ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2015, 53, 215–224
doi: 10.1093/mmy/myu088
Advance Access Publication Date: 30 December 2014
Original Article

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Translation elongation factor 1-α gene as a potential taxonomic and identification marker in dermatophytes

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Received 25 July 2014; Revised 12 October 2014; Accepted 30 October 2014

Abstract

Intra- and interspecies variations of the translation elongation factor 1-α (*Tef-1α*) gene were evaluated as a new identification marker in a wide range of dermatophytes, which included 167 strains of 30 species. An optimized pan-dermatophyte primer pair was designed, and the target was sequenced. Consensus sequences were used for multiple alignment and phylogenetic tree analysis and the levels of intra- and interspecific nucleotide polymorphism were assessed. Between species, the analyzed part of the *Tef-1α* gene varied in length from 709 to 769 nucleotides. Significant numbers of species including *Trichophyton rubrum, T. tonsurans*, *T. schoenleinii*, *T. concentricum*, *T. violaceum*, *Epidermophyton floccosum*, *Microsporum ferrugineum, M. canis*, *M. audouinii*, *T. equinum*, *T. eriotrephon*, and *T. erinacei* were invariant in *Tef-1α* and had sufficient barcoding distance with neighboring species. Although overall consistency was found between ITS phylogeny as the current molecular marker of dermatophytes and *Tef-1α*, a higher discriminatory power of *Tef-1α* appeared particularly useful in some clades of closely related species such as the *A. vanbreuseghemii*, *T. rubrum*, *A. benhamiae*, and *A. otae* complexes. Nevertheless, we stress that a single gene can not specify species borderlines among dermatophytes and multiple lines of evidence based on a multilocus inquiry may ascertain an incontrovertible evaluation of kinship.

Key words: dermatophytes, translation elongation factor 1-α, identification marker.

Introduction

Dermatophytes are a unique, highly specialized, and closely interrelated group of filamentous fungi that share the peculiar ability to digest and grow on keratinized tissue, enabling them to invade skin, nails and hair of living human and animal hosts [1–3]. Phylogenetically they comprise a homogeneous entity with relatively low genetic variation, combined with a high phenotypic diversity [4,5]. Based on their natural ecological habitats, dermatophytes are categorized into geophilic, anthropophilic, and zoophilic species [1,3], and taxonomically they are classified in the genera *Trichophyton*, *Microsporum,* and *Epidermophyton*. Dermatophyte infections are the most common superficial mycosis and are among the most common infectious diseases. About 30 out of 40 known species of dermatophytes have been observed to cause infections in humans and other mammals [6].

For a long time, phenotypic methods including morphology, physiology, and biochemistry were the basis of taxonomy and identification of dermatophytes, but these are time-consuming, inaccurate, and in many cases insufficiently conclusive to identify the less-common taxa or isolates potentially representing novel species [7,8].

Over the last two decades, DNA sequencing and molecular systematics have generated new species concepts in dermatophytes. This caused a reduction in the number of recognized taxa [4] and, on the other hand, has led to the discovery of some cryptic and novel species [7–9]. However, definition of taxonomic borderlines of some species has still remained debatable, for example, *T. mentagrophytes* complex species [5]. During the last decade progress has been made with modern systematics of dermatophytes using a number of targets for molecular classification and identification; internal transcribed spacer 1 (ITS1) and ITS2 regions of rDNA [4], partial β-tubulin (*BT2*) [10], and chitin synthase 1 (*CHS1*) genes [11]. Of these, ITS has proven to be the most informative [4], and currently sequence diversity in ITS provides the taxonomic basis of species identification in dermatophytes as found through sequencing [12], restriction fragment length polymorphism (RFLP) analysis [13,14], and use of specific primers or probes [15]. However, for fine resolution of closely related species, ITS-rDNA has its limits [13,14]. To unify sequence-based and classical concepts of species, confirmation and refinement using other genes is overdue.

The nucleotide sequence of translation elongation factor 1-α (*Tef*-1α) gene encoding a part of the protein translation machinery, was first used in fungi in *Fusarium* [16]. The gene appears to be consistently single-copy and shows a high level of sequence polymorphism among related species so that it was considered as an alternative to rDNA [17] and to have desirable properties for phylogenetic inference in other groups of pathogenic fungi [18].

The usefulness of *Tef-1α* gene in the systematics of dermatophytes has not been reported, and therefore we provided sequence information for reference and clinical isolates including wide range of common and rare pathogenic species. Our key aims in the present study were characterization of *Tef-1α* as a new marker to evaluate intra- and interspecies variation, as well as to understand relationships and species boundaries among dermatophyte fungi. The data could also be useful to improve diagnostic detection and differentiation of dermatophytes in clinical and epidemiological settings.

Materials and methods

Strains and Isolates

A total of 167 strains consisting 30 species of dermatophytes, including 144 reference and 23 clinical isolates (Table 1) were used in the partial sequence analysis of the *Tef-1α* gene. The reference strains were obtained from the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, and Teikyo University Institute of Medical Mycology (TIMM), Tokyo, Japan. Clinical isolates were recovered from a variety of specimens including skin, nail, and hair of proven cases of dermatophytosis, which were submitted to two medical mycology laboratories in Tehran, Iran. Species names used for clinical isolates were in concordance with the molecular ITS-based taxonomy introduced by Gräser *et al.* [4].

Primer Design

Initial primer pairs were selected according to a multiple alignment of *Tef-1α* sequences of various fungal species including dermatophytes and nondermatophyte fungi obtained from GenBank. After application and optimization of some primer sets for polymerase chain reaction (PCR) amplification of DNAs extracted from different species of dermatophytes, and analysis the obtained sequences, a pan-dermatophyte primer pair was designed as follows: EF-DermF (5-CACATTAACTTGGTCGTTATCG-3) and EF-DermR (5-CATCCTTGGAGATACCAGC-3). Primer design was performed manually. ITS1 and ITS4 primers [19] were used as reference for clinical isolates. All primers were synthesized by Sigma-Aldrich (Sigma-Aldrich Co., USA).

DNA Extraction and PCR

DNA was extracted and purified from the colonies by a method previously described [12]. Briefly, about 10–20

Table 1. Dermatophyte strains used in this study, *Tef-1α* gene fragment size, sequence differences and percentage identity between species.

Species (number of tested strains)	Strains (accession numbers)	Range of fragment size (mean)	Range of difference count within the species (mean)	Range of identity percent within the species (mean)
T. rubrum (14)	CBS 304.60 (KM678081), CBS 734.88 (KM678115), CBS 100237 (KM678123), CBS 130808 (KM678156), CBS 130817 (KM678160), CBS 130821 (KM678162), CBS 130825 (KM678165), CBS 130927 (KM678055), CBS 130933 (KM678169), CS 329 (KM678174), CS 534 (KM678183), CS 669 (KM678189), NBRC 5467 (KM678201), NBRC 5808 (KM678202)	738	0	100
T. violaceum (7)	CBS 459.61 (KM678102), CBS 118536 (KM678132), CBS 120319 (KM678139), CBS 120320 (KM678140), CBS 130937 (KM678056), CS 352 (KM678175), NBRC 31064 (KM678211)	743	0	100
T. mentagrophytes (4)	CBS 106.67 (KM678063), CBS 318.56 (KM678083), CBS 764.84 (KM678116), CBS 101546 (KM678051)	761	0–2 (1)	99.7– 100 (99.8)
T. schoenleinii (12)	CBS 335.32 (KM678087), CBS433.63 (KM678096), CBS 434.63 (KM678097), CBS435.63 (KM678098), CBS 458.59 (KM678101), CBS 855.71 (KM678120), CBS 114328 (KM678129), CBS 118537 (KM678133), CBS 118538 (KM678134), CBS 130812 (KM678053), NBRC 8191 (KM678209), NBRC 8192 (KM678210)	762	0	100
T. simii (4)	CBS 150.66 (KM678067), CBS 417.65 (KM678090), CBS 448.65 (KM678099), CBS 520.75 (KM678104)	759–761 (760.5)	0–14 (7.3)	98.1–100 (99)
M. racemosum (3)	CBS 423.74 (KM678093), CBS 102175 (KM678125), CBS 130935 (KM678171)	752–755 (753)	7–24 (17.6)	96.8–99 (97.6)
M. cookei (4)	CBS 129.67 (KM678064), CBS 202.66 (KM678071), NBRC 7862 (KM678208), CS 779 (KM678212)	752–755 (753.5)	0–10 (6.6)	98.6–100 (99)
M. gallinae (3)	CBS 133.89 (KM678066), CBS 215.38 (KM678072), CS 778 (KM678194)	709	0–15 (10)	97.8–100 (98.5)
T. interdigitale (*A. vanbreuseghemii)* (31)	CBS 130823 (KM678163), CBS 102.68 (KM678062), CBS 116916 (KM678130), CBS 119445 (KM678137), CBS 130788 (KM678142), CBS 130789 (KM678143), CBS 130790 (KM678144), CBS 130791 (KM678145), CBS 130792 (KM678146), CBS 130794 (KM678147), CBS 130796 (KM678149), CBS 130799 (KM678152), CBS 130803 (KM678153), CBS 130804 (KM678154), CBS 130810 (KM678157), CBS 130816 (KM678159), CBS 130824 (KM678164), CBS 130923 (KM678166), CBS 130930 (KM678168), CBS 130940 (KM678173), CS 365 (KM678178), CS 407 (KM678180), CS 437 (KM678181), CS 490 (KM678182),CS 559 (KM678184), CS 575 (KM678185),CS 655 (KM678187), NBRC 5466 (KM678200), NBRC 5812 (KM678203), NBRC 5974 (KM678206), JCM 1891 (KM678058)	767–769 (768.7)	0–6 (1.54)	99.3–100 (99.7)
T. tonsurans (8)	CBS 238.33 (KM678074), CBS 130814 (KM678158), CBS 130822 (KM678054), CBS 130924 (KM678167), NBRC 5928 (KM678204), CS 768 (KM678193), CS 782 (KM678195), NBRC 5945 (KM678205)	757	0	100
T. equinum (4)	CBS 270.66 (KM678075), CBS 634.82 (KM678112), CBS 100080 (KM678122), NBRC 31610 (KM678061)	744	0	100
M. canis (*A. otae*) (10)	CBS 132.88 (KM678065), CBS 277.62 (KM678076), CBS 566.80 (KM678106), CBS 101514 (KM678124), CBS 130795 (KM678148), CBS 130797 (KM678150), CBS 130798 (KM678151), CBS 130811 (KM678052), CS 674 (KM678190), NBRC 9182 (JN662936.1)	720	0	100

Table 1. (Continued)

Species (number of tested strains)	Strains (accession numbers)	Range of fragment size (mean)	Range of difference count within the species (mean)	Range of identity percent within the species (mean)
M. ferrugineum (7)	CBS 427.63 (KM678095), CBS 457.80 (KM678100), CBS 118546 (KM678135), CBS 118547 (KM678136), CS 650 (KM678186), NBRC 5831 (JN662934.1), NBRC 6081 (KM678059)	720	0	100
M. audouinii (4)	CBS 280.63 (KM678077), CBS 332.68 (KM678086), CBS 119448 (KM678138), NBRC 6074 (JN662935.1)	721	0	100
A. benhamiae (6)	CBS 280.83 (KM678078), CBS 623.66 (KM678110), CBS 807.72 (KM678118), CBS 809.72 (KM678119), JCM 1885 (KM678198), CBS 808.72 (KM678050)	737–739 (737.5)	0–19 (8.8)	97.4–100 (98.7)
T. verrucosum (1)	CBS 563.50 (KM678049)	763	–	–
T. erinacei (3)	CBS 344.79 (KM678088), CBS 511.73 (KM678103), CS 379 (KM678179)	763	0	100
T. eriotrephon (3)	CBS 220.25 (KM678073), CS 361 (KM678176), CS 363 (KM678177)	765	0	100
T. concentricum (4)	CBS 173.40 (KM678068), CBS 109404 (KM678126), CBS 109405 (KM678127), CBS 109407 (KM678128)	737	0	100
E. floccosum (5)	CBS 767.73 (KM678117), CS 657 (KM678188), CS 739 (KM678191),CS 750 (KM678192), NBRC 9045 (KM678060)	733	0	100
T. ajelloi (3)	CBS 180.64 (KM678070), CBS 572.80 (KM678108), IFO 31978 (KM678197)	717	4–7 (5.3)	99–99.4 (99.2)
T. eboreum (1)	CBS 117155 (KM678131)	745	–	–
M. gypseum (*A. gypseum*) (5)	CBS 130820 (KM678161), CBS 130939 (KM678172), CBS 424.66 (KM678094), IFO 5948 (KM678196),IFO 8228 (KM678057)	749	0–1 (0.6)	99.8–100 (99.9)
M. gypseum (*A. incurvatum*) (2)	CBS 174.64 (KM678069), CBS 548.82 (KM678105),	734	0	100
M. fulvum (4)	CBS 287.55 (KM678079), CBS 599.66 (KM678109), CBS 130805 (KM678155), CBS 130934 (KM678170)	734–749 (744.7)	0–38 (19)	94.9–100 (97.4)
M. praecox (2)	CBS 288.55 (KM678080), CBS 673.89 (KM678113)	728	0	100
M. persicolor (4)	CBS 421.74 (KM678091), CBS 422.74 (KM678092), CBS 871.70 (KM678121), NBRC 5975 (KM678207)	734–736 (734.5)	0–3 (1.5)	99.5–100 (99.7)
M. duboisii (1)	CBS 349.49 (KM678089)	731	–	–
M. nanum (7)	CBS 314.54 (KM678082), CBS 321.61 (KM678084), CBS 322.61 (KM678085), CBS 569.80 (KM678107), CBS 633.82 (KM678111), CBS 728.88 (KM678114), JCM 1907 (KM678199)	738–740 (739)	0–5 (2.4)	99.4–100 (99.7)
M. amazonicum (1)	CBS 121947 (KM678141)	733	–	–

cubic millimeters of the fresh colonies were added to 300 µl of lysis buffer (200 mM Tris-HCl pH 7.5, 25 mM EDTA, 0.5% w/v SDS, and 250 mM NaCl) in a 1.5 ml tubes and crushed with a conical grinder (Micro Multi Mixer, IEDA Co. Ltd., Tokyo, Japan) for 1 min, incubated in a boiling water bath for 10 min, mixed with 150 µl of 3.0 M sodium acetate, kept at −20°C for 10 min, and centrifuged at 12,000 rpm for 10 min. The supernatant was extracted once with phenol/chloroform/isoamyl alcohol (25:24:1) and once more with chloroform. The DNA in the supernatant was precipitated with 250 µl isopropanol, washed with 300 ml of 70% ethanol, air dried, rehydrated in 50 µl ultrapure water, and stored in −20°C until using for PCR amplification.

PCR mixture contained 12.5 µl of 2× *Taq* DNA Polymerase Master Mix Red (Ampliqon, Copenhagen, Denmark), 0.5 µM of the forward and reverse primers, 1µl of extracted DNA, and enough ultrapure water to adjust the final reaction volume to 25 µl. The PCR were programmed for preheating at 96°C for 6 min and 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min and a final extension step at 72°C for 10 min, by a Takara thermal cycler

(Takara Co., Tokyo, Japan). Five microliters of the PCR products were electrophoresed onto 1.5% agarose gel in TAE buffer (Tris 40mM, acetic acid 20mM, EDTA 1mM), stained with 0.5 μg/ml of ethidium bromide, and observed and photographed under UV irradiation.

Sequencing and Sequence Analysis

ITS-rDNA sequence data, obtained as already described [10], were compared in GenBank using BLASTn and compared with information provided by CBS. *Tef-1α* gene PCR products were purified using QIAquick purification kit (Qiagen, Valencia, CA, USA), subjected to ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA), and sequenced in both directions, using the same forward and reverse primers as for the primary PCR, by an automated DNA sequencer (ABI Prism™ 3730 Genetic Analyzer, Applied Biosystems). The data were edited with GENEIOUS software (http://www.geneious.com), and the consensus sequences were used for further analysis. All sequences were deposited in GenBank (Table 1).

Pair-wise comparisons and multiple alignment were performed to calculate the levels of intra- and interspecific nucleotide polymorphism by using BioEdit software version 7.0.5 [20]. The bioinformatics data were analyzed and used for assessment of the inter-/intraspecies nucleotide variation of *Tef-1α*.

Phylogenetic Analyses

For tree construction, consensus sequences were imported into MEGA5 software [21]. The phylogenetic tree was built, and the tree topology were evaluated visually for congruence of species-rank clades with the following conditions: The methodology used was Maximum Likelihood, phylogram stability was evaluated by parsimony bootstrapping with 1000 simulations, a bootstrap percentage value as good support was regarded above 70%, and the substitution model was Tamura-Nei and uniform rates among sites. Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value.

Results

The *Tef-1α* partial gene was successfully amplified in all strains, yielding a single band ranging in size from about 700 to 770 base pair (bp). Sequence analysis using MEGA5 showed significant interspecies size polymorphism ranging from 709 nucleotides (nt) for *M. gallinae* to 769 nt for some strains of *T. interdigitale* (Table 1). Some species like *M. canis* / *M. ferrugineum* and *T. verrucosum*/*T. erinacei* had identical nucleotide lengths. Figure S1(supplementary file) shows the multiple DNA sequence alignment of *Tef-1α* in the most common dermatophytes. The alignment reveals significant divergences, including substitutions and insertions/deletions (indel). Comparison of sequences using Bioedit showed that many species including *T. schoenleinii*, *T. concentricum*, *T. violaceum*, *T. rubrum*, *E. floccosum*, *M. ferrugineum, M. canis*, *M. audouinii*, *T. tonsurans*, *T. equinum*, *T. eriotrephon*, and *T. erinacei* had invariant *Tef-1α* sequences, while in other species some intraspecific variations were detected. Interspecies divergence ranged from 0% (0 nt) between some strains of *T. mentagrophytes*/*T. schoenleinii* and *M. cookei*/*M. racemosum* and 23.9% (184 nt) between *T. verrucosum* and *T. ajelloi* (Table 2). Intraspecies differences of 0–6, 0–19, and 0–5 nt, leading to four distinct *Tef-1α* genotypes, were found within strains of *T. interdigitale*, *A. benhamiae* and *M. nanum*, respectively (Table 1). Overall, the interspecies divergence was higher between *Microsporum* than between *Trichophyton* species (Table 1).

Rates of population divergence within strains of *T. mentagrophytes*, *T. simii*, *T. ajelloi*, *M. gallinae, M. cookei*, *M. racemosum*, *M. nanum,* and *M. persicolor* were computed as 0–2, 0–14, 4–7, 0–15, 0–10, 7–24, 0–5, and 0–3 nt, respectively. A broad range of intraspecific polymorphisms of 0–38 nt was calculated within four stains of *M. fulvum*. The diversity within the *A. gypseum* strains was 0–1 nt, while variation was significantly higher between *A. gypseum* and its sister species *A. incurvatum* (75bp). Figure 1 shows the *Tef-1α* taxonomic naming framework, which is computed by MEGA5 software. The topology was similar to that observed in the ITS tree (data not shown) and most species, in particular closely related taxa, clustered in similar, strongly supported clades (≥70% bootstrap values). However, the backbone of the tree had low bootstrap values and the interspecies correlations were not obvious in some clades.

Discussion

During the last decades, taxonomy of dermatophytes has gone through significant changes, which in part are still a matter of controversy [22–24]. The validity of genetic differentiation for species delimitation was evaluated in different scenarios [4,10–12,23]. Recently, the dermatophytes were reclassified based on the data inferred from ITS sequences, but the species-recognition scheme as gold-standard does not always provide sufficient support in some species groups. For example, the discrimination between the closely related taxa *T. tonsurans*/*T. equinum* and

Table 2. Sequence differences based on pairwise sequence comparison of *Tef-1α* gene between dermatophyte species investigated in this study.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	CBS 129.67 - *M. cookei*	ID																													
2	CBS 173.40 - *T. concentricum*	150	ID																												
3	CBS 220.25 - *T. eriotrephon*	145	54	ID																											
4	CBS 288.55 - *M. praecox*	128	140	131	ID																										
5	CBS 321.61 - *M. nanum*	134	133	124	97	ID																									
6	CBS 349.49 - *M. duboisii*	120	133	123	84	88	ID																								
7	CBS 417.65 - *T. simii*	139	75	64	133	123	138	ID																							
8	CBS 511.73 - *T. erinacei*	139	47	14	128	114	122	61	ID																						
9	CBS 548.82 - *A. incurvatum*	130	132	124	97	103	49	139	118	ID																					
10	CBS 563.50 - *T. verrucosum*	143	52	4	131	121	125	62	12	123	ID																				
11	CBS 572.80 - *T. ajelloi*	162	177	182	165	168	156	179	183	159	184	ID																			
12	CBS 599.66 - *A. fulvum*	124	143	130	99	95	59	129	129	76	129	155	ID																		
13	CBS 808.72 - *A. benhamiae*	153	21	54	143	136	135	81	56	136	52	178	147	ID																	
14	CBS 101546 - *T. mentagrophytes*	139	80	69	134	128	131	7	66	136	67	176	129	84	ID																
15	CBS 117155 - *T. eboreum*	143	176	179	178	163	159	169	179	160	179	115	164	182	172	ID															
16	CBS 121947 - *M. amazonicu*	132	127	117	95	86	72	127	116	90	115	167	80	125	128	167	ID														
17	CBS 130811 - *M. canis*	154	155	155	138	132	135	162	156	138	157	144	154	160	159	157	133	ID													
18	CBS 130812 - *T. schoenleinii*	134	79	68	133	127	130	6	65	135	66	175	128	85	1	171	127	159	ID												
19	CBS 130822 - *T. tonsurans*	145	90	77	125	115	126	39	75	137	75	180	122	96	42	171	115	158	41	ID											
20	CBS 130927 - *T. rubrum*	131	62	56	123	119	112	70	52	121	54	164	120	64	72	171	99	145	71	72	ID										
21	CBS 130935 - *M. racemosum*	22	156	150	137	140	125	144	144	130	148	163	128	159	141	151	142	160	136	143	139	ID									
22	CBS 130937 - *T. violaceum*	130	56	51	117	111	105	66	47	114	49	162	111	58	68	166	93	142	67	68	11	137	ID								
23	CS 778 - *M. gallinae*	129	169	165	138	134	123	158	168	131	165	129	150	172	160	140	132	96	159	156	140	142	138	ID							
24	IFO 8228 - *M. gypseum*	122	142	131	83	98	69	125	128	75	129	155	72	145	123	165	89	147	122	125	115	126	111	141	ID						
25	JCM 1891 - *A. vanbreuseghe*	145	89	80	130	121	131	36	78	146	78	178	130	93	37	177	122	153	36	19	75	144	68	158	126	ID					
26	NBRC 5975 - *M. persicolor*	125	144	133	86	102	63	141	126	75	133	167	90	149	143	168	79	129	142	135	124	134	116	136	88	141	ID				
27	NBRC 6074 - *M. audouinii*	152	157	157	137	132	136	166	158	140	157	141	153	164	162	159	133	8	161	164	150	158	146	97	149	158	128	ID			
28	NBRC 6081 - *M. ferrugineum*	156	158	158	141	134	137	167	159	142	158	144	154	165	163	162	134	10	162	162	150	161	147	99	147	157	131	10	ID		
29	NBRC 9045 - *E. floccosum*	131	143	137	101	102	90	140	134	106	135	165	95	141	136	172	74	141	136	130	121	134	113	148	106	129	103	142	143	ID	
30	NBRC 31610 - *T. equinum*	143	94	82	124	118	125	46	80	134	80	172	120	99	48	170	115	155	47	14	73	140	69	154	121	31	134	161	159	124	ID

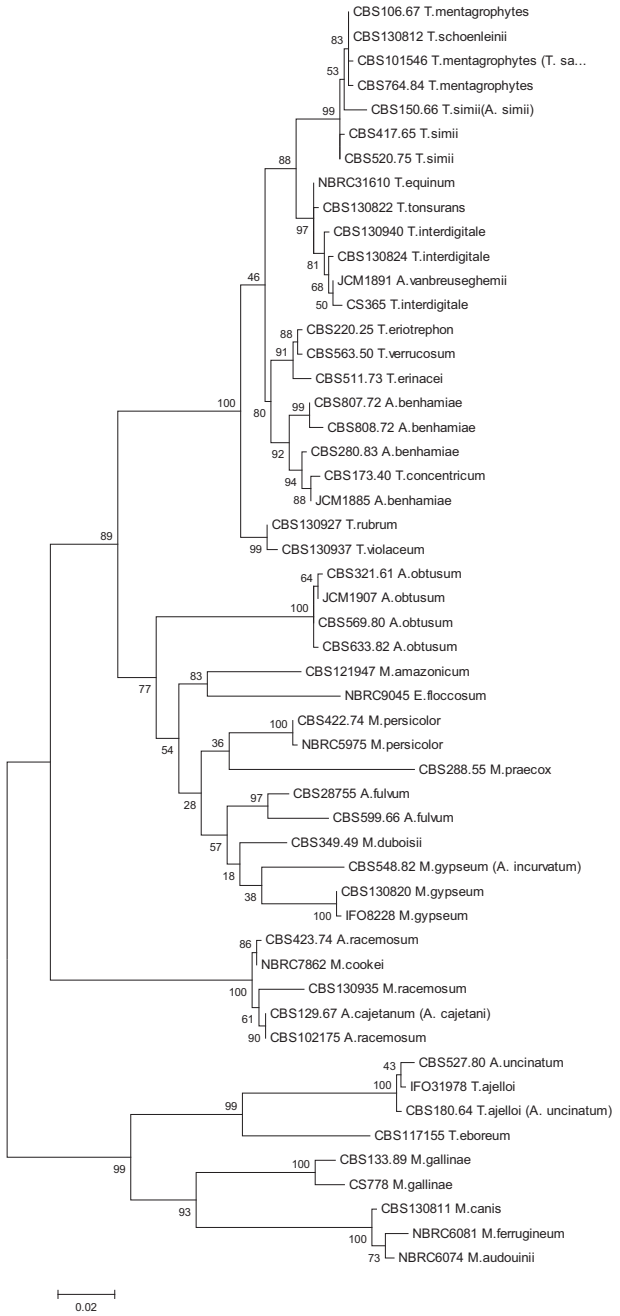

Figure 1. Phylogenetic tree of 52 dermatophyte strains, representatives of 30 species, based on analysis of translation elongation factor 1 – α sequence.

M. canis/*M. ferrugineum* were known to be limited as a single- and two-base exchange in ITS1 and ITS2 regions, respectively, which was judged to be insufficient for their identification [3,13,14]. In the present study the taxonomic value of partial translation elongation factor 1 alpha (*Tef-1α*) gene to specify species boundaries among dermatophytes was investigated.

Phylogeny of the *A. benhamiae* complex based on *Tef-1α* data was informative and notable interspecies variation rate of 4–56 bp was observed between taxa of the complex. *Trichophyton eriotrephon* had a high similarity with *T. verrucosum* and *T. erinacei*, and these three species were located on the same internode of the *Tef-1α* tree. This observation strengthens the probability that the taxon has an animal-associated ancestry. In contrast, *T. concentricum* and *A. benhamiae* strains had a separate internode. Interestingly, the phylogenies obtained from partial sequencing of actin (*ACT*) and topoisomerase-II (*TOP-II*) loci in the study of Kawasaki *et al.* indicated that *T. concentricum* was most closely related to *A. benhamia,* and likewise *T. verrucosum* and *T. erinacei* shared a common branch[23]. While the lengths of ITS and *BT2* in all members of the complex were shown to be almost identical [10], sizes of *Tef-1α* for the three former species were apparently longer than those of *T. concentricum* and *A. benhamiae* (Table 1), as reflected in the alignment as 1 to 23 bp indels.

As detailed in our earlier study [10], the strains of *T. erinacei* show intraspecific polymorphisms in ITS and *BT2*, whereas no *Tef-1α* variation was found within this zoophilic species. The gap between *T. erinacei* and *A. benhamiae* in *Tef-1α* (43–56 nt) was higher than that found in ITS and *BT2*. Like *BT2*, topoisomerase II (*TOP-II*) and actin (*ACT*) genes [23], *Tef-1α* showed high similarity between *T. concentricum* and *A. benhamiae*. However, *Tef-1α* provided higher species resolution, and three species-specific "signature nucleotides" corresponding to *T. concentricum* were found. For *T. verrucosum* only a single strain (CBS 563.50) was available, and more investigation is needed. With respect to size polymorphisms and the number and patterns of barcode sequences, *Tef-1α* potentially represents more effective signals for species recognition in the *A. benhamiae* complex.

The partial *Tef-1α* data provided sufficient resolution of species boundaries for the *A. vanbreuseghemii* complex, three anamorphic species within the complex having interspecies polymorphisms. *Trichophyton interdigitale* (formerly known as *T. mentagrophytes*) is a unique species encompassing strains with various morphological traits, and has zoophilic as well as anthropophilic traits [4,22]. Furthermore, the taxon is known to have high intraspecific genetic variation [10,25] with no correlations between genotypes, phenotypes or mating types of the strains [22]. In this study such genetic diversity was reflected with four *Tef-1α* genotypes among 31 strains of *A. vanbreuseghemii*/*T. interdigitale*.

The *Tef*-1α length variation between *T. interdigitale* and *T. tonsurans*/*T. equinum* strains (10–25 bp) was found to be significantly higher than with use of other loci like *ACT*, *TOP-II*, ITS and *BT2* (data not shown). *Trichophyton interdigitale* differed from *T. tonsurans* and *T. equinum* in some single nucleotide substitution plus four and five

sets of insertions, respectively. It has been proven that the interspecific difference between two ecologically distant species, *T. equinum* and *T. tonsurans*, is just a single base pair (99.8% similarity in ITS1/*BT2*), but a large *Tef-1α* distance including a 13 nucleotide indel and a signature substitution nucleotide corroborated the usefulness of this gene.

Within the *T. rubrum* complex several phenotypic species and varieties have been described that recently were reduced to single monophyletic group consisting of two species, *T. rubrum* and *T. violaceum* [26]. In contrast to *T. rubrum* strains, which are almost invariant, the latter species is polymorphic showing variable numbers of TA repeats (TATA box) in the ITS2 region [10,26]. The two species had no *BT2* length polymorphism but differed in two "signature nucleotides" [10]. With *Tef-1α*, strains of the two species differed in both length (Table 1) and in nucleotide composition with three indels and four signature sets of single-nucleotide polymorphisms (SNPs). Mean *Tef-1α* distance between all tested strains of *T. rubrum* and *T. violaceum* was 11 nt, which is considerably more than the 2 nt found for *BT2* [10] and *ACT* [23,27]. Given to the invariability of *Tef-1α* within the two species, these indels and SNPs provide a much higher diagnostic signal than ITS for species distinction of *T. rubrum* and *T. violaceum*.

The type species of the genus *Epidermophyton*, *E. floccosum*, was found to have no intraspecies variation [10,28]. Contrary to the statement [29] that the species is closer to anthropophilic *Trichophyton* species, our recent *BT2* investigation [10] and the current *Tef*-1α-based analysis clearly demonstrate that the species is more related to *Microsporum*.

The complex of *A. simii* is known to comprise three closely related taxa. The anthropophilic species, *T. schoenleinii,* which causes favus-type tinea capitis worldwide [4,30], *T. simii* associates with monkeys, which its geographical distribution extends beyond the Indian subcontinent [31], and the camel-associated species, *T. mentagrophytes,* which previously contained several species or variants like *T. papillosum*, *T. depressum*, *T. langeronii*, *T. sarkisovii,* and *T. mentagrophytes* var. *quinckeanum,* have all been reduced to synonymy with *T. mentagrophytes sensu stricto* [4,30]. On the basis of *Tef-1α* data, the relationships among the three species could not be resolved. The difference between *T. mentagrophytes s. str.* and *T. schoenleinii* was the lowest (0–1 bp); however, the two species were distinct from *T. simii*. These findings were consistent with earlier analyses based on *BT2* [10] and *ACT* genes [24] and support the hypothesis that *T. schoenleinii* possibly originated from camels [30]. We also found that *T. simii* reveals high intraspecific variability in *Tef-1α*. In concordance with this finding, Beguin *et al.*, based on phylogenies inferred from ITS and actin, found *A. simii* as a sister group of the clade containing *T. quinckeanum* and *T. schoenleinii* [24].

The complex of *M. gypseum* is recognized for encompassing two morphologically indistinguishable entities, *M. gypseum* and *M. fulvum*. Unlike *M. fulvum* (teleomorph: *A. fulvum*), *M. gypseum* is more frequently involved in dermatophytosis and connected with two teleomorphic stages known as *A. gypseum* and *A. incurvatum*. Despite large phenetic similarities in anamorphic states, the three teleomorphs are genetically distant [4,10,32]. For the complex, the phylogeny inferred from*Tef-1α* sequences provided stronger phylogenetic signals but was topologically congruent with ITS. The intra-species homology within *A. gypseum* and *A. incurvatum* was high (99.8–100%). However, relatively high intrapopulation variability (5.1%) was detected within *M. fulvum* strains, while substantial interspecies variation rates (9.1−11.1%) were detected between the three species. Recently, *Microsporum aenigmaticum* was described as a sister species to *M. gypseum*, based on ITS/*BT2* sequences [8], but this could not be included in our assessment.

The taxonomic situation of members of the *M. cookei* clade has become more complicated based on the topology inferred from *Tef*-1α. Both tested strains of *M. cookei* grouped amidst *M. racemosum* strains (Fig. 1). A relatively high rate of intraspecific variation (1–3.2% for *M. racemosum* and 1.4% for *M. cookei*) along with a high degree of interspecies overlap (96.5–100% similarity) were observed with *Tef-1α* pairwise comparison. These findings agree with those of Choi *et al.* [9] and Rezaei-Matehkolaei *et al.* [10] who found that both ITS and *BT2* datasets have low resolution for delimitating the taxa in this clade. Considering all molecular evidences, it seems that the species status should be revisited in the *M. cookei* clade as incorporating two species into one.

Representing different *Tef-1α*-genotypes, strains of the less human-pathogenic species *M. nanum*, *T. ajelloi*, *M. persicolor,* and *M. gallinae* were found to possess enough interspecies diversity to locate them in separate branches (Fig. 1).

The present study was carried out in line with ongoing evaluations of relationships and boundaries in dermatophytes using a new genetic locus, *Tef*-1α. Overall, consistency was found between ITS and *Tef-1α* in most areas, but the specificity and discriminatory power of *Tef-1α* was found to be higher than ITS, which proved particularly useful in some closely interrelated species groups such as *A. vanbreuseghemii*, *T. rubrum*, *A. benhamiae,* and *A. otae* complexes.

Main remaining questions concern the selection of the most appropriate genetic locus for routine diagnostics, and

what is the threshold of genetic differentiation to define a species in dermatophytes. As found in our earlier investigation [10], we stress that a single gene is insufficient to define all species borderlines among dermatophytes and multiple lines of evidence are needed to ascertain kinship stable taxonomic framework.

Acknowledgements

This work was financially supported by both Tehran University of Medical Sciences (TUMS), grant no. 90-04-27-15673, Tehran, Iran, and Teikyo University Institute of Medical Mycology (TIMM), Tokyo, Japan. We thank all personnel in Molecular Biology Laboratory in TIMM and in the reference collection of Centraalbureau voor Schimmelcultures (CBS), The Netherlands.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Supplementary Material

Supplementary material is available at *Medical Mycology* online (http://www.mmy.oxfordjournals.org/).

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