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Trichophyton indotineae sp. nov.: A New Highly Terbinafine-Resistant Anthropophilic Dermatophyte Species

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Abstract In this report, we describe the first isolation of two highly terbinafine (TRF)-resistant *Trichophyton interdigitale*-like strains from a Nepali patient and an Indian patient with tinea corporis in Japan. These strains (designated NUBS19006 and NUBS19007) exhibited a TRF minimal inhibitory concentration (MIC) of > 32 mg/L and contained a missense mutation (Phe397Leu) in squalene epoxidase (*SQLE*) gene. The internal transcribed spacer (ITS) region sequences amplified from the isolates (NUBS19006 and NUBS19007) were 99.5% identical to Japanese isolates of *T. interdigitale* and *T. interdigitale* strain CBS 428.63. The homology of region sequences were also 97.6% identical to *T. mentagrophytes* strain CBS 318.56. Moreover, the ITS sequences amplified from the isolates were 100% identical to highly TRF-resistant strains of *T. interdigitale*, which were isolated in Delhi, India, and harbored mutations in *SQLE*. The urease test on Christensen's urease agar was positive for *T. mentagrophytes* and *T. interdigitale* after 7 days of incubation. On the other hand, the type strain of *T. rubrum* CBS 100081 ^{T} and highly TRF-resistant strains (NUBS19006 and NUBS19007) were negative on Christensen urease agar after 7 and 14 days of incubation. Moreover, NUBS19006 and NUBS19007 were also negative reaction on the hair perforation test. To avoid confusion in the taxonomy of the *T. mentagrophytes*/*T. interdigitale* complex, we suggest that the highly TRF-resistant Indian strains be considered a new species independent of *T. interdigitale*, according to clinical and mycological features.

Keywords Dermatophytosis · Tinea corporis · Terbinafine resistance · *Trichophyton indotineae*

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Introduction

Trichophyton interdigitale is an anthropophilic species that is frequently isolated from tinea unguium and tinea pedis throughout the world [1, 2]. Itraconazole

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(ITZ) and terbinafine (TRF) have been used in the treatment of these infections for more than 20 years.

In recent years, highly TRF-resistant *T. interdigitale* infections were epidemic in North India and harbored a missense mutation (Phe397Leu) in the squalene epoxidase-encoding gene, *SQLE* [3, 4]. These Indian strains are morphologically and genetically close to *T. interdigitale,* but were isolated predominately from tinea corporis with severe lesions [3, 5]. This high level of TRF resistance of Indian dermatophyte isolates seems to be driving an ongoing outbreak of dermatophytosis in countries other than India [4, 5].

In this report, we describe the first isolation of two highly TRF-resistant *T. interdigitale*-like strains from a Nepali patient and an Indian patient with tinea corporis in Japan. These strains (designated NUBS19006 and NUBS19007) exhibited a TRF minimal inhibitory concentration (MIC) of > 32 mg/L and contained a missense mutation (Phe397Leu) in *SQLE* [6, 7]. In the present study, molecular and mycological investigations were performed on these isolates, which should be considered a new species independent of *T. interdigitale*.

Materials and Methods

Strains

Two *T. interdigitale*-like clinical isolates (NUBS19006 and NUBS19007) were obtained from two human cases of tinea corporis in Shinjuku, Tokyo and Urayasu, Chiba in Japan in 2019 (Table 1) [6, 7]. The patients have made regular trips between Japan and Nepal/India many times and have been treated with TRF in Japanese hospitals; however, no response was seen. After an antifungal susceptibility test, the patients were cured with ITZ or fos-ravuconazole (F-RVZ) administration. These strains were sub-cultured on potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) [1].

The type strain of *T. rubrum* CBS 100081 ^{T} and clinical isolates of *T. interdigitale* and *T. mentagrophytes* and examined in this study are also listed in Table 1.

Internal Transcribed Spacer (ITS) Region Sequences and Phylogenetic Analysis

Genomic DNA samples were isolated as reported previously [8]. Molecular characteristics of the strains were also identified by sequence analysis of the ITS region. The ITS region of isolates was amplified using the universal fungal primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGC) and ITS4 (5′-TCCTCCGCTTATTGATAGC) [9].

Thirty cycles of PCR amplification were performed with the following conditions: denaturation for 30 s at 95 °C, primer annealing for 30 s at 55 °C, and extension for 1 min at 72 °C in a total reaction volume of 30 µl amplification mixture (10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 mM deoxynucleotide triphosphate, 1.0 U *Taq* polymerase (Takara, Kyoto, Japan), and 50 M each primer). The resulting amplified DNA fragments were electrophoresed on a 2% (w/v) agarose gel with 1 × TAE buffer and visualized by ethidium bromide staining.

An approximately 550-bp-long DNA band for each strain was excised from the gel, purified with the ExoSAP-IT® kit (USB Corporation, Cleveland, OH, USA), and sequenced on an ABI PRISM 3130 DNA Analyzer (Thermo Fisher Scientific, Inc., Tokyo, Japan).

Comparative sequence analyses were carried out using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website.

The resulting sequences were compared with those of the type strains and isolates (Table 1) using the ClustalW multiple sequence alignment program [10]. The phylogenetic tree was constructed with the neighbor-joining method using the TreeView program [11]. Bootstrap analysis was performed on 1000 replicates of random samples and analyzed with the ClustalW program [12].

Strains that are Susceptible to TRF and Azoles

To assess the susceptibility of the isolates to TRF, clotrimazole (CTZ), luliconazole (LCZ), miconazole (MCZ), ITZ, and RVZ, the broth microdilution assay was performed based on the Clinical & Laboratory Standards Institute (CLSI) M38-A2 guidelines with modifications as previously described [13, 14].

Table 1 Strains, minimal inhibitory concentrations (MICs) (mg/L) of anti-fungal drugs and urease test

Species	Strain number	Source	TRF[a]	ITZ[b]	RVZ[c]	LCZ[d]	CTZ[e]	MCZ[f]	Urease test	
									7 days[g]	14 days
T. indotineae	NUBS[h] 19,006	tinea coporis of Nepali	> 32	0.03	0.5	< 0.03	4	8	−	−
T. indotineae	NUBS19007	tinea coporis of Indian	> 32	0.03	0.03	< 0.03	0.06	0.125	−	−
T. interdigitale	NUBS18016[i]	tinea pedis of Japanese	2	0.38	< 0.03				+	
T. interdigitale	14	tinea pedis of Japanese	< 0.03	1	< 0.03				+	
T. interdigitale	1	tinea pedis of Japanese	< 0.03	0.125	< 0.03				+	
T. interdigitale	7	tinea pedis of Japanese	< 0.03	0.75	0.0625				+	
T. interdigitale	10	tinea pedis of Japanese	< 0.03	0.064	0.125				+	
T. interdigitale	12	tinea pedis of Japanese	< 0.03	0.064	< 0.03				+	
T. interdigitale	13	tinea pedis of Japanese	< 0.03	0.125	< 0.03				+	
T. interdigitale	15	tinea pedis of Japanese	< 0.03	0.25	< 0.03				+	
T. interdigitale	20	tinea pedis of Japanese	< 0.03	0.75	< 0.03				+	
T. mentagrophytes	No. 11	guinea pig' hair	0.03	0. 03	< 0.03				+	
T. mentagrophyte	No. 21	guinea pig' hair	0.03	0.75	< 0.035				+	
T. rubrum	CBS 100081 [T]								−	−

[a]*TRF* terbinafine, [b]*ITZ* itraconazole, [c]*RVC* ravuconazole, [d]*LCZ* luliconazole, [e]*CTZ* clotrimazole, [f]*MCZ* miconazole

[g]7 days incubation at room temperature; [h]NUBS: Nihon University College of Bioresource Sciences; [i]Strain was previously reported [15]

Table 2 Primers used for amplification and sequencing of the *T. interdigitale SQLE* gene

Sense primer	Sense primer sequence	Position*	Reverse primer	Reverse primer sequence	Position*
SQEL1S	5′-ATGGTTGTAGAGGCTCCTCC	2356–2375	SQEL1R-2	5′-CGAAGGCGCATGATAAAGCG	2776–2795
SQEL2S	5′-CGGCCGCTTTATCATGCGCC	2772–2791	SQEL2R	5′-AGGGCCGAATACACTCTGGC	3216–3235
SQEL3S	5′-TGTAAAGGGTCACATGCGG	3176–3195	SQEL3R	5′-CAGTGGAACTTGGAGAGGCTG	3466–3485
SQEL3S	5′-GTGTAAAGGGTCACATGCGG	3176–3195	SQEL4R-2	5′-AAGTTCGGCAAATACGAAAG	3868–3887

*Positions reflect that of the *Trichophyton mentagrophytes* strain TIMM2789 squalene epoxidase (*SQLE*) gene (GenBank Accession No. KU242352)

For quality control, the strain *Candida parapsilosis* ATCC 22019 was used in CLSI M38-A2 to check the accuracy of drug dilution [13, 14].

Detection of the Mutation Hotspot (Phe397Leu) in SQLE

To sequence the mutation hotspot of *SQLE* in the isolates, primers were prepared based on the conserved sequence of *T. mentagrophytes SQLE* (GenBank accession no. KU242352). The primers SQEL3S (5′-GTGTAAAGGGTCACATGCGG; position 3176–3195) and SQEL4R-2 (5′-AAGTTCGGCAAATACGAAAG; position 3868–3887) were used. The PCR amplification conditions and sequence analysis were performed as described previously [15].

Sequencing of Full-Length SQLE

To sequence the full length of *SQLE* of the clinical isolate (NUBS19006), primers were prepared based on the conserved sequence of *T. mentagrophytes SQLE* (GenBank accession no. KU242352) (Table 2) [15].

Approximately, 100 mg mycelium sample was flash-frozen in liquid nitrogen and ground to homogeneity. Total RNA was extracted with the RNeasy total RNA kit (QIAGEN, Tokyo, Japan). Reverse transcription of poly(A) + RNA was performed using the Omniscript™ Reverse Transcriptase kit (QIAGEN). Aliquots (100 ng) of the resulting cDNA samples were then amplified with PCR.

The sample cDNA (50 ng) was amplified with PCR in a 30-µl total volume reaction mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 mM each deoxynucleoside triphosphate, 1.0 unit *Taq* polymerase (Takara), and 50 µmol each primer pair.

PCR amplification (GeneAmp® PCR System 9700; Thermo Fisher Scientific K.K.) was carried out for 30 cycles consisting of denaturation for 30 s at 94 C, primer annealing for 30 s at 57 C, and extension for 1 min at 72 °C. A final extension was performed at 72 C for 5 min. PCR products were detected with electrophoresis on 2% agarose gels followed by staining with ethidium bromide and visualization under UV light. PCR products were cloned into the pCRII vector (Invitrogen, San Diego, CA, USA). Plasmid DNA from more than three clones of each species was extracted using the QIAGEN plasmid kit

(QIAGEN, Valencia, CA, USA) and separately sequenced with the dideoxy chain termination method using an ABI PRISM 3130 DNA Analyzer (Thermo Fisher Scientific, Inc.).

PCR Analysis of the Alpha-Box and HMG Genes Specific to the Genomic DNA of NUBS19006 and NUBS19007

The primers TmMATa1S and TmMATa1R amplified a 471-bp fragment of the *T. mentagrophytes* alpha-box gene fragment. The primers TmHMG1S and TmHMG1R amplified a 524-bp fragment of the *T. mentagrophytes HMG* fragment [8].

Genomic DNA samples (100 ng) from the clinical isolates were amplified with PCR in a volume of 30 µl, using a reaction mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 mM each deoxynucleoside triphosphate, 1.0 unit *Taq* polymerase (Takara), and 50 nM of the pair of primers. Amplification was carried out over 35 cycles consisting of template denaturation (1 min, 94 C), primer annealing (1 min, 55 C), and polymerization (2 min, 72 C). The PCR products were detected with electrophoresis on 2% agarose gels followed by staining with ethidium bromide and visualization under UV light.

Physiological Investigation for Urease Activity and Hair Perforation Tests

The type strain of *T. rubrum* ($CBS10081^T$) and clinical isolates of *T. mentagrophytes* (No. 11 and 21), *T. interdigitale* (strain 1 to 20), NUBS19006, and NUBS19007 (Table 1) were cultured on Christensen's urea agar (0.1% peptone, 0.1% glucose, 0.5% sodium chloride, 0.2% monopotassium phosphate, 2% urea, 0.0012% phenol red, and 1.5% agar) at room temperature [1, 16, 17].

The hair perforation test was also performed for 4 weeks at room temperature [1, 16, 17].

Mating Experiment

For the mating test, NUBS19006 was mated with (+) and (−) tester strains of *A. vanbreuseghemii* (VUT 77007 = RV27960 (+) and (−) VUT 77008 = RV 27961 (−)) [18] on soil hair medium for 3 weeks at room temperature [1].

Results

Morphological Characteristics

Colonies of the isolates (NUBS19006 and NUBS19007) were flat, white in color, with a suede-like surface, and yellowish to brown reverse pigment on SDA at 24 C for 2 weeks (Fig. 1). Similarly, colonies of the isolates (NUBS19006 and NUBS19007) were flat, white to cream in color, with a powdery surface, and yellowish to brown reverse pigment on PDA at 24 C for 2 weeks (Fig. 2).

Numerous subspherical to pyriform microconidia and occasional spiral hyphae were present (Fig. 3). Macroconidia were abundant and cigar- to club-shaped, with three to four septa, and were smooth and thin-walled, measuring 6–8 × 20–50 µm with narrow attachment bases (Fig. 3).

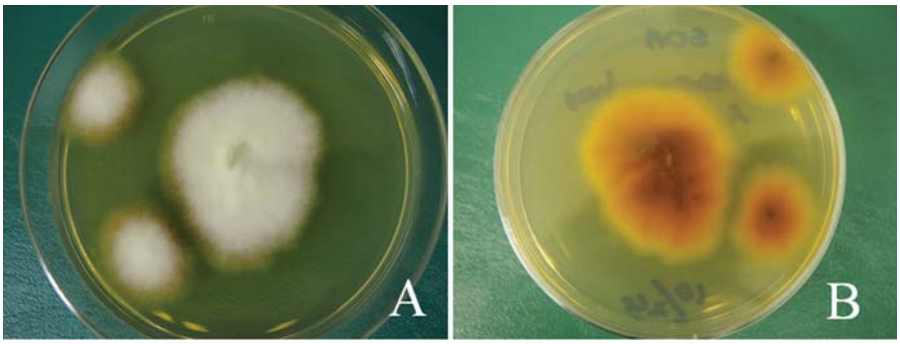


Fig. 1 Colonies of NUBS19006 were flat, white in color, with a suede-like surface, (**a**) and yellowish to brown reverse pigment (**b**) on SDA at 24C for 2 weeks

ITS Identity

Comparative nucleotide sequence analysis using the BLAST algorithm on the NCBI website showed that the ITS sequences amplified from the isolates (NUBS19006 and NUBS19007) were 99.5% identical to Japanese isolates of *T. interdigitale* (GenBank accession nos. LC428133 and LC404027) and *T. interdigitale* strain CBS 428.63 (GenBank accession No. NR_144900). The homology of region sequences was also 97.6% identical to *T. mentagrophytes* strain CBS 318.56 (GenBank accession No. MH857656).

Moreover, the ITS sequences amplified from the isolates were 100% identical to highly TRF-resistant strains of *T. interdigitale* (GenBank accession Nos. MH990853, MH990852, and MH990851), which were isolated in Delhi, India, and harbored mutations in *SQLE*.

In the ITS region, the isolates (NUBS19006 and NUBS19007) and all Indian TRF-resistant strains of *T. interdigitale* harbored three single polymorphisms (SNPs) at position 94 (C), 125 (T) and 462 (T), whereas all strains with SNPs at position 94 (A), 125 (C), and 462 (C) were Japanese isolates and CBS 428.63 of *T. interdigitale* (Fig. 4).

The ITS sequences determined in this study have been deposited in the GenBank database: *Trichophyton interdigitale* strain NUBS19006, GenBank-LC508024 and *T. interdigitale* strain NUBS19007, GenBank accession no. LC508728.

Fig. 4 Comparison of the ITS regions of the nucleotide ▶ sequences of the isolate of NUBS19006 (GenBank accession no. LC508024), VPCI 390/P/17 (MH990848), *T. interdigitale* CBS 428.63 (NR_144900), Japanese former isolates of *T. interdigitale* strain *T. interdigitale* strain 1 (LC508729) and IMF63291 (LC317810). An asterisk indicates identity with the nucleotide found in the NUBS19006 ITS sequence. Boxes indicate specific SNP between high TRF-resistance strains and other reference strains of *T. interdigitale*

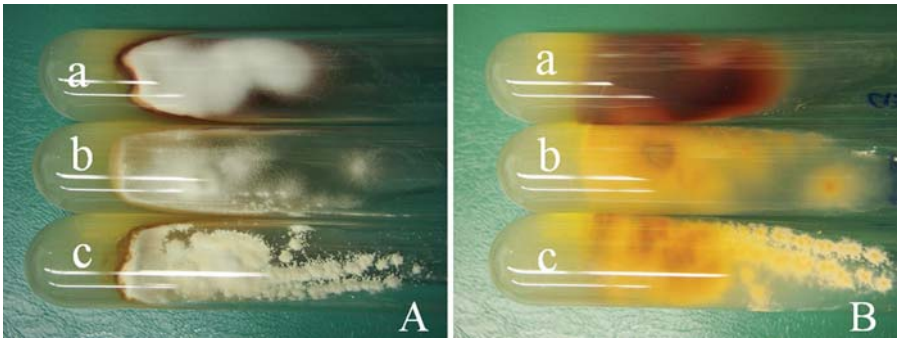


Fig. 2 On PDA, colonies were flat, white in color, with a suede-like surface (A:b) or powdery surface (A:c). Colonies had yellowish to brown reverse pigment (B:b and c) or red reverse pigment (B:a). **a**; *T. rubrum* CBS 100081 ^{T}; **b**, NUBS19006; **c**, *T. mentagrophytes* strain 10

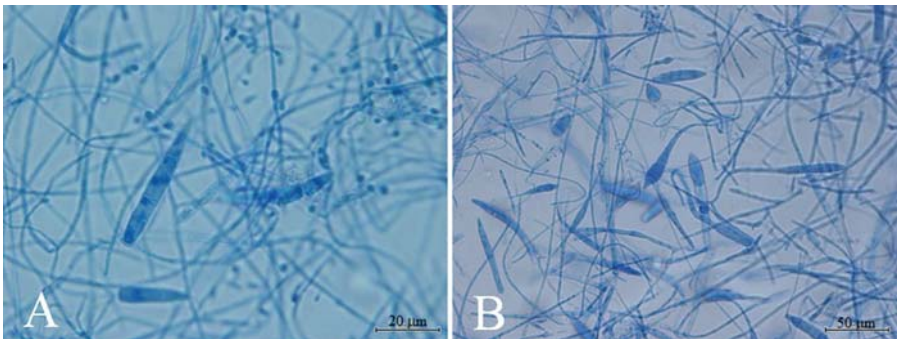
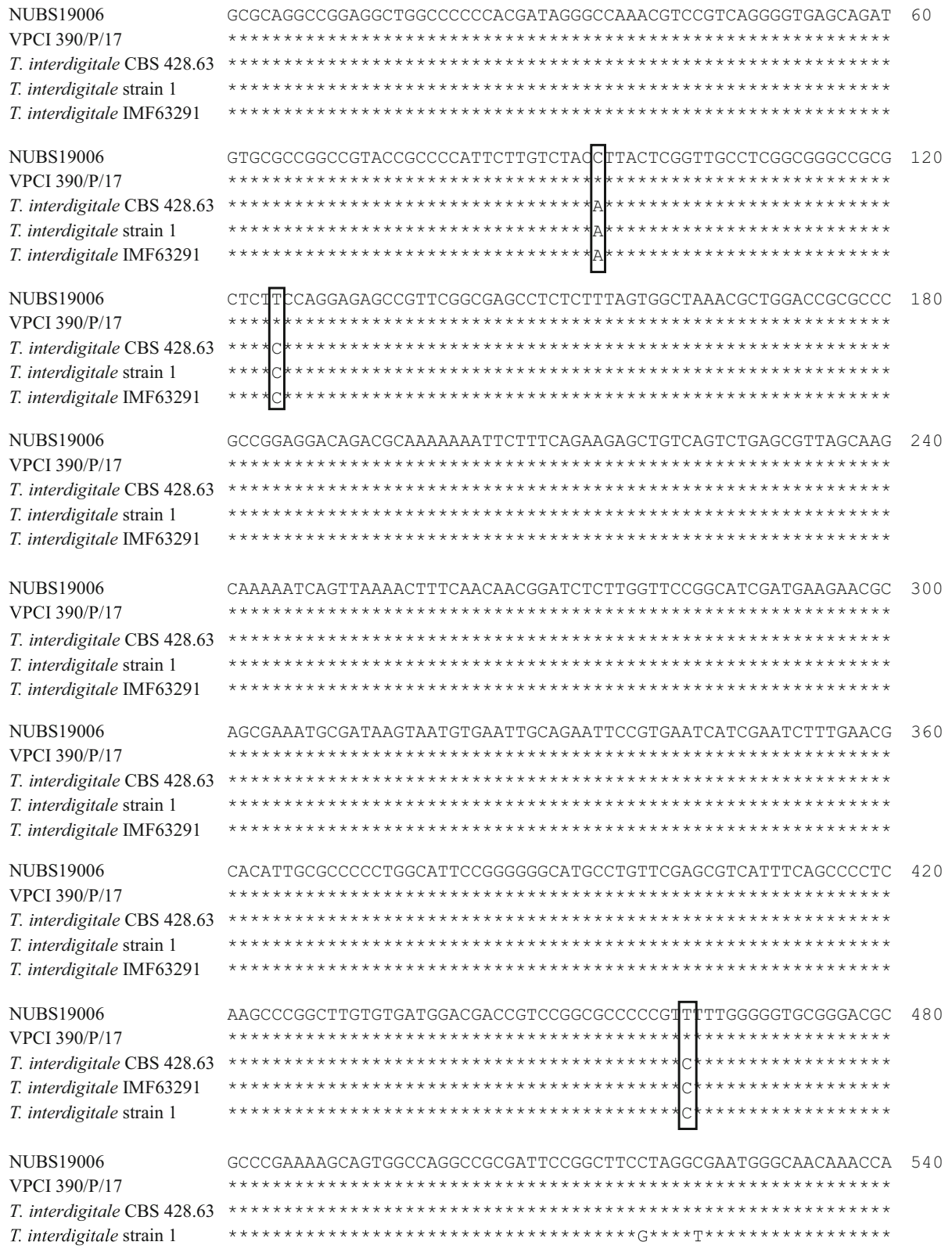


Fig. 3 Numerous subspherical to pyriform microconidia and occasional spiral hyphae were present. Macroconidia were abundant and cigar- to club-shaped, with three to four septa, and were smooth and thin-walled, measuring 6–8 × 20–50 µm with narrow attachment bases (**a** and **b**)



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T. interdigitale IMF63291   **********************************************************

NUBS19006                   GCGCCTCCAGGACCGGCCGCCCTGGCCTCAAAATCTGTTTTATACTTATC  590
VPCI 390/P/17               **************************************************
T. interdigitale CBS 428.63 **************************************************
T. interdigitale strain 1   **************************************************
T. interdigitale IMF63291   **************************************************
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Fig. 4 continued

Phylogenetic Relationships

This study was performed to clarify the phylogenetic relationships among the highly TRF-resistant clinical isolates (NUBS19006 and NUBS19007), *T. interdigitale* strain CBS 428.63, *T. mentagrophytes* strain CBS 318.56, *T. benhamiae* strain CBS 624,66 (MH858898), *T. rubrum* strain CBS 392.58 (XM_003239433), *T. tonsurans* strain CBS 385.68, Japanese isolates of *T. interdigitale*, and Indian strains. Phylogenetic analysis of the ITS region sequences revealed that the isolates (NUBS19006 and NUBS19007) were grouped in a cluster of Indian strains and were independent of the cluster of the *T. interdigitale* strain CBS 428.63, *T. mentagrophytes* strain CBS 318.56, *T. benhamiae* strain CBS 624,66 (MH858898), *T. rubrum* strain CBS 392.58 (XM_003239433), *T. tonsurans* strain CBS 385.68, and Japanese isolates of *T. interdigitale* (Fig. 5).

MICs of Antifungal Drugs

The MICs for NUBS19006 were $>$ 32 mg/L for TRF, 4 mg/L for CTZ, 8 mg/L for MCZ, $<$ 0.03 mg/L for LCZ, 0.03 mg/L for ITZ, and 0.5 mg/L for RVZ (Table 1). Similarly, the MICs for NUBS19007 were $>$ 32 mg/L for TRF, 0.06 mg/L for CTZ, 0.125 mg/L for MCZ, $<$ 0.03 mg/L for LCZ, 0.03 mg/L for ITZ, and 0.03 mg/L for RVZ (Table 1).

The mutation hotspot (Phe397Leu) of SQLE

In *SQLE*, the highly TRF-resistant strains (NUBS19006 and NUBS19007) encoded Leu at codon 397 instead of Phe (Phe397Leu) in the *T. mentagrophytes* strain TIMM2789 (GenBank accession number, KU242352).

Full Length of SQLE in the Isolate (NUBS19006)

The sequence of *SQLE* in NUBS19006 was determined to be 1470 bp, which encoded a protein of 489 amino acids, beginning with a putative initiating methionine (ATG).

The amino acid sequence of *SQLE* of NUBS19006 shared approximately 100% sequence similarity with the highly TRF-resistant *T. interdigitale* isolates in Delhi, India, harboring mutations in *SQLE* (GenBank accession number: AVU05318) in the conserved region.

The sequences determined in this study have been deposited in the GenBank database (*Trichophyton interdigitale* NUBS19006 squalene epoxidase (*SQLE*), complete cds: GenBank accession no. LC510258).

PCR Detection of Alpha-Box and HMG Genes Specific to NUBS19006 and NUBS19007

HMG was detected in NUBS19006 and NUBS19007, whereas the alpha-box gene was not detected in the isolates. Therefore, both strains were presumed to show the ($+$) mating type reaction in the mating test.

Urease Test and Hair Perforation Tests

The urease test on Christensen urease agar was positive for *T. mentagrophytes* and *T. interdigitale* after 7 days of incubation (Table 1 and Fig. 6). On the other hand, the type strain of *T. rubrum* CBS 100081 ^{T} and highly TRF-resistant strains (NUBS19006 and NUBS19007) were negative on Christensen urease agar after 7 and 14 days of incubation (Table 1 and Fig. 6).

T. rubrum CBS 100081 ^{T} and highly TRF-resistant strains (NUBS19006 and NUBS19007) were negative in the in vitro hair perforation tests. However, TRF-

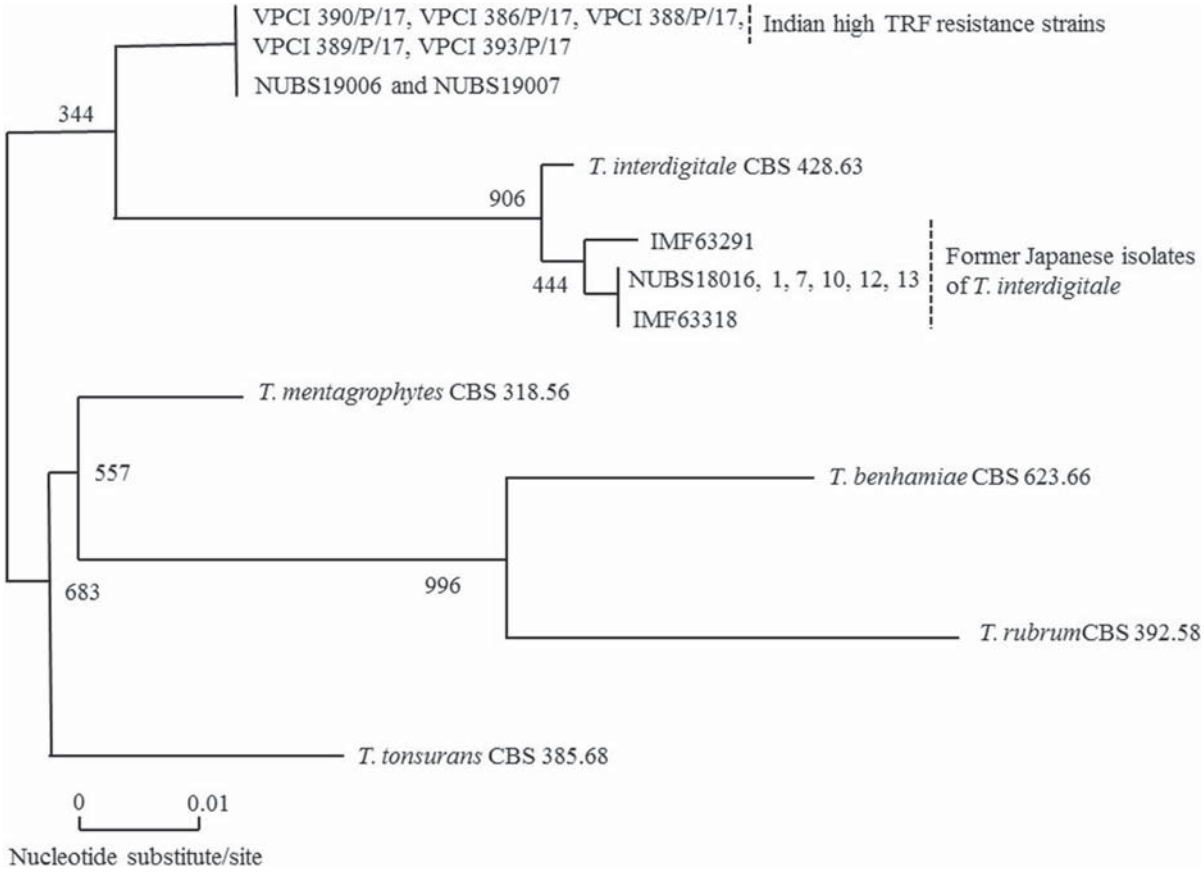

Fig. 5 A phylogenetic tree created with the neighbor-joining method showing the molecular taxonomy of the ITS regions. The numbers at the branches were determined by the bootstrap value and indicate the times in 1000 repeat sub-samples in monophylogenic grouping. TRF resistance of Indian dermatophyte strains VPCI 390/P/17 (GenBank accession no. MH990848), VPCI 386/P/17 (MH990848), VPCI 388/P/17 (MH990850), VPCI 389/P/17 (MH990851), and VPCI 393/P/17 (MH990853). Highly TRF-resistant isolates of NUBS19006 (LC508024) and NUBS19007 (LC508728). *T. mentagrophytes* CBS 318.56 (MH857656), *T. interdigitale* CBS 428.63 (NR_144900), *T. benhamiae* CBS 624,66 (MH858898), *T. rubrum*CBS 392.58 (XM_003239433), *T. tonsurans* CBS 385.68 (MH859163), Japanese former isolates of *T. interdigitale* strain IMF63291 (LC317810), *T. interdigitale* strain IMF63318 (LC31781), *T. interdigitale* strain 1 (LC508729), *T. interdigitale* strain 7 (LC508730), *T. interdigitale* strain 10 (LC508731), *T. interdigitale* strain 12 (LC508732), and *T. interdigitale* strain 13 (LC508733)

susceptible strains of *T. interdigitale* (1, 10, and 14) were positive.

Mating Activity

NUBS19006 produced several abortive gymnothecia in a cross with the (−) tester strain of *A. vanbreuseghemii* VUT 77,008 = RV 27,961 (−), and thus reacted as the (+) mating type.

Discussion

In this report, we described the isolation of highly TRF-resistant *T. interdigitale*-like strains from Nepali and Indian patients with tinea corporis in Japan. These strains (designated NUBS19006 and NUBS19007) exhibited a TRF MIC of > 32 mg/L but remained susceptible to ITZ and RCZ. These strains were isolated from patients who had been treated with TRF in Japanese hospitals; however, no response was seen. No highly TRF-resistant dermatophyte strain has been previously isolated in Japan. In our previous study, antifungal susceptibility testing was performed on *T.*

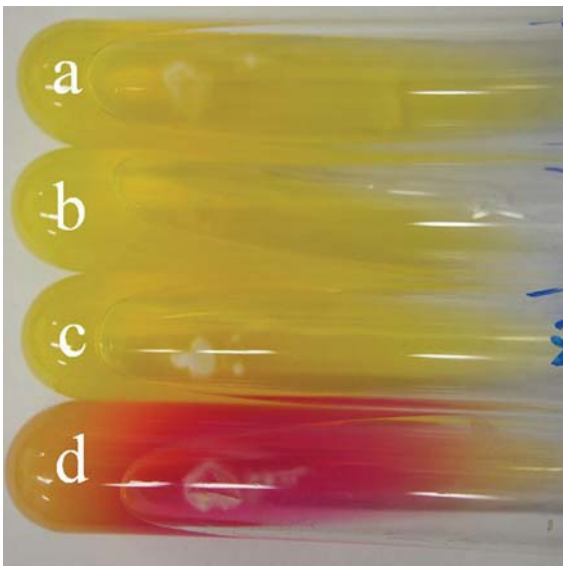

Fig. 6 The urease test on Christensen urease agar after 7 days of incubation. **a**, *T. rubrum* CBS 100081 ^{T}; **b**, NUBS19006; **c**, NUBS19007; **d**, *T. interdigitale* NUBS18016. The type strain of *T. rubrum* CBS 100081 ^{T} and highly TRF-resistant strains (NUBS19006 and NUBS19007) were negative on Christensen urease agar after 7 days of incubation

interdigitale isolates from Japanese patients (isolated in 2017–2018; 24 strains) to assess TRF susceptibility of these strains [15]. CLSI M38-A2 determinations revealed that the mean TRF MIC of 23 of the 24 strains was < 0.03 mg/L. Among these strains, one strain (NUBS18016) had a TRF MIC of 2 mg/L, confirming its resistance to TRF [15]. The predicted amino acid sequence of *SQLE* from the TRF-resistant strain (NUBS18016) was 100% identical to the *SQLE* sequence of the reference strain, *T. interdigitale*, indicating that no gene mutations were present in NUBS18016 [15].

On the other hand, NUBS19006 and NUBS19007 were genetically close (100% homology) to the TRF-resistant Indian dermatophyte strains and were recognized as harboring a missense mutation (Phe397Leu) in *SQLE*. Therefore, we identified these isolates as TRF-resistant Indian dermatophyte strains.

We investigated the molecular and mycological characteristics of the strains, whose morphology was the same as that of *T. interdigitale*. PCR detection of both *MAT* genes specifically indicated that NUBS19006 and NUBS19007 encoded only *HMG* and were presumed to have the (+) mating type reaction. Singh et al. reported that all high TRF-resistance Indian strains had *HMG gene* in their genomes and anticipated mating type (+) [4]. We also performed a mating test for NUBS19006 with (+) and (−) tester strains of *A. vanbreuseghemii*. Several abortive gymnothecia were observed in a cross with the (−) tester strain, and thus reacted as the (+) mating type. This mating reaction was also the same as that of *T. interdigitale* [18].

ITS sequence homology indicated that the strains were 99.5% identical to *T. interdigitale* strain CBS 428.63 and to Japanese isolates of *T. interdigitale.* At first glance, the strains were genetically within the intra-species level. However, the phylogenetic tree indicated that the strains were grouped in a cluster of Indian strains, and were independent of the cluster of *T. interdigitale* strains. Moreover, Singh et al. reported the sequence of the ITS region of the ribosomal DNA and determined that Indian strains are genetically distinct from other reference strains worldwide [3]. Singh et al. also performed genome sequencing and phylogenetic analysis of these Indian strains and concluded that they are distinct from the strains of *T. mentagrophytes/T. interdigitale* species of the genus [4]. They also treated the strains as a clonal population of "*Trichophyton* sp." [4].

T. tonsurans and *T. equinum* have been treated as different species [19] however, are indistinguishable from each other when tested by the standard morphological and physiological tests [20]. Kander et al. reported that 40 isolates (26 of *T. equinum* and 14 of *T. tonsurans*) harbored a T-SNP at position 18; all strains with a C-SNP at this position were human isolates of *T. tonsurans* [21]. The authors concluded the evolution of *T. tonsurans* and *T. equinum* must be relatively recent and the speciation process might not yet be complete [21].

In the ITS regions of this study, the isolates (NUBS19006 and NUBS19007) and all Indian TRF-resistant strains of *T. interdigitale* harbored three SNPs at position 94 (C), 125 (T) and 462 (T), whereas all strains with SNPs at position 94 (A), 125 (C) and 462 (C) were Japanese isolates and CBS 428.63 of *T. interdigitale* (Fig. 4). Our study indicated that three specific SNP sites were observed between Indian strains and other reference strains of *T. interdigitale.*

In this study, we observed negative urease activity and negative results in the hair perforation test for the strains. The urease test and the hair perforation test are classical phenotyping methods to differentiate *T.*

rubrum from *T. mentagrophytes*/*T. interdigitale* [1, 16, 17]. These results suggested that the strains may be regarded as a new clonal offshoot species from *T. mentagrophytes*/*T. interdigitale* species.

We also hesitated regarding whether to consider the strains as a variety of *T. interdigitale*. Historically, *T. interdigitale* had been treated as a variety of *T. mentagrophytes,* because *T. mentagrophytes* is close to *T. interdigitale*. *T. interdigitale* was subsequently separated from *T. mentagrophytes* following molecular analysis in 2017 [19]. To avoid confusion in the taxonomy for the *T. mentagrophytes*/*T. interdigitale* complex, we suggest that the highly TRF-resistant Indian strains should be considered a new species independent of *T. interdigitale* based on clinical and mycological features.

We expect that high-level resistance to other antifungal drugs will develop in *T. indotineae* strains that can readily be transmitted from human to human. Dermatologists should be cautious about the prevalence of dermatophytosis due to antifungal drug-resistant strains. Anti-fungal susceptibility of clinical isolates could be a major determinant of treatment outcome.

Taxonomy

Trichophyton indotineae R. Kano, U Kimura, M Kakurai, J Hiruma, H Kamata, Y Suga & K Harada, sp. nov. MycoBank number: MB 833488.

The species epithet "indotineae" refers to dermatophytosis in the country of India, where this species was epidemic.

Morphology

Good and rapid growth (approximately 5–6 cm in diameter) at 24 °C on SDA. On SDA, colonies were flat, white in color, with a suede-like surface, and yellowish to brown reverse pigment. Numerous subspherical to pyriform microconidia and occasional spiral hyphae were present.

Similarly, colonies of the isolate were flat, white to cream in color, with a powdery surface, and yellowish to brown reverse pigment on PDA. Numerous subspherical to pyriform microconidia and occasional spiral hyphae were present. Macroconidia were abundant and cigar- to club-shaped, with three to four septa, and were smooth and thin-walled, measuring 6–8 × 20–50 µm with narrow attachment bases.

Physiology

The urease test on Christensen urease agar was negative after 7 and 14 days of incubation at room temperature. The in vitro hair perforation test was negative after 4 weeks of incubation at room temperature.

Mating Type Test

NUBS19006 reacted as the (+) mating type but could not produce ascospores.

Molecular Characteristics (Type Strain)

The ITS region sequence of our isolate was deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (*Trichophyton interdigitale* NUBS19006 genes for ITS1, 5.8S rRNA, ITS2, partial and complete sequence: GenBank accession no. LC508024).

Habitat and Ecology

Trichophyton indotineae is an anthropophilic species that is frequently isolated from tinea corporis in India, Japan, and elsewhere. This species is highly TRF resistant and is epidemic in North India. A missense mutation (Phe397Leu) is recognized in squalene epoxidase (*SQLE*). These Indian strains are morphologically and genetically close to *T. interdigitale,* but were isolated predominately from tinea corporis with severe lesions.

Deposits

The holotype was isolated from human skin in Urayasu, Chiba, Japan and is preserved in the collection (herbarium) of the Medical Mycology Research Center (MMRC), Chiba University, Japan (IFM 66168). Ex-type cultures are deposited in public collections of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS 146623) = Postgraduate Institute of Medical Education & Research, Chandigarh, India (NCCPF IL4163) – culture ex-holotype.

Strains Studied

Ex-type strain NUBS19006 = IFM 6616 = CBS 146623 = NCCPF IL4163, paratypes = NUBS19007 = IFM 66169 = CBS 146624 = NCCPF 4164.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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