Mycopathologia (2017) 182:487–493
DOI 10.1007/s11046-017-0110-3

Relationship Between Phenotypic and Genotypic Characteristics of *Trichophyton mentagrophytes* Strains Isolated from Patients with Dermatophytosis

I. Dhib · I. Khammari · A. Yaacoub · F. Hadj Slama · M. Ben Saïd · R. Zemni · A. Fathallah

Received: 15 October 2016 / Accepted: 4 January 2017 / Published online: 18 January 2017

Abstract According to epidemiological, clinical and mycological criteria, it has long been admitted that the *Trichophyton mentagrophytes* species includes two varieties: a zoophilic variety (var. *mentagrophytes*) and an anthropophilic variety (var. *interdigitale)* that involve the upper and the lower part of the body, respectively. The further application of molecular techniques to the characterization of dermatophyte strains showed that this classification is unreliable. The aim of our study was to assess the usefulness of PCR–RFLP (restriction fragment length polymorphism) and sequencing in the characterization of *T. mentagrophytes* strains taken from Tunisian patients. The study was carried out in 2008 in the laboratory of Parasitology–Mycology of Farhat Hached University Hospital, Sousse, Tunisia. A total of 133 strains were isolated from 133 patients addressed to the laboratory for dermatological lesions very evocative of dermatomycosis. Eighty strains were isolated from lesions located on the lower part of the body (onychomycosis, tinea pedis) and 53 strains from the upper part of the body (tinea capitis, tinea corporis). All strains were submitted to mycological examination (direct microscopic examination and culture on Sabouraud medium) and further investigated by using RFLP analysis of the PCR-amplified ITS1-5.8 s-ITS2 region of the ribosomal DNA and the *MvaI* restriction enzyme. In addition, 62 strains were further submitted to a sequencing of the ITS1-5.8 s-ITS2 region. On the basis of mycological criteria, all strains were diagnosed as *T. mentagrophytes*. All strains produced the same RFLP pattern and were identified as *T. mentagrophytes interdigitale* regardless of the location of lesions. Out of the 62 sequenced strains, 16 were found anthropophilic and 46 were zoophilic. In conclusion, all strains provisionally diagnosed as *T. mentagrophytes* on the basis of mycological criteria were shown to belong to *T. interdigitale* by using PCR–RFLP and sequencing irrespective of the site of lesions. The predominance of zoophilic strains needs further investigation.

Keywords Dermatophytes · *Trichophyton mentagrophytes* · PCR–RFLP · Sequencing · Phenotypic features

I. Dhib (✉) · I. Khammari · A. Yaacoub · M. Ben Saïd · A. Fathallah
Parasitology-Mycology Laboratory, Faculty of Medicine, Farhat Hached University Hospital, Mohamed El Karoui Street, 4002 Sousse, Tunisia
e-mail: dhib.imen@yahoo.fr

F. Hadj Slama · R. Zemni
Immunology and Genetic Laboratory, Faculty of Medicine, Sousse, Tunisia

Introduction

Dermatophytes are the main causal agents of superficial mycoses in humans and animals. They are

usually identified on the basis of macroscopic appearance, together with microscopic examination of cultures. According to epidemiological, clinical and mycological criteria, it has long been admitted that the *T. mentagrophytes* species includes two varieties: a zoophilic variety (var. *mentagrophytes*) and an anthropophilic variety (var. *interdigitale*) that involve the upper and the lower part of the body, respectively [2, 7, 16]. The further development of molecular tools and techniques for the characterization of strains of the *T. mentagrophytes* complex [11] and the comparison between phenotypic and genotypic findings [14] showed that this classification was not appropriate [4, 6]. The aim of our study was to assess the usefulness of PCR–RFLP (*restriction fragment length polymorphism*) and sequencing in the identification of *T. mentagrophytes* strains taken from different body lesions in patients originating from Tunisia.

Materials and Methods

Fungal Strains

The study was carried out in 2008 in the laboratory of Parasitology-Mycology of Farhat Hached University Hospital, Sousse, Tunisia. It included 133 strains of the *T. mentagrophytes* complex: 129 were isolated from 129 patients originating from Sousse, addressed to the laboratory for dermatological lesions very evocative of dermatomycosis, 2 strains from 2 patients originating from Tunis (Northern Tunisia) and 2 from Sfax (Southern Tunisia).

Two reference strains from CBS center (Central Bureau voor Schimmel cultures CBS Utrecht, Netherlands) were included in the study: *T. interdigitale* CBS 165.66 and *T. mentagrophytes* CBS 106.67.

Fungal strains were divided into two groups: 80 strains were isolated from lesions located on the lower part of the body (toes onychomycosis, tinea pedis), designed Ginf, and 53 strains from the upper part of the body (tinea capitis, tinea corporis), designed Gsup.

Morphological Identification

The identification of *T. mentagrophytes* strains was made on the basis of macroscopic and microscopic growth criteria. All isolates were cultured in tubes on Sabouraud agar with chloramphenical (0.5 g/L) and cycloheximide (0.5 g/L) at 27 °C for 3 weeks. The cultures were examined for macroscopic characters including texture colony and pigmentation. Microscopic examination was performed according to the standard procedure using adhesive tape and lactophenol blue stain. Strains were identified as members of the *T. mentagrophytes* complex according to the following criteria:

- The powdery to cottony texture.
- The color of colonies and reverse pigmentation.
- The production of round to broadly clavate microconidia.
- The formation of cigar-shaped macroconidia and spiral hyphae.

Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) of ITS Regions

Extraction of genomic DNA was performed using the rapid mini preparation method previously described by Liu et al. [10] with minor modifications. In brief, a small lump of mycelia was added to 500 µL of lysis buffer (400 mM Tris–HCl pH 8; 60 mM EDTA pH 8; 150 mM NaCl; 1% SDS) and 150 µL of potassium acetate 5 M.

The tube was vortexed and centrifuged at 6000 t/min × 3 min. Isopropyl alcohol was added to the supernatant (w/w) and the solution was centrifuged at 6000 t/min × 3 mn. The resultant DNA pellet was washed in 70% ethanol and dissolved in 50 µL Tris–EDTA buffer.

PCR was performed using the universal primers Mas 266 (5′ GCA TTC CCA AAC TCG ACTC 3′) and V9D (5′ TTA CGT CCC TGC CCT TTG TA 3′) amplifying a DNA fragment of approximately 1 Kb.

For RFLP, the amplicon was digested with the restriction enzyme *MvaI* (Promega, Madison, WI, USA) for 2 h at 60 °C. The resulting restriction fragments were separated electrophoretically on 3% agarose gel for 60 V at 90 min and visualized under UV light after ethidium bromide staining. Gel photography was obtained using camera (Biometra, Germany).

Sequencing

Out of the 133 strains, 62 were submitted to sequencing of the ITS1-5.8S and ITS2 regions ITS of rDNA.

They include 22 strains from toes onychomycosis, 6 from tinea pedis, 12 from tinea capitis and 22 from tinea corporis. Fifty-eight isolates were from patients living in Sousse, 2 in Sfax and 2 in Tunis. Animal contact could be documented for 43 of them: 9 with cats, 3 with chicken, 12 with dogs, 10 with rabbits and 9 with sheep. PCR products were purified using the Big-Dye® Terminator v1.1 cycle sequencing kit (Applied Biosystems). The sequence analysis was performed using the ABI seq-scape 2.0 software (Applied Biosystems) (http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html) [18].

Statistical Analysis

In order to compare the phenotypic and genotypic features of *T. mentagrophytes* strains, all the collected data were processed in the SPSS database computer program version 11.5 (SPSS Inc, Chicago, USA). The results were analyzed using the Chi-square test. A P value of $<0.05\%$ was considered to be significant.

Results

Mycological Identification of Strains

Detailed morphological characteristics of the 133 strains are shown in Table 1. All clinical isolates from both Ginf and Gsup groups were provisionally identified as *T. mentagrophytes* on the basis of macroscopic and mycological features. Colonies of most strains were powdery ($P < 0.001$) with no difference between both groups (88.1 for Gsup vs. 87.5% for Ginf). Colonies yielded by strains of both groups were mostly white (84.9 for Gsup vs. 85% for Ginf). The reverse of most strains was colorless or had a yellow pigment. A red to brown reverse pigment was present in 20.7 and 31.1% of strains of groups Gsup and Ginf, respectively. Microconidia were abundant to very abundant in strains of both groups. They were spherical in 77.3 and 66.3% of strains of Gsup and Ginf, respectively. Macroconidia were present in nearly 50% of strains (45.4 for Gsup vs. 42.5% for Ginf). They were abundant in 35.8% of Gsup strains and 30% of Ginf strains. Thus, the phenotypic features of strains isolated from Gsup and Ginf were very similar.

RFLP and Sequencing

PCR–RFLP

All the 133 strains gave the expected 1-kb band and produced the same RFLP pattern after digestion with *MvaI* (Fig. 1). They were identified as *T. interdigitale*, whatever the location of the lesion (superior or inferior part of the body).

Sequencing

The sequences of all 62 strains showed an identity with the ITS regions of the *T. interdigitale* strain CBS165.66 registered in NCBI database. On the other hand, the sequence alignment of the ITS regions of 16 *T. mentagrophytes* strains (2 from Tunis, 2 from Sfax and 12 from Sousse) showed 100% homology with the anthropophilic *T. interdigitale* strain AF506033. The sequence alignment of 46 *T. mentagrophytes* strains from Sousse showed 100% homology with the zoophilic type III* *T. interdigitale* strain FM986758 (Fig. 2). The sequences of 20 strains are registered in GenBank with accession numbers from KU921371 to KU921390.

Relationship Between Phenotypic and Genotypic Characters

RFLP analysis of all the 133 strains yielded a unique pattern composed of five bands (Fig. 1). This pattern is identical to that of the *T. interdigitale* CBS165.66 reference strain, regardless of the phenotypic features and the location of lesions (Gsup or Ginf). Furthermore, the 62 sequenced strains were confirmed as *T. interdigitale*. The comparison of phenotypic traits showed no differences between the 16 anthroponotic and the 46 zoonotic strains isolated from the upper and the lower parts of the body.

Discussion

In this study, 133 strains of dermatophytes isolated from Tunisian patients with variable dermatophyte lesions, provisionally identified as *T. mentagrophytes* on the basis of morphological criteria, were further characterized by PCR–RFLP and sequencing of ITS regions which are reported to be the more

Table 1 Phenotypic features of 133 *T. mentagrophytes* strains: 53 from Gsup lesions and 80 from Ginf

Colony	Lesion site morphology	Gsup N (%)	Ginf N (%)	*P*
Texture of colonies	Powdery cottony	47 (88.7)	70 (87.5)	⋘3.84 (NS)
		6 (11.3)	10 (12.5)	
Color of colonies	White	45 (84.9)	68 (85)	
	Cream	8 (15.1)	12 (15)	
Reverse pigment	Red to brown	11 (20.7)	25 (31.1)	
	Colorless to yellow	42 (79.3)	55 (68.7)	
Microconidia shape	Spherical	41 (77.3)	53 (66.3)	
	Pyriform + spherical	12 (22.7)	27 (33.7)	
Abundance of microconidia	+/++	15 (28.3)	27 (33.7)	
	+++/4+	38 (71.7)	53 (66.3)	
Abundance of macroconidia	Absent	24 (45.4)	34 (42.5)	
	± to +	10 (18.8)	22 (27.5)	
	++/+++	19 (35.8)	24 (30)	
Spiral hyphae	Absent	3 (5.6)	6 (7.5)	
	Spiral hyphae	50 (94.4)	74 (92.5)	
Abundance of spiral hyphae	+/++	40 (80)	66 (89)	
	+++/++++	10 (20)	8 (11)	

NS no significant value. ± to + rare; +/++ or ++/+++ abundant; +++/4+ very abundant

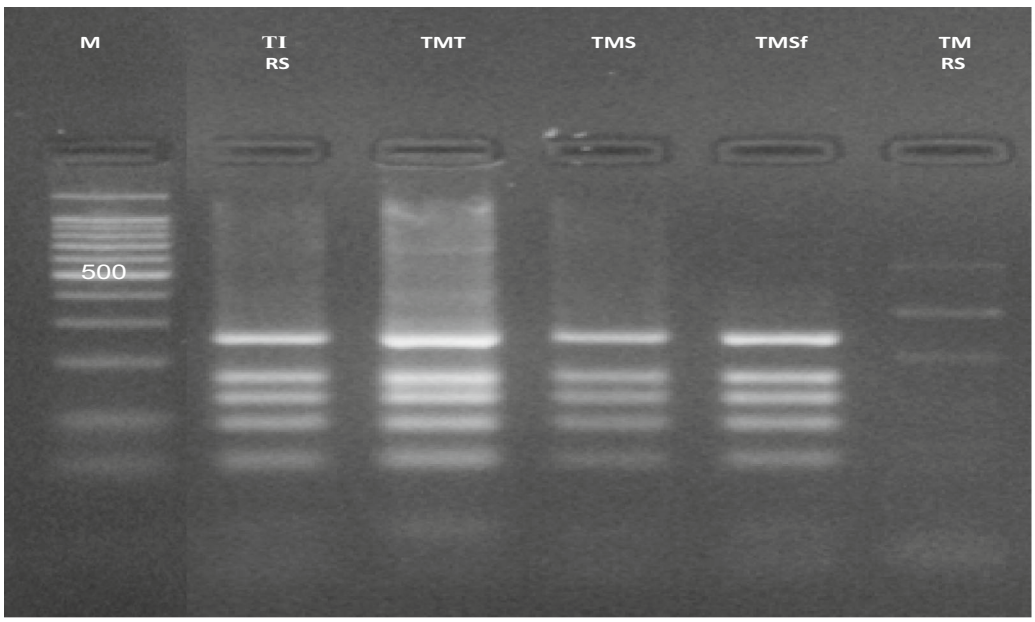

Fig. 1 Restriction fragment length polymorphism patterns of internal transcribed spacer-polymerase chain reaction products digested with the restriction enzyme *MvaI* of *T. mentagrophytes*. *M* molecular size marker 100 pb, *TI RS* reference strain of *T. interdigitale*, *TMT T. mentagrophytes* strains from Tunis, *TMS T. mentagrophytes* strains from Sousse, *TMSf T. mentagrophytes* strains from Sfax, *TM RS* reference strain of *T. mentagrophytes*

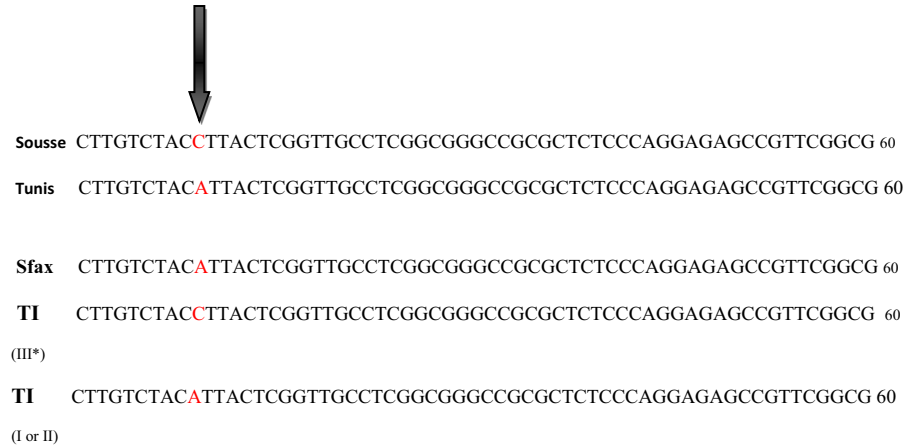

Fig. 2 Sequence alignment of *T. mentagrophytes* strains from Sousse, Tunis and Sfax with *T. interdigitale*: FM986758 (type III*) and *T. interdigitale*: AF506033 and AF506036 (type I and II). Shows the A > C mutation between anthropophilic and zoophilic sequences

suitable techniques for the characterization of dermatophyte strains [11, 14, 15, 21]. All types of strains were identified as *T. interdigitale* irrespective of the lesion site. Our findings are in agreement with most recent reports and with the new classification criteria of dermatophytes causing pathology in humans [4, 8, 16]. The ancient distinction between the strains involving the upper part of the body regarded as zoophilic and named *T. mentagrophytes* var. *mentagrophytes* and those involving the lower part of the body considered as anthropophilic and named *T. mentagrophytes* var. *interdigitale* [2, 12, 20] is no more valid. So that nearly all human strains of the *T. mentagrophytes* complex are now considered to belong to *T. interdigitale* species [3, 4, 13, 16].

In our study, mycological characteristics of strains (texture and color of colonies, reverse pigment, abundance of conidia and of spiral hyphae) obtained from lesions located in the upper part of the body (Gsup) and those obtained from lesions located in the lower part of the body (Ginf) were very similar and no statistical difference could be demonstrated between both groups. Our results are in agreement with those of Takashi [14] and Nenoff [16] who found no relation between morphology of strains and the location of the lesions (lower vs. upper part of the body).

Our findings are, however, in contrast with some other previous studies where the *T. mentagrophytes* var. *interdigitale* strains were reported to be cottony as compared to those of the *T. mentagrophytes* var. *mentagrophytes* characterized by a much more granular texture [2, 9]. These conflicting results argue for the unreliability of morphological criteria and for the need of molecular techniques for the correct characterization of strains and species.

In our study, no variability between strains was demonstrated as all 133 *T. mentagrophytes* strains showed the same profile in RFLP which was identical to the *T. interdigitale* CBS165.66 strain. Gupta [5] described two different profiles Tm1 and Tm2 by using RFLP of the amplified 18rDNA and ITS regions. Takashi [14] divided *T. mentagrophytes* in 12 types (P1–P12) by using Southern blot PCR–RFLP of NTS region and established a correlation between types and macroscopic features. At the same time, Ninet [17], by sequencing ITS regions of rDNA, identified three types (I, II, III) in *T. mentagrophytes* strains but without difference in phenotypic traits between the three types. Later, Heidmann [6] reported that *T. interdigitale* strains could be divided into five different types (I, II, III, III* and IV) on the basis of the sequences of ITS regions; I and II types being anthropophilic and III, III* and IV being zoophilic.

In our study, the sequencing of 62 out of the 133 *T. mentagrophytes* strains showed that 46 were identical to the zoophilic *T. interdigitale* FM986758 strain, while the 16 remaining strains were identical to the anthropophilic *T. interdigitale* AF506033 strain. It is to note that the four strains from Tunis and Sfax were anthropophilic, while the majority of strains isolated from Sousse patients were zoophilic. This finding argues for the occurrence of regional differences between strains causing dermatophytosis in Tunisia. It requires, however, further investigations by analyzing additional strains. Our results are somewhat in contrast with previous reports where it is stated that most

strains of *T.interdigitale* causing lesions in humans are of anthropophilic origin [2, 3].

Kim in [8] reported that RAPD profiles of *T. mentagrophytes* strains were different, according to the colonies' texture of studied isolates and showed that colonies of strains of animal origin had a characteristic granular texture in contrast to the anthropophilic strains which were powdery or cottony. Similar findings were reported by Arabatzis in [1] and Van Rouij [19] who showed that zoophilic strains isolated from rabbits were phenotypically monomorph with granular colonies, red brown reverse, a higher number of microconidia and less macroconidia than anthropophilic strains. In contrast, Heidmann et al. [6] showed that colonies of *T. interdigitale* strains were either powdery or cottony and this, in the same proportion, whatever they were anthropophilic or zoophilic; and no relation between macroscopic characteristics and origin of strains was demonstrated. Our results are similar to those of Heidmann et al. [6] as the majority of our strains had a powdery texture irrespective of their origin, which could only be determined by sequencing.

Kac in [7], Kim in [8] and Takashi in [14] found no differences between strains' profiles in PCR RFLP and RAPD when lesion location was considered. In contrast, Ninet et al. [17] showed that toes' onychomycosis and tinea pedis are caused by type I and II strains, while lesions of the upper part of the body are caused by strains of type III. Similar findings were reported by Heidmann et al. [6] who showed that lesions of the upper part of the body are caused by the zoophilic type III and III* strains, while the anthropophilic strains of type I and II mainly causes toes' onychomycosis and tinea pedis. This study showed for the first time that tinea corporis and tinea pedis/onychomycosis are caused by ecologically different strains (zoophilic and anthropophilic origin). In our study, the sequencing of 62 strains showed that lesions are caused by either anthropophilic or zoophilic strains whatever their location (upper vs. lower part of the body).

Conclusion

Our study confirms that mycological criteria are not reliable for the characterization of strains of the *T. mentagrophytes* complex and that PCR RFLP and sequencing are much more appropriate for this purpose. Actually, all of our strains identified as *T. mentagrophytes* on the basis of morphological criteria were shown to belong to the *T. interdigitale* species whatever the site of lesion. The high proportion of zoophilic strains needs further confirmation.

Acknowledgements This work was supported by the 04/UR/08-05. Research Unit, from the Ministry of Health, Tunisia.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Arabatzis M, Xylouri E, Frangiadaki I, Tzimogianni A, Milioni A, et al. A rapid detection of *Arthroderma vanbreuseghemii* in rabbit skin specimens by PCR-RFLP. Eur Soc Vet Dermatol. 2006;17:322–6.
2. Badillet G. Dermatophytosis and dermatophytes. *Encycl Méd Chir* 1994 (Paris-France), maladies infectieuses, 8-614-A-10, Pédiatrie, 4-110-A-10, 14p [in French, Dermatophyties et dermatophytes].
3. De Baere T, Summerbell R, Theelen B, Boekhout T, Vaneechoutte M. Evaluation of internal spacer 2-RFLP analysis for the identification of dermatophytes. J Med Microbiol. 2010;59:48–54.
4. Gräser Y, Scott J, Summerbel R. The new species concept in dermatophytes- a polyphasic approach. Mycopathologia. 2008;166:239–56.
5. Gupta AK, Kohli Y, Summerbell RC. Variation in restriction fragment length polymorphism among serial isolates from patients with *Trichophyton rubrum* infection. J Clin Microbiol. 2001;39:3260–6.
6. Heidmann S, Monod M, Gräser Y. Signature polymorphisms in the internal transcribed spacer region relevant for the differentiation of zoophilic and anthropophilic strains of *Trichophyton interdigitale* and other species of *T mentagrophytes*. Br J Dermatol. 2010;162:282–95.
7. Kac G, Bougnoux ME, Feuilhade DCM, Sene S, Derouin F. Genetic diversity among *Trichophyton mentagrophytes* isolates using random amplified polymorphic DNA method. Br J Dermatol. 1999;140:839–44.
8. Kim JA, Takahashi Y, Tanaka R, Fukushima K, Nishimara K. Identification and subtyping of *Trichophyton mentagrophytes* random amplified polymorphic DNA. Mycoses. 2001;44:157–65.
9. Koenig H. Dermatophytes in "medical mycology instructions". Ellipse-Edition 1995;97–134 [In French, Guide de mycologie médicale].
10. Liu D, Coloe S, Baird R, Pederson J. Rapid mini-preparation of fungal DNA for PCR. J Clin Microbiol. 2000;38:471.
11. Makimura K, Takashi M, Atsuhiko H, Katsuhisa U, Hiuga S, et al. Phylogenetic classification of *Trichophyton mentagrophytes* complex strains based on DNA sequences of

nuclear ribosomal internal transcribed spacer 1 region. J Clin Microbiol. 1998;36:2629–33.
12. Masri-Fridling GD. Dermatophytosis of the feet. In: Mervyn L, Elgart MD, editors. Dermatologic clinics, cutaneous mycology, vol. 14. W.B Saunders company; 1996. Pp. 33–36.
13. Mochizuki T, Watanabe S, Uehara M. Genetic homogeneity of *Trichophyton mentagrophytes* var. interdigitale isolated from geographically distant regions. J Med Vet Mycol. 1996;34:139–43.
14. Mochizuki T, Ishizaki H, Barton RC, Moore MK, Jackson CJ, et al. Restriction fragment length polymorphism analysis of ribosomal DNA intergenic regions is useful for differentiating strains of *Trichophyton mentagrophytes*. J Clin Microbiol. 2003;41:4583–8.
15. Monod M, Bontems O, Zaugg C, Léchenne B, Fratti M, et al. Fast and reliable PCR/sequencing/RFLP assay for identification of fungi in onychomycoses. J Med Microbiol. 2006;55:1211–6.
16. Nenoff P, Herrmann J, Gräser Y. *Trichophyton mentagrophytes* sive *interdigitale*? A dermatophyte in course of time. J Dtsch Dermatol Ges. 2007;5:198–202.
17. Ninet B, Jan I, Bontems O, et al. Identification of dermatophyte species by 28S ribosomal DNA sequencing with a commercial Kit. J Clin Microbiol. 2003;41:826–30.
18. Tatusova TA, Madden TL. Blast 2 sequences, a new tool for comparing protein and nucleotide sequences. FEMS Microbiol Lett. 1999;174:247–50.
19. Van Rooij P, Detandt M, Nolard N. *Trichophyton mentagrophytes* of rabbit origin causing family incidence of kerion: an environmental study. Mycoses. 2006;49:426–30.
20. Weitzman I, Arvind A. Dermatophytes: gross and microscopic. In: Mervyn L, Elgart MD, editors. Dermatologic clinics, cutaneous mycology, vol. 14. W.B Saunders company; 1996. Pp. 9–12.
21. White TC, Oliver BG, Gräser Y, Henn MR. Generating and testing molecular hypotheses in the dermatophytes. Eukaryot Cell. 2008;7:1238–45.