in silico using NCBI Primer-BLAST, available at <http://www.ncbi.nlm.nih.gov>, to ensure target specificity and to avoid cross-reactivity with closely related dermatophytes species. Primer expected amplicon sizes were further confirmed through preliminary PCR optimization experiments prior to analysis. The characteristics of each primer used in the present study are summarized in (Table 1).

2.3. PCR amplification reaction

Genomic DNA was extracted from fungal growth of suspected dermatophytes isolates according to the protocol of the Wizard Genomic DNA purification kit (Promega/USA). The quality and concentration of extracted DNA were evaluated using a Nano Drop spectrophotometer (Thermo Scientific, USA).

All PCR amplifications were carried out in a Thermal cycler (Bio Rad, USA), were set up with a total reaction volume of 25 µL comprising 13 µL of Taq mix (Promega /USA) and 1 µL of each primer, followed by 3 µL of extracted DNA. PCR cycling conditions consisted of: 95°C for 5 min followed by 35 cycles of 30s at 95°C, 1

min at 58°C and 45s at 72°C and a final extension step of 10 min at 72°C. The PCR products were resolved by agarose gel electrophoresis (1% w/v) at 90 V for 1 h. Nuclease free water served as a negative control in each PCR run. DNA was visualized by UV light after staining with ethidium bromide.

2.4. Phylogenetic analysis

In order to assess the degree of genetic relatedness among the isolates, multiple sequence alignments of the ITS region were performed using the MEGA (version 11) software program. Phylogenetic tree analysis was conducted using the Neighbor-Joining method. The robustness of the tree was evaluated by bootstrap analysis with 1000 replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Bootstrap values greater than 50% are shown next to the branches. Evolutionary distances were computed using the Kimura 2-parameter model and are expressed as the number of base substitutions per site. This analysis included 25 nucleotide sequences. All positions were considered (non-coding region). All ambiguous positions were removed using the pairwise deletion option. *T. mentagrophytes* was used as an out-group to root the tree.

2.5. Ethical approval

All procedures performed in the present study were in accordance with the ethical standards of the scientific and ethics committee at the College of Medicine/University of Basrah Ref. No. 1 on February 11/2024.

2.6. Statistical Analysis

Due to the uniform detection of most genes among isolates, data were summarized using descriptive statistics including frequencies and percentages of gene.

3. Results

The present findings indicated that, all clinical isolates of *T. indotineae* were positive for the investigated SUB-related and MEP-related genes, with the exception of SUB5 which was present in 60% of isolates as shown in (Table 2). PCR technique demonstrated successful amplification of the targeted virulence associated genes, the SUB1 and SUB2 genes yielding amplicons of 197bp and 770bp, respectively, as shown in (Figure 1). The SUB3

genes generated a large amplicon of 1070bp (Figure2). While, the SUB4, SUB5, SUB6, and SUB7 genes produced fragments of 270bp, 380bp, 905bp and 237bp, respectively, (Figure3-6). In addition, the MEP3 and MEP4 genes were successfully amplified, producing smaller amplicons of (183bp) and (105bp) respectively (Figure 7).

To investigate the genetic relationship between local Iraqi isolates and global strains, a phylogenetic tree was constructed using the neighbor-joining method implemented in the MEGA program (version 11). The analysis was based on ITS region sequences. The results showed limited genetic variation among the isolates, indicating a close genetic relatedness. Eight local Iraqi isolates identified as *T. indotineae* were analyzed and compared with a reference strain available in the Genbank database using the Basic Local Alignment Search Tool (BLASTN) with Pairwise alignment. Sequencing analysis showed high

4. Discussion:

Studies investigating virulence genes in *T. indotineae* remain limited. This may be partly due to the relatively recent recognition of this species and the lack of extensive molecular investigation compared with other fungal pathogens. Moreover, the complete genome sequence of *T. indotineae* has only recently become available, which may have restricted in depth characterization of its virulence profile. The present study provides molecular data on the distribution of subtilisin and fungalysin genes among clinical isolates of *T. indotineae*. PCR results showed that all human isolates were positive for SUB1, SUB2, SUB3, SUB4, SUB6, SUB7, MEP3, and MEP4, while SUB5 was detected in 60% of the isolates as shown in

sequence similarity to a reference USA strain under accession number [ID: OR483785.1] with four isolates displaying 100% nucleotide identity and four isolates exhibited slightly lower similarity ranging from 99.83% to 99.49%. Similarly, three Iraqi isolates displaying 100% nucleotide identity with Turkish strain under accession number [ID: ON528187.1]. Whereas one isolate showed 100% identity with an Iranian and UAE reference strain under accession number [ID: OR417015.1] and [ID: OR626642.2], respectively. The remaining isolates exhibiting slightly lower similarity (99.83% - 97.58%) with strains reported from Iraq, India, Bangladesh, and Italy as presented in (Figure 8) and (Table 3). Furthermore, the multiple sequence alignment of ITS region sequences showed a high degree of sequence conservation among *T. indotineae* isolates and their corresponding reference strains as represented in (Figure 9).

(Table2). The successful amplification of MEP3 genes using primers designed in the present study confirms their specificity and reliability for molecular detection. The high frequency of SUB and MEP genes, suggests that the proteins encoded by these genes may be associated with the infection related process particularly in adherence to keratinized tissues and the establishment of infection. However, since only gene presence was assessed, their direct role in pathogenicity cannot be confirmed. Previous studies have shown that proteases of the SUB and MEP families may contribute to host-pathogen interactions and stimulate immune response, which could be associated with increased inflammatory

reactions and the recurrent nature of the infection [15,16].

In contrast, the lower detection rate of SUB5 suggests possible variability among isolates, similar findings have been reported in a previous study by Tran et al [17], indicating that not all proteases encoded by MEP and SUB genes are consistently or essential virulence factors in all dermatophytes. Similarly, a previous study suggested that, variability in SUB5 could be attributed to adaptive mechanisms occurring during infection [18]. Moreover, it has been reported that the absence of the SUB5 gene in certain isolates suggests that not all SUB genes are involved in the pathogenic processes, in addition, the level of gene expression and the proteolytic activity of SUB encoded proteases may be more closely associated with infection severity rather than the capacity to produce infection [13]. However, these aspects were not investigated in the present study. Previous study reported that although the amino acids sequences of dermatophytes proteases are highly conserved across species, their secretion level may vary [19]. This suggests that differences in genes expression rather than structural divergences, may contribute to functional diversity among isolates. Such variations may explain the detection of SUB5 from USA, Turkey, Iran, UAE, Iraq, India, Bangladesh and Italy. Furthermore, the multiple sequence alignment of ITS region sequences showed a high degree of sequence conservation among *T. indotineae* isolates and their corresponding reference strains. The consensus and identity profiles revealed minimal nucleotide variation across most positions, with only a limited number of polymorphic sites

in some *T. indotineae* isolates. In contrast, another study [13] reported the absence of SUB5 in *T. mentagrophytes* isolates, which may reflect genetic or regulatory differences between closely related species, this observation is consistent with the proposed evolutionary relationship between these species, as *T. indotineae* is considered an anthropophilic clonal offshoot of the zoophilic *T. mentagrophytes* [20]. These findings align with previous observations suggests that variability in expression and secretion of proteases may be associated with ecological adaptation which in turn could contribute to differences in host inflammatory responses [19]. Furthermore, variation in the secretion patterns of proteases may be associated with host adaptation, and the ability of dermatophytes to survive in a new host. The identification and characterization of secreted proteases may contribute to a better understanding of dermatophytes biology. Therefore, secretion profiles may serve as a valuable tool for differentiating closely related dermatophyte species.

Gene family may undergo genetic changes during infection which could influence enzymes secretion, the investigating of the genetic relationship between Iraqi isolates and global strains using a phylogenetic tree revealed that high sequence similarity to a reference strains observed, indicating a high degree of genetic conservation regardless of geographic origin as illustrated in (Figure 9). Similarly, short branch lengths and low genetic distances observed in the phylogenetic tree suggest limited sequence divergence and a high degree of genetic relatedness among most isolates, although minor variation was observed in some geographically distinct strains, this pattern is in

agreement with recent genomic epidemiological studies [21,22] which reported high genetic similarity among *T. indotineae* isolates from different countries. For instance, Rhodes et al [21] described the rapid international spread of terbinafin-resistant *T. indotineae*, with limited genomic variations, while dos Santos et al [22] found that *T. indotineae* isolates from 14 countries were genetically closely related and did not cluster according to their geographic origin. In line with these observations, the findings of the current study may suggest a relatively recent introduction and possible regional spread of this species in Iraq rather than long-term local evolutionary divergence. In addition, the presence of *T. indotineae* in Basrah may be attributed to increased international travel and the influx of foreign workers in recent years, both of which could have facilitated the introduction and dissemination of this emerging dermatophyte species. Furthermore, Kubaisi reported that having domestic animals, particularly Shirazi cats, may carry new species of dermatophytes. However,

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it should be noted that the present analysis was based on a small sample size and limited genetic markers and further large scale genomic and epidemiological studies are required to confirm these patterns and to better understand the factors influencing dermatophyte diversity and genes expression under both in vivo and in vitro conditions [23].

Conclusion:

This study provides the first molecular data on the distribution of SUB and MEP genes in *T. indotineae* isolates from Basrah, Iraq. The widespread detection of these genes and the high genetic similarity between Iraqi and global isolates suggest their potential role in dermatophyte pathogenicity and support the recent regional dissemination of this emerging species. However, the detection of these genes does not necessarily reflect their functional activity. Therefore, further studies investigating gene expression, protease activity, and larger epidemiological datasets are required to clarify their role in virulence and disease transmission. **Conflicts of interest:** The authors declare that they have no conflicts of interest.

References:

1. Hashoosh QH, Al-Aaraji AMY, Determination of Metalloprotease Gene in a Few Dermatophyte Species and Its Relationship with Antifungal Resistance. *J Commun Dis.* 2024;56(1):127-134.DOI: [10.24321/0019.5138.202417](https://doi.org/10.24321/0019.5138.202417)
2. Martinez D, Oliver BG, Gräser Y, Goldberg JM, Li W, Martinez-Rossi NM, Monod M, Shelest E, Barton RC, Birch E, Brakhage AA, Chen Z, Gurr SJ, Heiman D, Heitman J, Kostı I, Rossi A, Saif S, Samalova M, Saunders CW, Shea T, Summerbell RC, Xu J, Young S, Zeng Q, Birren B, Cuomo C, White T. Comparative genome analysis of *Trichophyton rubrum* and related dermatophytes reveals candidate genes involved in infection. *mBio.* 2012;3(5):e00259-12. DOI: [10.1128/mBio.00259-12](https://doi.org/10.1128/mBio.00259-12)
3. Abdel-Rahman, S.M. Polymorphic exocellular protease expression in clinical isolates of *Trichophyton tonsurans*. *Mycopathologia.* 2001;150(3):117-120. DOI: [10.1023/a:1010977014536](https://doi.org/10.1023/a:1010977014536)
4. Kaplan E, Gonca S, Kandemir H, Döğen A, Hilmioğlu-Polat S, Ilkit M, Tanaka R, Yaguchi T, Uhrlaß S, Nenoff P. Genes Encoding Proteolytic Enzymes Fungalysin and Subtilisin in Dermatophytes of Human and Animal Origin: A Comparative Study. *Mycopathologia.* 2020; 185(1): 137–144. DOI: [10.1007/s11046-019-00367-2](https://doi.org/10.1007/s11046-019-00367-2)
5. Alhamdi D, Alhamdi K. Recurrent dermatophytosis, new treatment modality. *Journal of Pakistan Association of Dermatologists.* 2024; 34(1): 46-50. DOI: <https://doi.org/10.66344/jpad.34.1.2024.2402>
6. Chanyachailert P, Leeyaphan C, Bunyaratavej S. Cutaneous Fungal Infections Caused by Dermatophytes and Non-Dermatophytes: An Updated Comprehensive Review of Epidemiology, Clinical Presentations, and Diagnostic Testing. *J. Fungi.* 2023; 9(6): 669. [https://doi.org/ 10.3390/jof9060669](https://doi.org/10.3390/jof9060669)
7. Kano R, Kimura U, Kakurai M, Hiruma J, Kamata H, Suga Y, Harada K. *Trichophyton indotineae* sp. nov.: A new highly terbinafine-resistant Anthropophilic Dermatophyte species. *Mycopathologia.* 2020; 185(6): 947–958. doi: [10.1007/s11046-020-00455-8](https://doi.org/10.1007/s11046-020-00455-8)
8. Sonogo B, Corio A, Mazzeletti V, Zerbato V, Benini A, di Meo N, Zalaudek I, Stinco G, Errichetti E, Zelin E. *Trichophyton indotineae*, an Emerging Drug-Resistant Dermatophyte: A Review of the Treatment Options. *J. Clin. Med.* 2024; 13(12): 3558. <https://doi.org/10.3390/jcm13123558>
9. Mohamed MF, Naser El-din A, Abu El-hamd M. Isolation, Identification, and In Vitro Antifungal Susceptibility Testing of Dermatophytes from Clinical Samples at Sohag University Hospital in Egypt. *Electron. Physician.* 2016; 8(6): 2557-2567. <https://doi.org/10.19082/2557>
- 10.

11. Singh A, Masih A, Monroy-Nieto J, Kumar Singh P, Bowers J, Travis J, Khurana A, Engelthaler DM, Meis JF, Chowdhary A. A Unique Multidrug-Resistant Clonal *Trichophyton* Population Distinct from *Trichophyton mentagrophytes/Trichophyton interdigitale* Complex Causing an Ongoing Alarming Dermatophytosis Outbreak in India: Genomic Insights and Resistance Profile. *Fungal Genet. Biol.* (2019); 3: 103266. DOI: [10.1016/j.fgb.2019.103266](https://doi.org/10.1016/j.fgb.2019.103266)
12. Tang C, Ahmed SA, Deng S, Zhang L, Zoll J, Al-Hatmi AMS, Meis JF, Thakur R, Kang Y and de Hoog GS. Detection of emerging genotypes in *Trichophyton mentagrophytes* species complex: A proposal for handling biodiversity in dermatophytes. *Front. Microbiol.* 2022; 13(960190):1-11. doi: [10.3389/fmicb.2022.960190](https://doi.org/10.3389/fmicb.2022.960190)
13. Tang C, Kong X, Ahmed SA, Thakur R, Chowdhary A, Nenoff P, Uhrlass R, Verma SB, Meis JF, Kandemir H, Kang Y, de Hoog S. Taxonomy of the *Trichophyton mentagrophytes/T. interdigitale* species complex harboring the highly virulent, multiresistant genotype *T. indotineae*. *Mycopathologia.* 2021; 186(3): 315–326. doi: [10.1007/s11046-021-00544-2](https://doi.org/10.1007/s11046-021-00544-2)
14. Khedmati E, Hashemi-Hazaveh SJ, Kachuei R, Bayat M, Amini K. Identification of subtilisin genes as a potential virulence factor in *Trichophyton mentagrophytes* isolated from human and bovine dermatophytosis lesions in Iran. *Comparative Clinical Pathology.* 2023; 32:217–224. <https://doi.org/10.1007/s00580-022-03429-y>
15. Datt S, Das S, Ansari MA, Sharma R, Datt T, Bhattacharya SN. Gene expression profiling of protease and non-protease genes in *Trichophyton mentagrophytes* isolates from dermatophytosis patients by qRT-PCR analysis. *Nature.* 2021; 11(403): 1-10. doi: [10.1038/s41598-020-79839-1](https://doi.org/10.1038/s41598-020-79839-1)
16. Brouta F, Descamps F, Vermout S, Monod M, Losson B, Mignon B. Humoral and cellular immune response to a *Microsporum canis* recombinant keratinolytic metalloprotease (r-MEP3) in experimentally infected guinea pigs. *Med Mycol.* 2003; 41(6):495–501. <https://doi.org/10.1080/13693780310001615385>
17. Descamps F, Brouta F, Vermout S, Monod M, Losson B, Mignon B. Recombinant expression and antigenic properties of a 31.5-kDa keratinolytic subtilisin-like serine protease from *Microsporum canis*. *FEMS Immunol Med Microbiol.* 2003; 38(1):29–34. [https://doi.org/10.1016/S0928-8244\(03\)00101-9](https://doi.org/10.1016/S0928-8244(03)00101-9)
18. Tran VD, Feuermann DM, Schmid-Siebert E, Bağcı E, Mignon B, Waridel P, Peter C, Pradervand S, Pagni M, Monod M. RNA Sequencing based genome reannotation of the dermatophyte *Arthroderma benhamiae* and characterization of its secretome and whole gene expression profile during infection. *mSystems.* 2016;1(4):e00036–16. Doi: [10.1128/mSystems.00036-16](https://doi.org/10.1128/mSystems.00036-16)

19. Khedmatia E, Hazaveh SJH, Bayata M, Amini K. Identification of subtilisin virulence genes (SUB1-7) in *Epidermophyton floccosum* isolated from patients with dermatophytosis in Iran. *Gene Reports*. 2020;20(100748). <https://doi.org/10.1016/j.genrep.2020.100748>
20. Giddey K, Favre B, Quadroni M, Monod M. Closely related dermatophyte species produce different patterns of secreted proteins. *FEMS Microbiol Lett*. 2007; 267(1): 95–101. Doi: [10.1111/j.1574-6968.2006.00541.x](https://doi.org/10.1111/j.1574-6968.2006.00541.x)
21. Kumar P, Das S, Tigga R, Pandey R, Bhattacharya SN, Taneja B. Whole genome sequences of two *Trichophyton indotineae* clinical isolates from India emerging as threats during therapeutic treatment of dermatophytosis. *3 Biotech*. 2021;11(402). <https://doi.org/10.1007/s13205-021-02950-1>
22. Rhodes J, Hui ST, Dellièrè S, Summerbell RC, Scott JA, Kaur A, Barton RC, Leitao R, Hemmings S, Goiriz R, Lambourne J, Farrer RA, Schelenz S, Hay RJ, Borman AM, Chowdhary A, Abdolrasouli A, Fisher MC. Emerging terbinafine-resistant *Trichophyton indotineae* between 2018 and 2023: a multinational genomic epidemiology study. *The lancet microbe*. 2026;7(2):1-9. DOI: [10.1016/j.lanmic.2025.101273](https://doi.org/10.1016/j.lanmic.2025.101273).
23. dos Santos AR, Uhrlaß S, Nenoff P, Gold JA, Bhuiyan MS, Goturu S, Gade L, Bagal UR, Peterson JG, Wiederhold NP, Lockhart SR, Järv H, Hameed AF, Szepletowski J, Vasani R, Singal A, Parnell L, Schwem B, Chiller T, Litvintseva AP, Chow NA, Verma SB. Global Emergence of Antifungal-Resistant Dermatophytosis Caused by *Trichophyton indotineae* (Formerly *T. mentagrophytes* ITS Genotype VIII): A Genomic Investigation Involving 14 Countries. *Mycoses*. 2025; 68(8): e70101. doi: [10.1111/myc.70101](https://doi.org/10.1111/myc.70101).
24. Kubaisi T.A. Resistant dermatophytosis: A Stubborn habit and major challenge in Iraq. *AMJ*, 2023; 19 (2): 78-80. DOI: [10.33091/amj.2023.141295.1225](https://doi.org/10.33091/amj.2023.141295.1225)

Table 1: The characteristics and product size of primers used in the PCR technique for detection of SUB and MEP genes in *T. indotineae*

Gene	Forward (5'→ 3')	Reverse (5'→3')	Amplicon size base pair(bp)
Sub1	TGG GTG TTT TCA GAT TCA TTTC	ACG GCG GGT GAT GTT ATG G	197
Sub2	AGA AGG CGT GTG GGC ATA C	ACC CTT CCA GCA TGA CTT GA	770
Sub3	TCA AGG TTA TCT CCG TCT TC	AAA GAG GAC TTC TGG TCA TC	1070
Sub4	TCG CTG CTG GTA ATG ACA ACG	GGA GCA TAG ATG TCA ACT GAAG	270
Sub5	TCT GGC GGC ATT GTC TGT	TGG GAG ATG ATA GCG TCT G	380
Sub6	GCT CAT ACA ACT TGG CTT AG	TCA GAG GCA GGA GAA GAG T	905
Sub7	CGG CAT CTG TCA TCA ACG	AGT GAC CAG AGT ATC CCT T	237
MEP 3	ACAGAGCCCCAACCTCATCT	AGTTACCAGCAGCCTCGTTG	183
MEP 4	AGT CGG GAC ACC ATT CTT CAG	ATT TGG GCT TCT ATG CTC TAC G	105

Table 2: Frequency of SUB and MEP genes among *T. indotineae* isolates (n=25)

Genes	<i>T. indotineae</i>	
	No. Positive	%
SUB1	25	100.0
SUB2	25	100.0
SUB3	25	100.0
SUB4	25	100.0
SUB5	15	60.0
SUB6	25	100.0
SUB7	25	100.0
MEP3	25	100.0
MEP4	25	100.0

Table 3. Percentage identity of ITS region sequences between local *T. indotineae* isolates and the reference strain retrieved from GenBank

GenBank accession No.	Sequence identity with local isolates								Reference country
	PX-062345	PX-062346	PX-062347	PX-062348	PX-062349	PX-0623	PX-06235	PX-062352	
	.1	.1	.1	.1	.1	50.1	1.1	.1	
OR48378 5.1	100.00 %	99.66%	100.00 %	100.00 %	100.00 %	99.83 %	99.66 %	99.49%	USA
ON52818 7.1	99.83%	99.51%	100.00 %	100.00 %	100.00 %	99.67 %	99.51 %	99.34%	Turkey
OR41701 5.1	100.00 %	99.67%	99.84%	99.84%	99.84%	99.84 %	99.67 %	99.51%	Iran
OR62664 2.2	100.00 %	99.67%	99.84%	99.84%	99.84%	99.84 %	99.67 %	99.51%	UAE

OQ78964 4.1	99.83%	99.51%	99.67%	99.67%	99.67%	99.67 %	99.51 %	99.34%	Iraq
PQ39617 4.1	98.48%	97.58%	97.58%	97.58%	97.58%	97.88 %	97.72 %	97.88%	India
PQ35766 3.1	99.65%	99.49%	99.66%	99.66%	99.66%	99.49 %	99.32 %	99.14%	Banglad esh
PP898437 .1	99.66%	99.35%	99.51%	99.51%	99.51%	99.51 %	99.35 %	99.18%	Italy
PQ45153 1.1	99.49%	98.70%	98.86%	98.86%	98.86%	98.86 %	98.70 %	98.69%	India

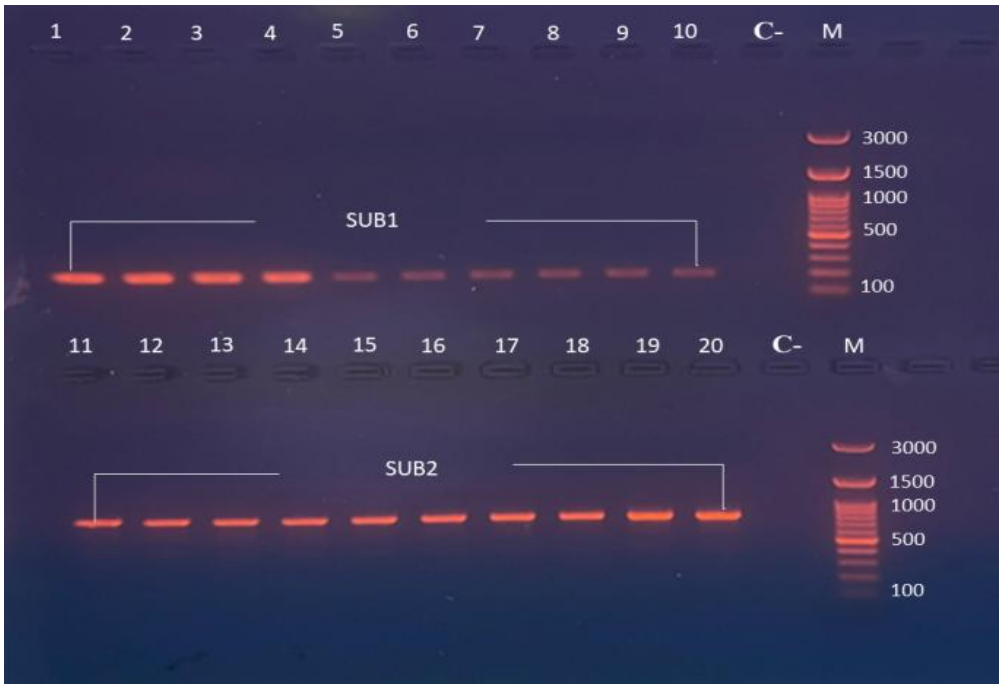


Figure 1: Agarose gel electrophoresis showing amplification of SUB1 (197 bp) and SUB2 (770 bp): Lanes 1–10 show positive amplification of SUB1, lanes 11–20 correspond to SUB2 while C- correspond to negative control. M: DNA molecular weight marker (100–3000 b

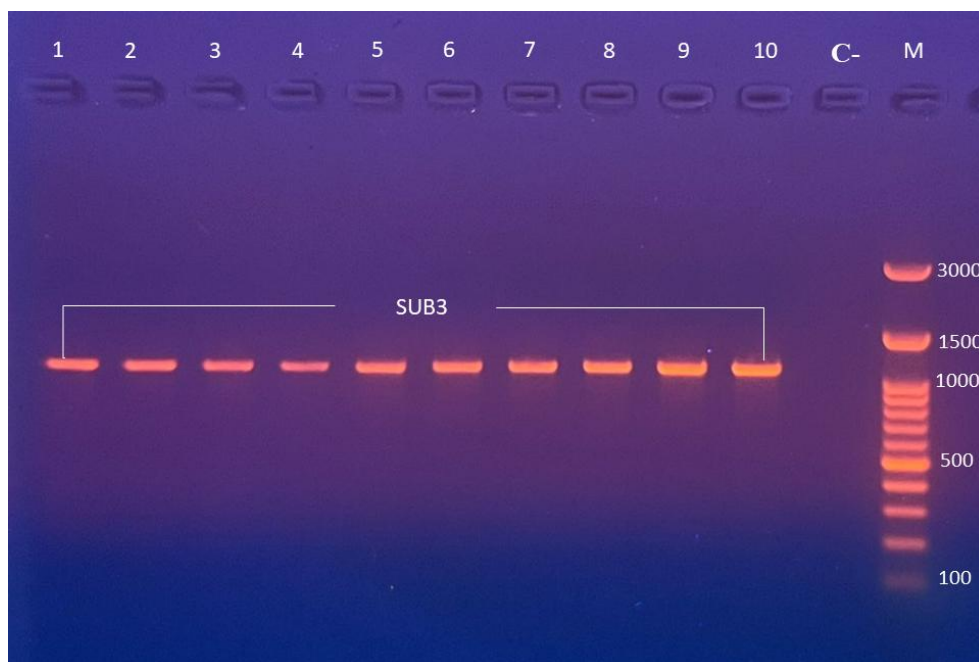


Figure 2: Agarose gel electrophoresis showing amplification of SUB3 (1070 bp): Lanes 1–10 show positive amplification of SUB3, while C- correspond to negative control. M: DNA molecular weight marker (100–3000 bp).

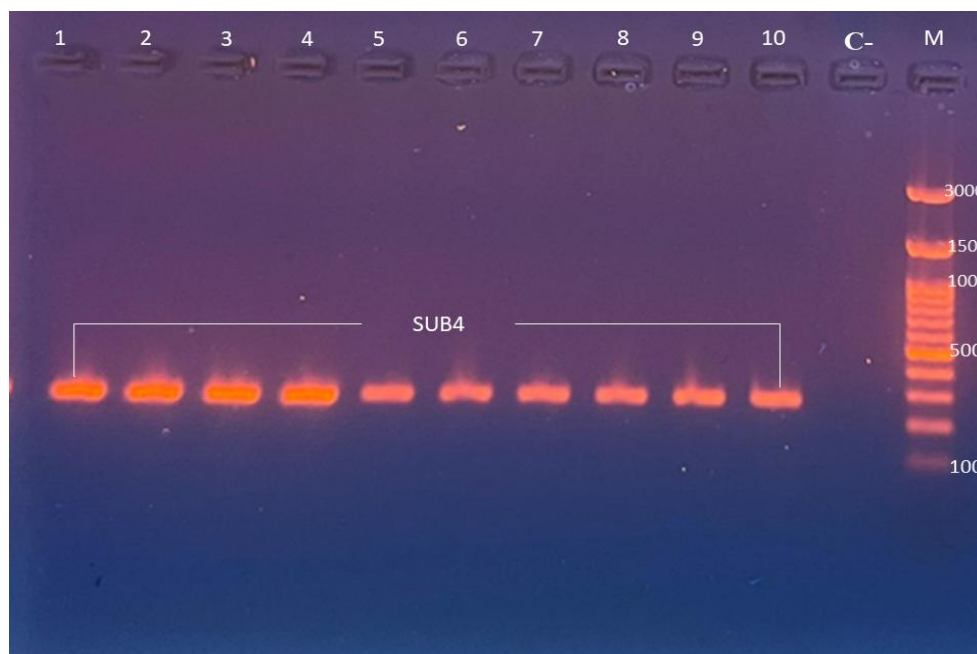


Figure 3: Agarose gel electrophoresis showing amplification of SUB4 (270 bp): Lanes 1–10 show positive amplification of SUB4, while C- correspond to negative control. M: DNA molecular weight marker (100–3000 bp).

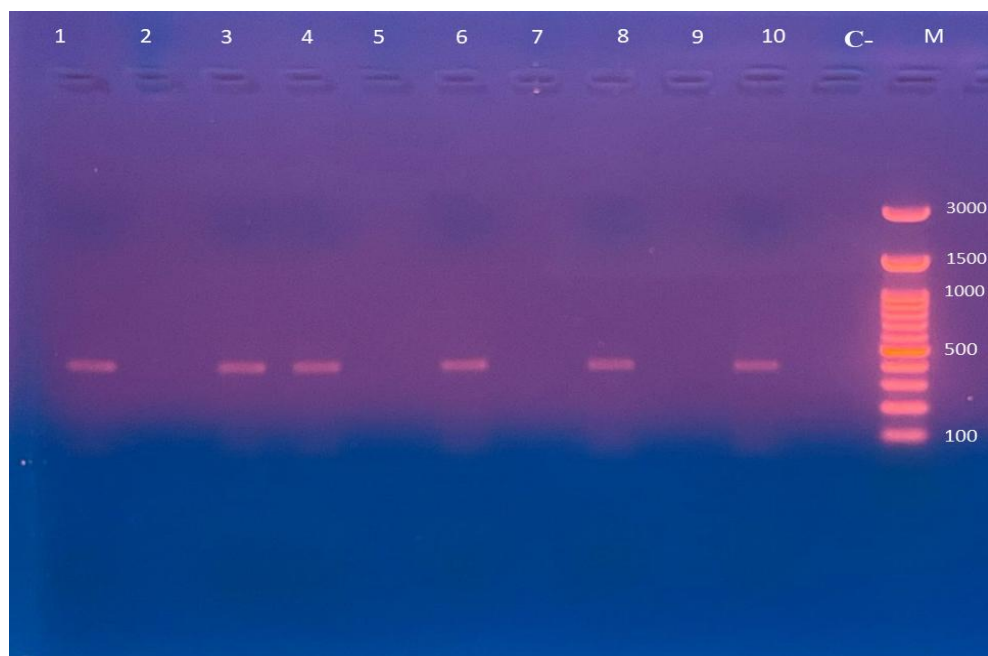


Figure 4: Agarose gel electrophoresis showing amplification of SUB5 (380 bp): Lanes 1,3,4,6,8,10 show positive amplification of SUB5, while C- correspond to negative control. M: DNA molecular weight marker (100–3000 bp).

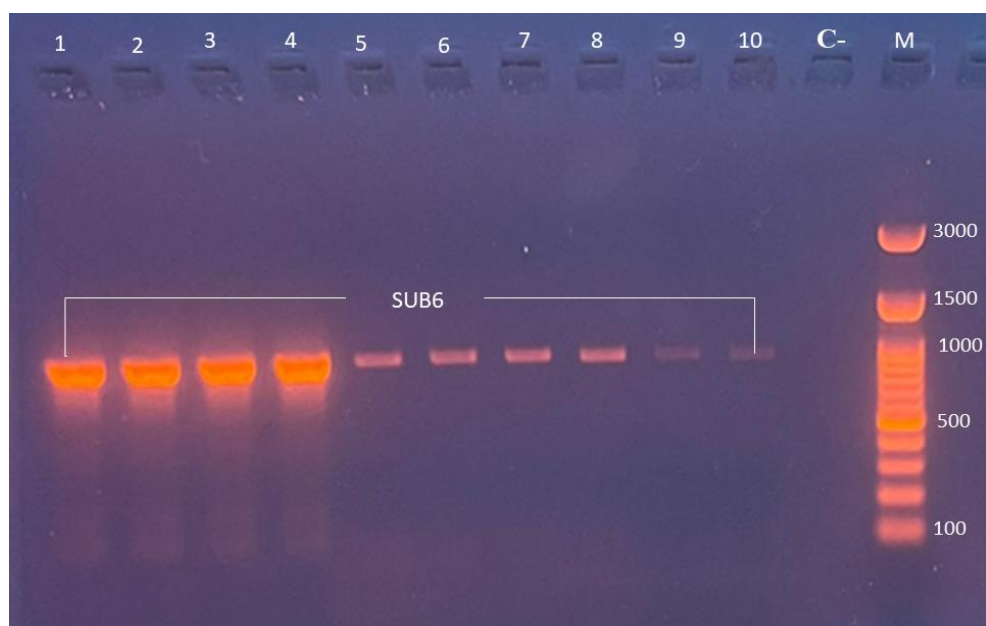


Figure 5: Agarose gel electrophoresis showing amplification of SUB6 (905 bp): Lanes 1–10 show positive amplification of SUB6, while C- correspond to negative control. M: DNA molecular weight marker (100–3000 bp).

Z



Z

Figure 6: Agarose gel electrophoresis showing amplification of SUB7 (237 bp): Lanes 1–10 show positive amplification of SUB7, while C- correspond to negative control. M: DNA molecular weight marker (100–3000 bp).

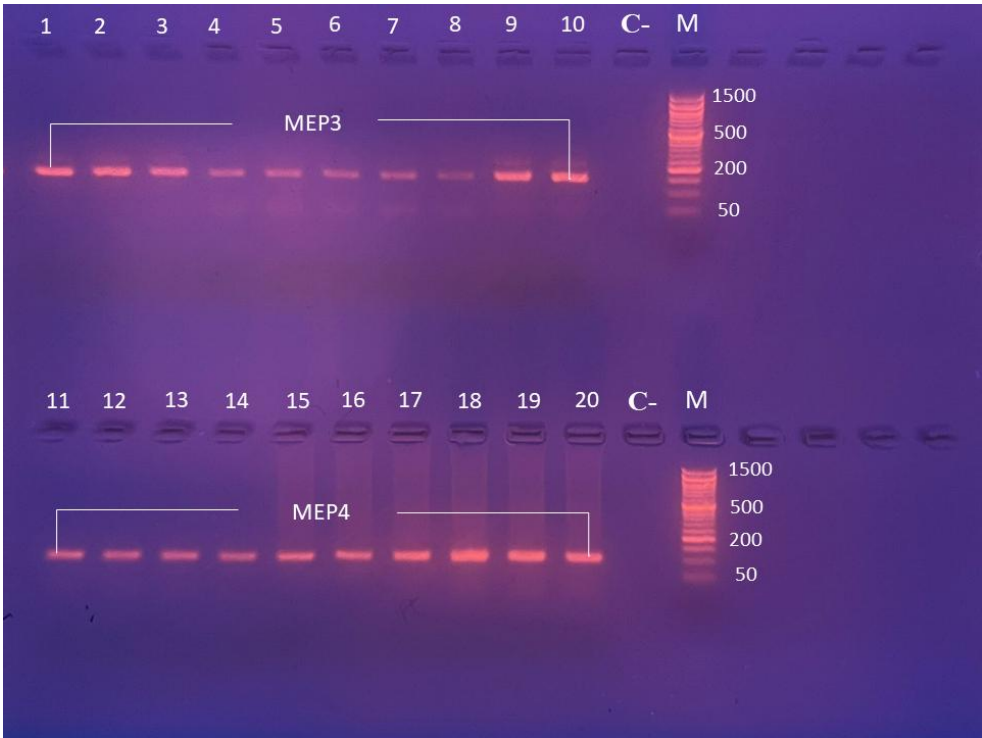
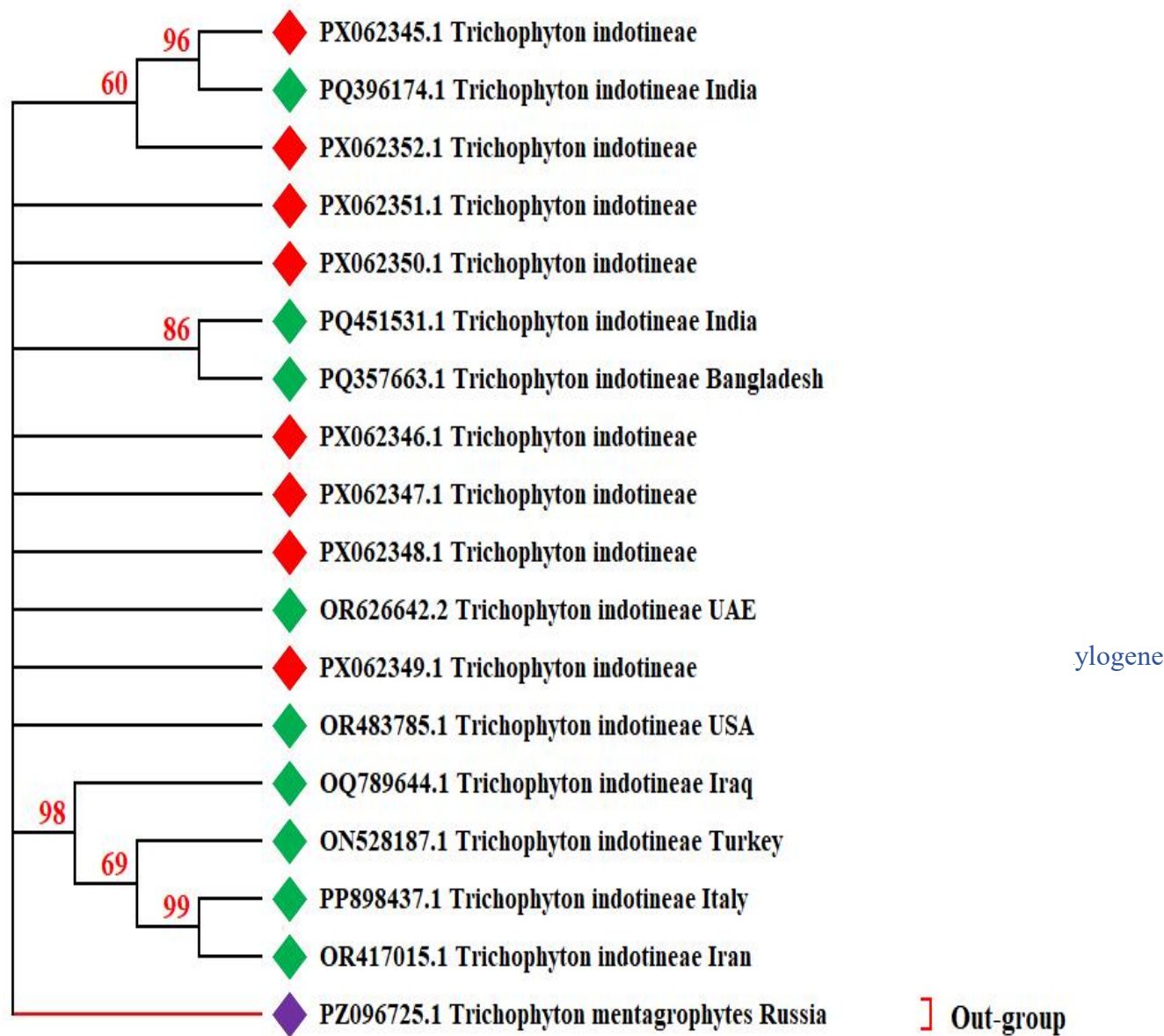


Figure 7: Agarose gel electrophoresis showing amplification of MEP3 (183 bp) and MEP4 (105 bp): Lanes 1–10 show positive amplification of MEP3 while lanes 11–20 correspond to MEP4 amplification. C⁻: negative control and M: DNA molecular weight marker (50–1500 bp).



tic tree based on ITS region sequence showing the evolutionary relationship between the Iraqi *T. indotineae* isolates and reference sequences retrieved from GenBank. Bootstrap values (>50%) based on 1000 replicates are indicated at branch nodes.

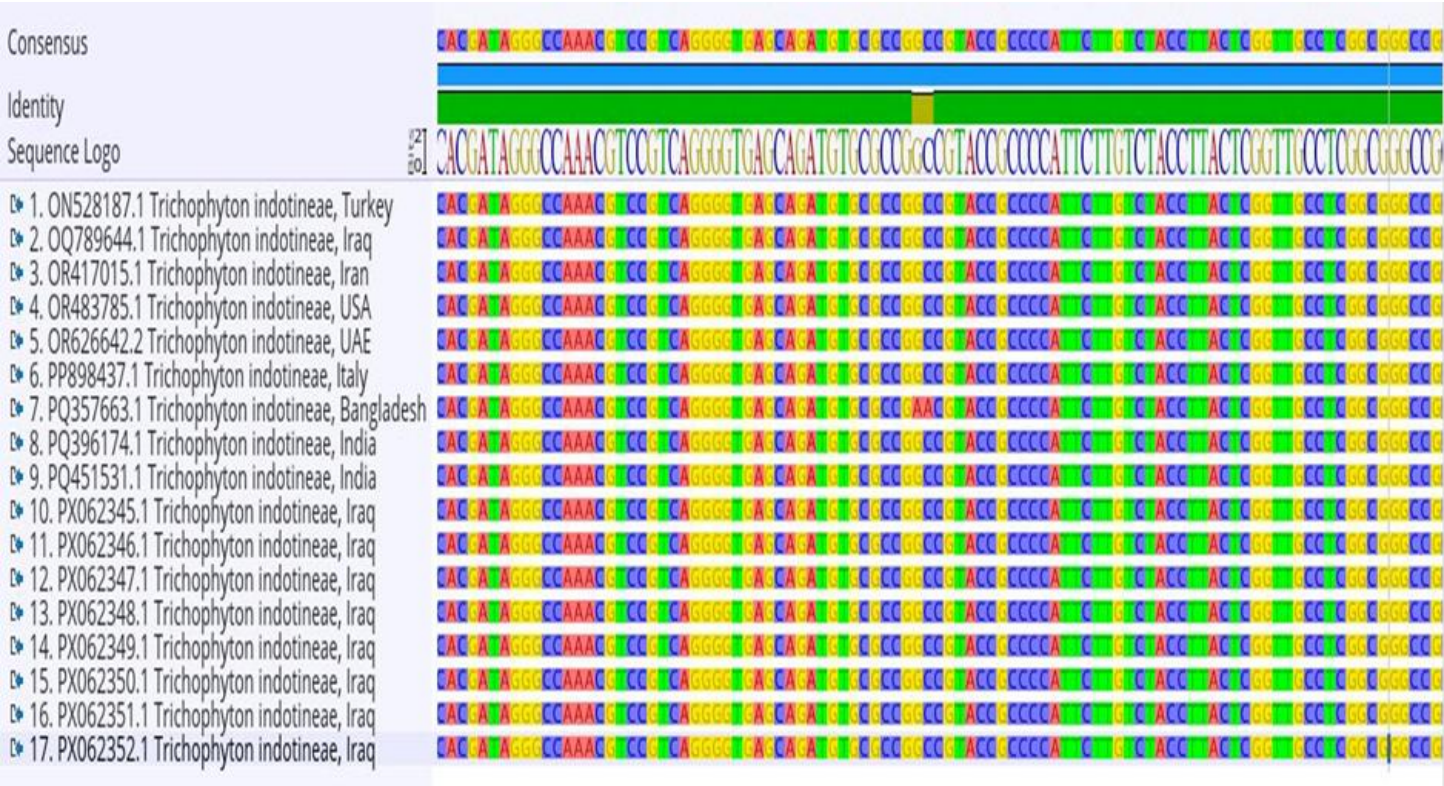


Figure 9. Multiple alignment of the nucleotide
zsequences of eight local Iraqi isolates *Trichophyton
indotineae* in this study [ID: PX062345.1,
PX062346.1, PX062347.1, PX062348.1,
PX062349.1, PX062350.1, PX062351.1, and
PX062352.1], nine isolate global [ID: OR417015.1,
OQ789644.1, PQ396174.1, PQ357663.1,
PP898437.1, OR626642.2, OR483785.1,
PQ451531.1, and ON528187.1] by Geneious program
version 2026.0.2.