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Diagnostic value of morphological, physiological and biochemical tests in distinguishing *Trichophyton rubrum* from *Trichophyton mentagrophytes* complex

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The two most frequently encountered dermatophyte etiologic agents of glabrous skin and nail dermatophytoses are *Trichophyton rubrum* and *T. mentagrophytes*. This study was aimed to discuss the efficacy of morphological, physiological and biochemical diagnostic tests commonly used in the identification of *T. rubrum* and members of the *T. mentagrophytes* complex. In this study, we evaluated; hydrolysis of urea in broth and on urea agar slants and Petri plates incubated at 22°C, 28°C and 37°C, *in vitro* hair perforation (blond child, sheep and goat hair), pigment production on cornmeal dextrose agar (CMDA) and bromcresol purple-milk solids-glucose agar (BCP-MS-G), Tween opacity, sorbitol assimilation, and salt tolerance. Additionally, the production of micro- and macroconidia was investigated by using brain heart infusion agar (BHIA), Christensen's urea agar in Petri plates (UPA), CMDA, Lowenstein-Jensen agar (LJA), malt extract agar, oatmeal agar, Oxoid chromogenic Candida agar, and potato dextrose agar. All cultures were incubated at 28°C, and conidial production was compared on days 5, 10 and 15. It was found that the urea hydrolysis test yielded more rapid and significant results when urea medium was prepared in Petri plates and incubated at 28°C ($P < 0.01$). LJA supported the highest production of microconidia after 15 days ($P < 0.001$). Additionally, it was found that *T. rubrum* strains produced red pigment on CMDA ($P < 0.01$) and BCP-MS-G, while strains of the *T. mentagrophytes* species complex did not. A special algorithm containing the various test procedures employed in these studies is presented which was found to be useful in the differentiation of *T. rubrum* strains from *T. mentagrophytes* complex. Our results revealed that UPA, CMDA, BCP-MS-G, LJA, and BHIA may be used as common mycological agars in routine practice.

Keywords Dermatophytes, Lowenstein-Jensen agar, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, urea Petri agar

Introduction

Currently there has been a rapid and striking change in dermatophyte taxonomy as a result of the introduction of new techniques such as the sequencing of the internal transcribed spacer region. Most of the earlier varietal names were found to have been invalidly described or to have been reported on the basis of minor characteristics such a color variations in their *in vitro* appearance. Furthermore, some of these names are now considered to represent species rather than varieties [1–5]. In other studies volatile fingerprints [6] and enzyme activities [7] were used with significant success in the rapid identification of *Trichophyton* spp.

In most mycology laboratories, these keratinophilic fungi are identified to species on the basis of colony

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characteristics, microscopic morphologies, growth requirements, and physiological and biochemical test results [8–11]. Due to the resources requirements of various molecular identification procedures and equipment, they are of little practical use except in mid-to-high level reference laboratories [11]. Furthermore, as changes are observed for a single dermatophyte species in its morphology (colony pattern, pigments and growth rate) and physiology (hydrolysis of urea and *in vitro* hair perforation), as well as in its biochemistry (sorbitol assimilation) and genotype (rRNA and mating patterns), the reliability of the conventional methods noted above are questioned [8–11]. Moreover, such isolates are problematic with respect to their proper identification in a clinical laboratory. Many dermatophyte species maintained under laboratory conditions lose their original colonial features and ability to form macro- and microconidia. This is especially true for *Trichophyton rubrum* and members of the *T. mentagrophytes* complex when recovered from chronic infections being treated with various antifungal agents as they often do not manifest their typical colonial morphology, pigmentation and production of micro- and macroconidia.

Recent studies have demonstrated that *T. rubrum* is composed of two anamorphic taxa, i.e., *T. rubrum,* and *T. violaceum* and that genotypes of *T. rubrum* show a worldwide distribution excluding the African continent [4,5]. On the other hand, such microorganisms like *T. rubrum* with *fischeri, kanei,* and *raubitschekii* morphotypes are genetically indistinguishable [4]. Gräser *et al*. [12] studied a set of *T. rubrum* strains including phenotypic varieties like *T. rubrum* var. *nigricans,* and by using molecular tools, could not detect any DNA differences among the varieties. On the other hand, the *T. mentagrophytes* complex is a group of related species formerly identified under this familiar name, but is now distinguishable through sequence-based studies into three distinct phylogenetic complexes, i.e., *Arthroderma vanbreuseghemii/T. interdigitale, A. benhamiae/T. erinacei* and *T. mentagrophytes* per se (now taxonomically classified as the former *T. mentagrophytes* var. *quinckeanum*) [2,3,13].

One of the prominent problems observed in mycology laboratories is distinguishing *T. rubrum* strains from members of the *T. mentagrophytes* species complex by using conventional diagnostic methods. The present study aimed to (i) discuss morphological, physiological and biochemical procedures used in the identification of both species, and (ii) measure the reliability of such procedures in routine laboratory practices.

Material and methods

Fungal strains

Table 1 lists the 18 *T. rubrum* isolates, 12 members of the *T. mentagrophytes* complex and 4 *Arthroderma* strains used in this study, as well as their origins and reference numbers in the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands. All strains were subcultured on Sabouraud's glucose agar (SGA, Merck, Darmstadt, Germany) at 26°C for 3 weeks. All tests were carried out from August 2006 to December 2007 at the Division of Mycology, Department of Microbiology, Faculty of Medicine, University of Cukurova.

Hydrolysis of urea

The ability to hydrolyse urea provides additional data to aid in distinguishing the typical, cosmopolitan form of *T. rubrum* (urease-negative) from; (1) members of the *T. mentagrophytes* complex (typically urease-positive, except *T. erinacei* known as hedgehog fungus), (2) the urease-positive 'Afro-Asiatic' or 'granular' variety of *T. rubrum*, formerly often called *T. raubitschekii*, and (3) *T. rubrum* with '*megninii*' morphotype [8–11,14,15]. In general, *T. mentagrophytes* hydrolyses urea ($\leq$7 days) much earlier than *T. rubrum* ($>$7 days) [15]. In this study, Christensen's urea agar (Oxoid, Hampshire, England) both in slants and in Petri plates, as well as urea broth were used. After the urea media were inoculated, the cultures were incubated at 22°C, 28°C, and 37°C for up to 4 weeks. The color change of the media from orange or pale pink to purple-red indicated positive results, i.e., the presence of urease. The data from the three different media and three incubation temperatures were compared with each other. In addition, the micromorphology of colonies on urea Petri agar (UPA) were examined.

In vitro *hair perforation test*

This test distinguishes between atypical isolates of the *T. rubrum* and *T. mentagrophytes* complex. Hairs exposed to *T. mentagrophytes* complex members show wedge-shaped perforations perpendicular to the hair shaft (a positive test result), whereas isolates of *T. rubrum* lack this ability to form perforations. Short strands of blond hair from children under 5 years of age, as well as hair samples from sheep and goats were placed in Petri plates and autoclaved at 121°C for 10 min. Twenty-five ml of sterile distilled water and 2 or 3 drops of a sterile solution of 10% yeast extract were then added to the plates. The plates were inoculated

Table 1 Strains analysed in this study.

Species	Reference no.	Strain-dependent deviation	Clinical picture	Geography
T. rubrum				
T. fischeri	CBS 100081	Abundant sporulation	Contaminant	–
T. fluviomunionse	CBS 592.68	–	Human, skin	Rio Muni, Guinea
T. kanei	CBS 289.86	Microconidia absent	Human, buttock	Ontario, Canada
T. kuryangei	CBS 517.63	–	Child, tinea capitis	Congo
T. kuryangei	CBS 518.63	–	–	–
T. kuryangei	CBS 422.67	–	Human, tinea capitis	Zaire
T. megninii	CBS 389.58	L–Histidine requirement	Man, nail	Utrecht, Netherlands
T. pervesii	CBS 303.38	–	Child, tinea capitis	D'Adrar Sahara, Mauretania
T. raubitschekii	CBS 202.88	Abundant MA; urease$^+$	Human, tinea pedis	Ontario, Toronto, Canada
T. raubitschekii	CBS 287.86	Abundant MA; urease$^+$	Human, skin	Toronto, Canada
T. raubitschekii	CBS 100084	Abundant MA; urease$^+$	Human, skin	Canada
T. raubitschekii	CBS 102856	Abundant MA; urease$^+$	Human thumb nail	Cameroon, Italy
T. rodhainii	CBS 376.49	–	Human, tinea cruris	Congo
T. rubrum	CBS 363.53	–	Man	–
T. rubrum	CBS 392.58	–	Human, tinea pedis	Rotterdam, Netherlands
T. rubrum	CBS 302.60	–	Man, skin	Netherlands
T. rubrum	CBS 363.62	–	Man, nail	Netherlands
T. r. var. *nigricans*	CBS 100237	Melanoid pigmentation	–	–
T. mentagrophytes complex				
T. asteroides	CBS 424.63	–	Man, arm	Haarlem, Netherlands
T. erinacei	CBS 344.79	Urease$^-$	Man, skin, arm	Netherlands
T. erinacei	CBS 511.73	Urease$^-$	*Erinaceus europaeus* (hedgehog)	New Zealand
T. erinacei	CBS 677.86	Urease$^-$	Man, nail	Germany
T. interdigitale	CBS 428.63	–	Human, skin of foot	Netherlands
T. interdigitale	CBS 558.66	–	Human, skin of foot	Netherlands
T. langeronii	CBS 764.84	–	Skin of camel	Saudi Arabia
T. mentagrophytes	CBS 318.56	–	Human, deep trichophytosis	Netherlands
*T. m.*var. *mentagrophytes*	CBS 110.65	–	Man, pubic hair	Netherlands
*T. m.*var. *mentagrophytes*	CBS 160.66	–	Man, skin, arm	Netherlands
T. papillosum	CBS 347.55	–	Human	France
T. quinckeanum	CBS 572.75	–	Man, skin of leg	Germany
***Arthroderma* strains**				
A. benhamiae (MT-)	CBS 807.72	–	Man	Spain
A. benhamiae (MT+)	CBS 808.72	–	Man	South Africa
A. simii (MT−)	CBS 417.65	–	Chicken	India
A. simii (MT+)	CBS 448.65	–	Poultry	India

MA, macroconidia; +, positive; −, negative.

with several fragments of the test fungi from SGA cultures. The test materials were incubated for 4 weeks at 28°C and hairs examined in lactophenol cotton blue (LCB, Fluka, France) mounts at regular intervals with a light microscope [8–11,16].

Pigment production on Cornmeal dextrose agar (CMDA) and Bromcresol purple-milk solids-glucose agar (BCP-MS-G)

CMDA (Difco, Detroit, USA) medium can be used to differentiate members of the *T. mentagrophytes* complex from *T. rubrum* on the basis of pigment production. Most *T. rubrum* isolates form deep wine-red reverse pigmentation when grown on this medium, while *T. mentagrophytes* complex colonies show variable pigmentation ranging from uncolored to reddish-brown [8,15]. The type of growth (profuse versus restricted) and a change in the pH indicator (BCP) indicating alkalinity are additional useful characteristics. On BCP-MS-G (Himedia, Mumbai, India), *T. rubrum* strains show restricted growth and produce no alkaline reaction, whereas members of the *T. mentagrophytes* complex typically show profuse growth and alkaline reactions [8,10,11,17,18].

Tween opacity test

The medium was prepared with 10 g peptone, 5 g of NaCl, 0.1 g $CaCl_2$, and 15 g of agar per liter distilled water. After the autoclaved medium cooled to about

50°C, 5 ml of autoclaved Tween 80 was added to it. The Tween opacity test was considered positive when a halo of crystals was detected macroscopically or microscopically around a colony after a period of 4 weeks [19].

Sorbitol assimilation

The medium was prepared with 3 g beef extract, 10 g peptone, 5 g NaCl, and 1 cm^3 bromthymol blue per liter distilled water with a final pH of 7.2, then autoclaved at 121°C for 15 min. Then a 10% sorbitol solution was prepared and added at 1/10 to the mentioned solvent [20].

Salt tolerance

Sabouraud glucose agar was supplemented with 3%, 5%, 7% and 9% NaCl, and was used to detect species-specific salt tolerance, as well as the stimulation of macroconidia formation [8,21].

Morphologic examination

The presence of micro- and macroconidia was investigated by inoculating a portion of growth from stock cultures to brain heart infusion agar (BHIA), UPA, CMDA, Lowenstein-Jensen agar (LJA), malt extract agar (MEA), oatmeal agar (OA), Oxoid chromogenic Candida agar (OCCA), and potato dextrose agar (PDA). All cultures were incubated at 28°C and conidial formation was compared on the media 5, 10 and 15 days after inoculation. Morphology was examined through light microscopic examination of LCB stained portions of growth from each of the media. All strains were screened twice for morphological, physiological and biochemical studies.

Statistical analysis

Statistical analysis was performed using the statistical package SPSS v 10.0 and Epi Info version 3.2. The categorical data between groups were analysed by using a Chi square test and Cochran's Q test. A P value of <0.05 was considered statistically significant.

Results

The results of urea hydrolysis in different media at various incubation temperatures are illustrated in Table 2. Data from the hair perforation, pigment production, Tween opacity, and sorbitol assimilation studies are found in Table 3. The production of micro- and macroconidia at each of the time points on each of the media can be seen in Table 4a,b. The results of the salt tolerance investigations and production of macroconidia on SGA supplemented with 3%, 5%, 7% and 9% NaCl are in Table 5.

Discussion

Identification of dermatophytes is often based on phenotypic characteristics of colonies grown in pure culture on SGA and their microscopic morphology. However, these criteria alone may be insufficient since colonial features may vary within a taxon or be similar to other fungi. Characteristic pigmentation may fail to develop and isolates, especially *Trichophyton* spp., may not sporulate. Special media may be required to induce pigment production, as well as to obtain sporulation, and physiologic tests in conjunction with morphological pattern could be needed for correct identification [11].

Recently, Guoling *et al*. [22] reported that *T. rubrum* shows a high degree of morphological diversity, including the presence or absence of reflexively branching hyphae, micro- and macroconidia, red colony pigmentation and urease activity. It has already been shown by microsatellite analysis that there is no group of genotypes which correlate to a special morphotype in *T. rubrum* [23]. Gräser *et al*. [23] noted an association between morphological features and genotypes in two populations, *T. rubrum* and *T. violaceum*. Phenotypically, the *T. rubrum* population was dominated by strains that did not produce urease and lacked reflexively branching hyphae (97%), whereas all isolates with reflexive hyphae were in *T. violaceum*. However, urease was expressed in strains of both populations, with or without the presence of reflexive hyphae.

Krempl-Lambrecht [24] investigated hydrolysis of urea on urea agar and reported that 75% of *T. mentagrophytes* strains were urease positive in 10 days, 5% in 20 days and 20% were negative. In contrast, 10% of *T. rubrum* strains were positive in 10 days, 70% in 20 days and 20% were negative. However, Kane and Fischer [14] noted that urea broth medium was preferred over agar based media because it was more sensitive. In our study, urea hydrolysis was best detected on UPA at 28°C and it was found to be statistically superior when compared to the other methods ($P < 0.01$, Fig. 1, Table 2). On the other hand, it is important to note that the usefulness of the urease test has a certain time limit. Therefore, when using the urease test, it should be read at 48–72 hours after inoculation. Most of the *T. mentagrophytes* isolates will hydrolyse urea within this period, but *T. rubrum* will hydrolyse urea more slowly and positive results will be found if the medium is incubated for $\geq$7–10 days (Table 2).

Table 2 Results of urea hydrolysis in different media and incubation temperatures according to days.

Species	CBS No	22°C			28°C			37°C		
		UB	USA	UPA	UB	USA	UPA	UB	USA	UPA
T. fischeri	CBS 100081	–	–	–	$18^{\pm}$	$2^{\pm}$, 5^{+}	7^{+}	–	$2^{\pm}$, 7^{+}	$7^{\pm}$
T. fluviomunionse	CBS 592.68	$2^{\pm}$, 7^{+}	$2^{\pm}$, 4^{+}	$5^{\pm}$, 17^{+}	4^{+}	$1^{\pm}$, 3^{+}	$4^{\pm}$, 5^{+}	$4^{\pm}$	$2^{\pm}$, 4^{+}	$3^{\pm}$, 7^{+}
T. kanei	CBS 289.86	–	–	–	–	$3^{\pm}$, 5^{+}	7^{+}	–	$4^{\pm}$	$7^{\pm}$
T. kuryangei	CBS 422.67	$1^{\pm}$, 2^{+}	2^{+}	$8^{\pm}$,	$10^{\pm}$, 12^{+}	$3^{\pm}$, 5^{+}	$1^{\pm}$, 4^{+}	1^{+}	1^{+}	$1^{\pm}$, 2^{+}
T. kuryangei	CBS 517.63	$1^{\pm}$, 2^{+}	$2^{\pm}$, 5^{+}	$2^{\pm}$, 4^{+}	$14^{\pm}$	$5^{\pm}$, 17^{+}	$1^{\pm}$, 2^{+}	1^{+}	1^{+}	1^{+}
T. kuryangei	CBS 518.63	1^{+}	2	$1^{\pm}$, 5^{+}	$4^{\pm}$, 6^{+}	$2^{\pm}$, 5^{+}	1^{+}	1^{+}	1	1^{+}
T. megninii	CBS 389.58	3^{+}	$6^{\pm}$, 10^{+}	$5^{\pm}$, 10^{+}	4^{+}	$6^{\pm}$, 10^{+}	10^{+}	4^{+}	$7^{\pm}$, 10^{+}	10^{+}
T. pervesii	CBS 303.38	–	–	$17^{\pm}$	–	$5^{\pm}$, 20^{+}	7^{+}	–	$4^{\pm}$,	–
T. raubitschekii	CBS 202.88	–	$10^{\pm}$, 24^{+}	$7^{\pm}$, 17^{+}	–	$9^{\pm}$, 24^{+}	$4^{\pm}$, 8^{+}	–	$1^{\pm}$, 4^{+}	–
T. raubitschekii	CBS 287.86	$2^{\pm}$, 4^{+}	$2^{\pm}$, 5^{+}	$7^{\pm}$	$4^{\pm}$, 5^{+}	$1^{\pm}$, 2^{+}	$1^{\pm}$, 4^{+}	$2^{\pm}$, 7^{+}	$2^{\pm}$, 4^{+}	$1^{\pm}$, 2^{+}
T. raubitschekii	CBS 100084	–	$2^{\pm}$, 6^{+}	$7^{\pm}$, 17^{+}	10^{+}	$2^{\pm}$, 5^{+}	$4^{\pm}$, 7^{+}	–	$3^{\pm}$	$3^{\pm}$
T. raubitschekii	CBS 102856	$7^{\pm}$, 11^{+}	$2^{\pm}$, 5^{+}	$5^{\pm}$, 6^{+}	2^{+}	$2^{\pm}$, 5^{+}	$3^{\pm}$, 7^{+}	7^{+}	$2^{\pm}$, 3^{+}	$3^{\pm}$
T. rodhainii	CBS 376.49	–	–	–	$18^{\pm}$	$2^{\pm}$, 5^{+}	$3^{\pm}$, 5^{+}	–	$3^{\pm}$, 7^{+}	–
T. rubrum	CBS 302.60	–	–	$5^{\pm}$, 17^{+}	10^{+}	$5^{\pm}$, 20^{+}	7^{+}	–	$3^{\pm}$	$7^{\pm}$
T. rubrum	CBS 363.53	–	–	–	3^{+}	$1^{\pm}$, 3^{+}	4^{+}	2^{+}	2^{+}	3^{+}
T. rubrum	CBS 363.62	$3^{\pm}$, 7^{+}	$3^{\pm}$, 5^{+}	$2^{\pm}$, 12^{+}	$18^{\pm}$	$2^{\pm}$, 5^{+}	$1^{\pm}$, 3^{+}	–	1^{+}	$3^{\pm}$
T. rubrum	CBS 392.58	–	–	$7^{\pm}$, 12^{+}	$18^{\pm}$	7^{+}	7^{+}	–	$2^{\pm}$, 7^{+}	$3^{\pm}$
T. rubrum var. *nigricans*	CBS 100237	–	$3^{\pm}$, 6^{+}	$7^{\pm}$, 17^{+}	$4^{\pm}$, 7^{+}	$3^{\pm}$, 5^{+}	$4^{\pm}$, 7^{+}	–	$1^{\pm}$, 4^{+}	$2^{\pm}$
T. asteroides	CBS 424.63	$2^{\pm}$, 4^{+}	$3^{\pm}$, 16^{+}	$3^{\pm}$, 17^{+}	–	$2^{\pm}$, 5^{+}	$2^{\pm}$, 3^{+}	1^{+}	1^{+}	$2^{\pm}$, 3^{+}
T. erinacei	CBS 344.79	$20^{\pm}$	$5^{\pm}$, 6^{+}	$6^{\pm}$, 10^{+}	$13^{\pm}$,17^{+}	$3^{\pm}$, 5^{+}	$5^{\pm}$, 6^{+}	$16^{\pm}$	$6^{\pm}$, 7^{+}	$6^{\pm}$, 7^{+}
T. erinacei	CBS 511.75	$20^{\pm}$	$4^{\pm}$, 6^{+}	$6^{\pm}$, 10^{+}	$16^{\pm}$	$3^{\pm}$, 5^{+}	$5^{\pm}$, 6^{+}	$18^{\pm}$	$6^{\pm}$, 7^{+}	$5^{\pm}$, 6^{+}
T. erinacei	CBS 677.86	$20^{\pm}$	$5^{\pm}$, 6^{+}	$6^{\pm}$, 10^{+}	$16^{\pm}$	$3^{\pm}$, 5^{+}	$5^{\pm}$, 6^{+}	$18^{\pm}$	$5^{\pm}$, 6^{+}	$5^{\pm}$, 6^{+}
T. interdigitale	CBS 428.63	2^{+}	$2^{\pm}$, 5^{+}	$2^{\pm}$, 5^{+}	$10^{\pm}$	$2^{\pm}$, 3^{+}	$1^{\pm}$, 3^{+}	1^{+}	1^{+}	1^{+}
T. interdigitale	CBS 558.66	1^{+}	$2^{\pm}$, 5^{+}	$2^{\pm}$, 5^{+}	2^{+}	$2^{\pm}$, 5^{+}	1^{+}	1^{+}	1^{+}	1^{+}
T. langeronii	CBS 764.84	$2^{\pm}$, 5^{+}	$1^{\pm}$, 4^{+}	$1^{\pm}$, 3^{+}	1^{+}	$1^{\pm}$, 2^{+}	$1^{\pm}$, 2^{+}	$2^{\pm}$, 4^{+}	$1^{\pm}$, 3^{+}	$1^{\pm}$, 3^{+}
T. mentagrophytes	CBS 318.56	1^{+}	$2^{h\pm}$, 3^{+}	$2^{h\pm}$, 3^{+}	1^{+}	$2^{h\pm}$, 1^{+}	$2^{h\pm}$, 1^{+}	1^{+}	$2^{h\pm}$, 1^{+}	$2^{h\pm}$, 1^{+}
*T. m.*var. *mentagrophytes*	CBS 110.65	$3^{\pm}$, 4^{+}	$7^{\pm}$	$8^{\pm}$,	3^{+}	$1^{\pm}$, 2^{+}	$1^{\pm}$, 2^{+}	$2^{\pm}$, 3^{+}	$2^{\pm}$, 4^{+}	$2^{\pm}$, 3^{+}
*T. m.*var. *mentagrophytes*	CBS 160.66	$2^{\pm}$, 9^{+}	$6^{\pm}$, 15^{+}	$7^{\pm}$, 17^{+}	3^{+}	$1^{\pm}$, 2^{+}	$3^{\pm}$, 5^{+}	$2^{\pm}$, 7^{+}	$1^{\pm}$,3^{+}	$3^{\pm}$, 4^{+}
T. papillosum	CBS 347.55	1^{+}	1^{+}	1^{+}	1^{+}	$1^{\pm}$, 3^{+}	1^{+}	1^{+}	1^{+}	1^{+}
T. quinckeanum	CBS 572.75	$2^{\pm}$, 7^{+}	$7^{\pm}$	$7^{\pm}$	3^{+}	$1^{\pm}$, 3^{+}	$2^{\pm}$, 4^{+}	$2^{\pm}$, 3^{+}	$1^{\pm}$	1^{+}
A. benhamiae (MT −)	CBS 807.72	$1^{\pm}$, 3^{+}	$1^{\pm}$, 3^{+}	$1^{\pm}$, 3^{+}	$10^{\pm}$, 12^{+}	$2^{\pm}$, 5^{+}	$1^{\pm}$, 3^{+}	2^{+}	$1^{\pm}$, 2^{+}	$2^{\pm}$, 3^{+}
A. benhamiae (MT+)	CBS 808.72	$7^{\pm}$, 11^{+}	$5^{\pm}$, 9^{+}	$5^{\pm}$, 8^{+}	8^{+}	$2^{\pm}$, 5^{+}	$4^{\pm}$, 7^{+}	11^{+}	$2^{\pm}$, 3^{+}	–
A. simii (MT −)	CBS 417.65	$2^{\pm}$, 3^{+}	$1^{\pm}$, 6^{+}	$1^{\pm}$, 5^{+}	$2^{\pm}$, 3^{+}	$2^{\pm}$, 5^{+}	$2^{\pm}$, 4^{+}	$2^{\pm}$, 3^{+}	$1^{\pm}$, 2^{+}	$1^{\pm}$, 3^{+}
A. simii (MT+)	CBS 448.65	$3^{\pm}$, 4^{+}	$2^{\pm}$, 5^{+}	$2^{\pm}$, 5^{+}	1^{+}	$1^{\pm}$, 2^{+}	$2^{\pm}$, 7^{+}	$2^{\pm}$, 3^{+}	$1^{\pm}$, 2^{+}	$2^{\pm}$, 5^{+}

UB, urea broth; USA, urea slant agar; UPA, urea Petri agar; h, hour; ±, weak; +, positive.

Table 3 Results of hair perforation, pigment production, Tween opacity and sorbitol assimilation tests according to days.

Species	CBS No	BCH	SH	CMDA	BCP-MS-G^{r}	BCP-MS-G^{y}	BCP-MS-G^{p}	TO	SA
T. fischeri	CBS 100081	–	–	12	$2^{\pm}$, 3^{+}	–	8^{+}	3	–
T. fluviomunionse	CBS 592.68	–	–	10	2^{+}	–	$5^{\pm}$, 8^{+}	5	7^{+}
T. kanei	CBS 289.86	–	–	10	$2^{\pm}$, 4^{+}	–	$5^{\pm}$, 8^{+}	5	$7^{\pm}$, 10^{+}
T. kuryangei	CBS 422.67	–	–	–	$3^{\pm}$, 5^{+}	–	7^{+}	8	–
T. kuryangei	CBS 517.63	–	–	–	$5^{\pm}$, 7^{+}	–	8^{+}	8	–
T. kuryangei	CBS 518.63	–	–	–	$2^{\pm}$, 3^{+}	–	$5^{\pm}$, 8^{+}	4	–
T. megninii	CBS 389.58	–	–	–	–	–	5^{+}	3	–
T. pervesii	CBS 303.38	–	–	7	–	2	$5^{\pm}$, 8^{+}	–	7^{+}
T. raubitschekii	CBS 202.88	–	–	–	$2^{\pm}$, 3^{+}	–	$5^{\pm}$, 8^{+}	4	7^{+}
T. raubitschekii	CBS 287.86	–	–	7	$2^{\pm}$, 3^{+}	–	$5^{\pm}$, 8^{+}	4	7^{+}
T. raubitschekii	CBS 100084	–	–	7	$2^{\pm}$, 3^{+}	–	$5^{\pm}$, 8^{+}	3	7^{+}
T. raubitschekii	CBS 102856	–	–	12	$2^{\pm}$, 5^{+}	–	$5^{\pm}$, 8^{+}	3	7^{+}
T. rodhainii	CBS 376.49	–	–	10	$2^{\pm}$, 3^{+}	–	$5^{\pm}$, 8^{+}	6	–
T. rubrum	CBS 302.60	–	13^{1}	12	$2^{\pm}$, 3^{+}	–	$5^{\pm}$, 8^{+}	3	7^{+}
T. rubrum	CBS 363.53	–	–	10	$2^{\pm}$, 3^{+}	–	$5^{\pm}$, 8^{+}	3	7^{+}
T. rubrum	CBS 363.62	–	–	7	$2^{\pm}$, 3^{+}	–	$5^{\pm}$, 8^{+}	4	7^{+}
T. rubrum	CBS 392.58	–	–	10	$2^{\pm}$, 3^{+}	–	$5^{\pm}$, 8^{+}	–	–
T. rubrum var. *nigricans*	CBS 100237	–	–	10	$2^{\pm}$, 3^{+}	–	$5^{\pm}$, 8^{+}	3	$7^{\pm}$, 10^{+}
T. asteroides	CBS 424.63	$5^{\uparrow}$	5^{1}, $6^{\uparrow}$	–	–	3	4^{+}	3	–
T. erinacei	CBS 344.79	5^{2}, $6^{\uparrow}$	$6^{\uparrow}$	–	–	3	4^{+}	3	$3^{\pm}$, 4^{+}
T. erinacei	CBS 511.73	$6^{\uparrow}$	6^{4},$7^{\uparrow}$	–	–	3	4^{+}	3	$3^{\pm}$, 4^{+}
T. erinacei	CBS 677.86	5^{3}, $6^{\uparrow}$	5^{5}, $6^{\uparrow}$	–	–	3	4^{+}	3	$3^{\pm}$, 4^{+}
T. interdigitale	CBS 428.63	$6^{\uparrow}$	–	–	–	–	4^{+}	3	7^{+}
T. interdigitale	CBS 558.66	$7^{\uparrow}$	15^{2}, $21^{\uparrow}$	–	–	5	$2^{\pm}$, 4^{+}	5	$7^{\pm}$, 10^{+}
T. langeronii	CBS 764.84	22^{1}	–	–	–	–	$2^{\pm}$, 5^{+}	3	7^{+}
T. mentagrophytes	CBS 318.56	$5^{\uparrow}$	$8^{\uparrow}$	–	–	2	4^{+}	3	7^{+}
*T. m.*var. *mentagrophytes*	CBS 110.65	$5^{\uparrow}$	$5^{\uparrow}$	–	–	4	4^{+}	3	7^{+}
*T. m.*var. *mentagrophytes*	CBS 160.66	$6^{\uparrow}$	$11^{\uparrow}$	–	–	4	4^{+}	3	7^{+}
T. papillosum	CBS 347.55	–	–	–	–	3	4^{+}	–	–
T. quinckeanum	CBS 572.75	$10^{\uparrow}$	$14^{\uparrow}$	–	–	2	4^{+}	3	7^{+}
A. benhamiae (MT −)	CBS 807.72	$6^{\uparrow}$	–	–	–	3	5^{+}	2	7^{+}
A. benhamiae (MT+)	CBS 808.72	$5^{\uparrow}$	13^{1}	–	–	3	5^{+}	3	7^{+}
A. simii (MT −)	CBS 417.65	5^{3},$6^{\uparrow}$	–	–	–	3	5^{+}	2	7^{+}
A. simii (MT+)	CBS 448.65	$6^{\uparrow}$	–	–	–	5	5^{+}	–	$7^{\pm}$, 10^{+}

BCH, blond child hair; SH, sheep hair; $^{n=1-9}$: number of perforation was between 1 to 9; $^{n\geq 10}$: number of perforation was $\geq$10.
CMDA, Cornmeal dextrose agar; BCP-MS-G, Bromcresol purple-milk solids-glucose agar; r, red; y, yellow; p, purple; $\pm$, weak; +, positive.
TO, Tween opacity test; SA, sorbitol assimilation.

Takahashi *et al*. [25] studied urease activity of 7 Japanese, 5 Kenyans and 6 European and New Zealand (including the CBS strains used in this study) *T. erinacei* isolates on Christensen's urea agar slant at 25°C for 7 days. The authors noted that only one of the Japanese isolates, and all of the Kenyans were urease-positive, whereas the Europeans strains (except CBS 108.91) were urease-negative. However, we observed that two of the Europeans (CBS 344.79 and CBS 677.86) and one New Zealand isolates (CBS 511.73) were urease positive on the same medium after 3–6 days of incubation at 22°C and 28°C. These results suggest that the urease activity of this dermatophyte is highly variable.

Sinski *et al*. [26] observed that the most reliable test for the differentiation of *T. mentagrophytes* from *T. rubrum* was the standard *in vitro* hair perforation test. While it is effective and easy-to-perform, hair perforation is time-consuming, requiring 1 to 4 weeks to complete. Although in our study more perforation was found with hair from blond children, the results of the tests using hair from this source and sheep were not statistically significant ($P > 0.05$, Fig. 2, Table 3). While the test was also performed with goat hair, the dark color of this hair under light microscope interfered with the examination.

Summerbell *et al*. [18] reported that *T. mentagrophytes* isolates had; (1) diffuse growth with a strong alkaline reaction visible within 7 days on BCP-MS-G, (2) a strong positive urease capability, and (3) perforated hair. On the other hand, *T. rubrum* strains were urease negative, did not perforate hair, and produced no alkalinity on BCP-MS-G after 7 days. Furthermore, the authors noted that *T. rubrum* with

Table 4a Production of micro- and macroconidia according to culture media on days 5, 10, and 15.

Species	CBS No	PDA		BHIA		MEA		LJA	
		MI	MA	MI	MA	MI	MA	MI	MA
		5/10/15	5/10/15	5/10/15	5/10/15	5/10/15	5/10/15	5/10/15	5/10/15
T. fischeri	CBS 100081	−/−/10	−/−/−	20/20/20	−/−/−	+/+/+	−/−/−	+/+/+	−/−/−
T. fluviomunionse	CBS 592.68	−/+/+	10/+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+
T. kanei	CBS 289.86	−/−/−	−/−/−	−/−/−	10/20/+	−/−/−	8/10/10	−/−/−	15/+/+
T. kuryangei	CBS 422.67	−/−+	−/−/5	+/+/+	−/−/3	+/+/+	−/−/−	+/+/+	−/2/3
T. kuryangei	CBS 517.63	−/−/+	−/−/−	−/−/+	−/−/−	−/10/24	−/2*/−	−/−/+	−/−/1
T. kuryangei	CBS 518.63	−/+/+	−/−/−	+/+/+	−/−/−	10/15/15	−/−/−	+/+/+	−/−/−
T. megninii	CBS 389.58	−/8/10	−/−/−	+/+/+	2/5/5	8/10/10	−/1/1	+/+/+	5/6/6
T. pervesii	CBS 303.38	30/+/+	−/−/3	+/+/+	1/2/5	−/20/30	−/−/−	+/+/+	3/3/5
T. raubitschekii	CBS 202.88	+/+/+	6/8/10	+/+/+	8/10/10	+/+/+	+/+/+	+/+/+	8/10/10
T .raubitschekii	CBS 287.86	+/+/+	−/10/10	+/+/+	5/15/15	+/+/+	1/2/4	+/+/+	+/+/+
T. raubitschekii	CBS 100084	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+
T. raubitschekii	CBS 102856	+/+/+	20/+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+
T. rodhainii	CBS 376.49	−/−/+	−/−/−	10/25/+	2/2/2	−/20/25	−/−/−	20/+/+	3/3/3
T. rubrum	CBS 302.60	+/+/+	−/−/−	+/+/+	4/7/+	−/+/+	3/4/+	+/+/+	6/7/+
T. rubrum	CBS 363.53	+/+/+	−/−/−	+/+/+	−/−/4	+/+/+	−/−/2	+/+/+	1/1/4
T. rubrum	CBS 363.62	−/−/−	−/−/−	+/+/+	+/+/+	−/−/−	+/+/+	+/+/+	+/+/+
T. rubrum	CBS 392.58	+/+/+	−/2/2	+/+/+	10/15/+	−/−/+	−/−/−	+/+/+	10/15/+
T. rubrum var. *nigricans*	CBS 100237	−/−/−	−/−/−	−/30/+	−/1/5	10/20/24	−/1/2	20/+/+	1/2/5
T. asteroides	CBS 424.63	+/+/+	−/−/−	+/+/+	−/−/−	−/+/+	−/−/−	+/+/+	1/3/3
T. erinacei	CBS 344.79	+/+/+	1/1/1	+/+/+	2/2/5	+/+/+	−/2/1	+/+/+	2/8/10
T. erinacei	CBS 511.73	+/+/+	2*/1*/2*	+/+/+	−/−/+	20/+/+	−/1*/5*	+/+/+	−/6/6
T. erinacei	CBS 677.86	+/+/+	1/2/1	15/+/+	1/3/10	20/+/+	−/2*/3*	+/+/+	+/+/+
T. interdigitale	CBS 428.63	−/−/+	−/−/−	+/+/+	−/2/3	+/+/+	−/1/2	+/+/+	−/2/3
T. interdigitale	CBS 558.66	+/+/+	3/4/4	+/+/+	2/2/3	+/+/+	2/2/3	+/+/+	3/3/3
T. langeronii	CBS 764.84	+/+/+	−/−/−	+/+/+	+/+/+	+/+/+	1/2/2	+/+/+	+/+/+
T. mentagrophytes	CBS 318.56	−/−/+	−/−/−	+/+/+	2/2/6	−/+/+	−/−/−	+/+/+	4/4/7
*T. m.*var. *mentagrophytes*	CBS 110.65	+/+/+	−/−/−	+/+/+	−/−/−	+/+/+	−/−/−	+/+/+	−/−/2*
*T. m.*var. *mentagrophytes*	CBS 160.66	+/+/+	−/−/2	+/+/+	8/8/10	+/+/+	4/8/8	+/+/+	10/10/12
T. papillosum	CBS 347.55	−/−/−	−/−/−	+/+/+	−/−/−	+/+/+	−/−/−	+/+/+	−/−/−
T. quinckeanum	CBS 572.75	20/+/+	−/−/−	+/+/+	1/4/5	+/+/+	−/−/−	+/+/+	4/5/5
A. benhamiae (MT −)	CBS 807.72	+/+/+	−/−/−	+/+/+	−/−/−	+/+/+	−/−/−	+/+/+	−/−/−
A. benhamiae (MT+)	CBS 808.72	+/+/+	−/−/2*	+/+/+	−/1*/4*	+/+/+	−/1*/3*	+/+/+	−/1*/4*
A. simii (MT −)	CBS 417.65	+/+/+	−/20/+	+/+/+	4/4/6	+/+/+	1/2/2	+/+/+	4/4/6
A. simii (MT+)	CBS 448.65	6/10/10	−/−/−	+/+/+	−/−/−	+/+/+	−/1/2	+/+/+	−/−/−

PDA, Potato dextrose agar; BHIA, Brain heart infusion agar; MEA, Malt extract agar; LJA, Lowenstein-Jensen agar; MI, microconidia; MA, macroconidia; +, 30↑; *, In all microscopic field; −, negative.

kanei, fischeri and *raubitschekii* morphotypes all showed a pattern of restricted growth, rapid development of red reverse pigmentation, and were alkaline positive on BCP-MS-G.

In this present study, while most of the *T. rubrum* strains produced (72.2%) red pigment on CMDA, members of the *T. mentagrophytes* complex and *Arthroderma* strains did not. Although this difference was found to be statistically significant ($P < 0.01$, Table 3), it should be again noted that only the majority of *T. rubrum* strains produced pigment. Our suggestion is that pigment production is not a reliable morphological character. In this investigation, somewhat better pigment formation was noted with BCP-MS-G medium. Most of the *T. rubrum* strains (88.8%) produced red pigment on BCP-MS-G, except *megninii* (uncolored, 5.6%) and *pervesii* (yellow, 5.6%) morphotypes. The current study has also demonstrated that features thought to be characteristic for a morphotype (e.g., *fischeri* or *megninii*) were not expressed (Table 3).

Our present observations are in agreement with those of Kane and Fischer [27] who reported that *T. rubrum* with *megninii* morphotype produced a spreading velvety growth. A darkening of the BCP milk dextrose agar indicated an increase in the pH and no reddish color was prominent around the colony. These authors noted that the aerial growth may show some pink color as the colony ages [27], while in our study, we found that most

Table 4b Production of micro- and macroconidia according to culture media and on days 5, 10, and 15.

Species	CBS No	OA		OCCA		CMDA		UPA	
		MI	MA	MI	MA	MI	MA	MI	MA
		5/10/15	5/10/15	5/10/15	5/10/15	5/10/15	5/10/15	5/10/15	5/10/15
T. fischeri	CBS 100081	−/10/6	−/−/−	+/+/+	−/−/−	−/−/10	−/−/−	−/−/10	−/−/−
T. fluviomunionse	CBS 592.68	+/+/+	+/+/+	10/+/+	10/+/+	+/+/+	6/8/8	6/8/8	−/−/3*
T. kanei	CBS 289.86	−/−/−	3*/−/−	−/−/−	−/−/7	−/−/−	−/−/−	−/−/−	5/12/+
T. kuryangei	CBS 422.67	+/+/+	−/1*/−	−/+/+	−/−/−	−/−/−	−/−/−	−/−/−	−/−/−
T. kuryangei	CBS 517.63	+/+/+	−/−/7	−/+/+	−/−/−	−/5/6	−/−/−	+/+/+	−/−/−
T. kuryangei	CBS 518.63	−/−/−	−/−/−	+/+/+	−/−/2*	−/−/−	−/−/−	−/−/−	−/−/−
T. megninii	CBS 389.58	+/+/+	−/−/1	+/+/+	−/−/−	+/+/+	−/−/−	−/20/+	−/−/−
T. pervesii	CBS 303.38	+/+/+	−/2/2	+/+/+	−/−/3	8/10/+	−/−/−	5/−/−	−/−/−
T. raubitschekii	CBS 202.88	−/−/−	−/5*/−	−/−/10	−/−/−	−/−/−	−/−/−	−/10/10	−/−/−
T. raubitschekii	CBS 287.86	+/+/+	4/10/−	+/+/+	+/+/+	+/+/+	−/3/4	+/+/+	−/2/2
T. raubitschekii	CBS 100084	−/+/+	−/10/15	+/+/+	−/15/20	+/+/+	5/8/8	−/20/+	−/10/+
T. raubitschekii	CBS 102856	+/+/+	3/−/−	+/+/+	7/20/+	+/+/+	2*/−/−	−/−/−	−/3/4
T. rodhainii	CBS 376.49	20/30/30	2/4/8	−/10/10	1/2/2	−/8/−	−/−/−	−/−/−	−/−/−
T. rubrum	CBS 302.60	+/+/−	−/−/−	+/+/+	−/−/−	24/20/20	−/−/−	−/+/+	−/3*/−
T. rubrum	CBS 363.53	−/−/−	−/−/−	−/−/−	−/−/−	−/−/−	−/−/−	−/8/−	−/−/−
T. rubrum	CBS 363.62	+/+/+	−/−/−	+/+/+	−/1/2	+/+/+	−/−/−	20/+/+	5/10/10
T. rubrum	CBS 392.58	+/+/+	4/4/−	−/−/−	−/−/−	−/+/+	−/−/−	10/+/+	−/−/1*
T. rubrum var. *nigricans*	CBS 100237	−/+/+	−/−/−	−/−/20	−/−/−	8/10/20	−/−/−	−/−/+	−/−/−
T. asteroides	CBS 424.63	+/+/+	5*/−/−	+/+/+	−/−/1*	−/+/+	−/−/−	−/+/+	−/−/2*
T. erinacei	CBS 344.79	30/+/+	−/1/1	+/+/+	4/+/+	+/+/+	−/−/2	20/+/+	−/−/−
T. erinacei	CBS 511.73	+/+/+	−/−/−	+/+/+	−/−/2*	20/+/+	−/−/−	+/+/+	−/−/−
T. erinacei	CBS 677.86	+/+/+	1/2/1	+/+/+	−/−/−	+/+/+	−/−/−	20/20/20	−/−/−
T. interdigitale	CBS 428.63	10/15/+	−/2*/−	−/10/+	−/−/−	20/20/20	−/−/−	8/10/10	−/−/−
T. interdigitale	CBS 558.66	20/30/30	−/−/−	+/+/+	−/2*/2*	8/−/−	−/−/−	+/+/+	−/2*/−
T. langeronii	CBS 764.84	10/+/+	−/−/−	10/10/+	1/3/3	−/−/−	−/−/−	8/8/4	2*/−/−
T. mentagrophytes	CBS 318.56	20/+/+	−/−/−	10/8/10	−/−/−	5/5/+	−/−/2	−/−/−	−/−/−
*T. m.*var. *mentagrophytes*	CBS 110.65	+/+/+	5*/−/−	+/+/+	5/10/10	+/+/+	2*/−/−	16/+/+	−/4/4
*T. m.*var. *mentagrophytes*	CBS 160.66	10/+/+	−/−/−	20/+/+	−/−/−	−/−/+	−/−/−	+/+/+	−/−/−
T. papillosum	CBS 347.55	−/−/−	−/−/−	−/−/−	−/−/2*	−/−/−	−/−/−	−/−/−	−/−/−
T. quinckeanum	CBS 572.75	6/+/−	−/−/−	+/+/+	−/1*/−	30/+/+	−/−/−	10/10/+	−/1*/−
A. benhamiae (MT−)	CBS 807.72	10/+/+	−/−/−	+/+/+	−/−/3*	−/−/−	−/−/−	−/−/−	−/−/−
A. benhamiae (MT+)	CBS 808.72	+/+/+	−/−/−	−/+/+	−/−/−	−/−/−	−/−/−	10/10/−	−/−/−
A. simii (MT−)	CBS 417.65	+/+/+	6/+/+	+/+/+	1*/8*/8*	+/+/+	1/2/2	+/+/+	2*/1*/2*
A. simii (MT+)	CBS 448.65	−/+/+	−/−/−	−/+/+	−/5*/5*	8/10/+	−/−/−	−/+/+	−/−/−

OA, Oat meal agar; OCCA, Oxoid chromogenic Candida agar; CMDA, Cornmeal dextrose agar; UPA, Urease Petri agar; MI, Microconidia; MA, Macroconidia; +, 30↑; *, In all microscopic field; +, positive; −, negative.

of the *T. mentagrophytes* complex (83.3%) and *Arthroderma* strains (100%) produced yellowish pigment. We observed that the growth of *T. mentagrophytes* complex and *Arthroderma* strains on BCP-MS-G caused an increase in the pH within a week of inoculation. However, such a pH change was observed around the colonies of *T. rubrum* after 5 days, and on all media after 8 days (Table 3).

Slifkin and Cumbie [19] noted in Tween opacity studies that all the isolates of *T. rubrum* tested produced macroscopic halos around the colonies 5–7 days after inoculation, while *T. mentagrophytes* var. *interdigitale* strains formed visible halos after 16–20 days of incubation. However, in our study, the efficacy of the Tween opacity test was not found to be statistically significant in differentiating between the two taxa ($P > 0.05$, Table 3). Rezusta *et al.* [28] reported that 147 *T. mentagrophytes* strains were able to assimilate sorbitol in API 20C AUX strips when read after 7 days of incubation at 30 °C, whereas 36 *T. rubrum* strains did not. However, in our study, the sorbitol assimilation test failed to distinguish both taxa from each other ($P > 0.05$, Table 3).

Yucel and Al-Ali [29] found that 69 *T. mentagrophytes* strains formed macroconidia on LJA (98.5%), Pai (96%), Lactrimel (63%), and SGA (25%) media. In line with their study, we demonstrated that the production of macroconidia was best on LJA after 15 days of

Table 5 Species-specific growth in SGA supplemented with 3%, 5%, 7% and 9% NaCl.

Species	CBS No	3%	5%	7%	9%
T. fischeri	CBS 100081	−	−	−	−
T. fluviomunionse	CBS 592.68	+	−	−	−
T. kanei	CBS 289.86	−	−	−	−
T. kuryangei	CBS 422.67	+	−	−	−
T. kuryangei	CBS 517.63	+	−	−	−
T. kuryangei	CBS 518.63	+	−	−	−
T. megninii	CBS 389.58	−	−	−	−
T. pervesii	CBS 303.38	+	−	−	−
T. raubitschekii	CBS 202.88	−	−	−	−
T. raubitschekii	CBS 287.86	−	−	−	−
T. raubitschekii	CBS 100084	−	−	−	−
T. raubitschekii	CBS 102856	−	−	−	−
T. rodhainii	CBS 376.49	−	−	−	−
T. rubrum	CBS 302.60	+	−	−	−
T. rubrum	CBS 363.53	−	−	−	−
T. rubrum	CBS 363.62	−	−	−	−
T. rubrum	CBS 392.58	−	−	−	−
T. rubrum var. *nigricans*	CBS 100237	−	−	−	−
T. asteroides	CBS 424.63	+	+	+	−
T. erinacei	CBS 344.79	+	+	+	−
T. erinacei	CBS 511.73	+	+	+	+
T. erinacei	CBS 677.86	+	+	−	−
T. interdigitale	CBS 428.63	+	−	−	−
T. interdigitale	CBS 558.66	+	+	+	+
T. langeronii	CBS 764.84	−	−	−	−
T. mentagrophytes	CBS 318.56	−	−	−	−
*T. m.*var. *mentagrophytes*	CBS 110.65	+	+	+	−
*T. m.*var. *mentagrophytes*	CBS 160.66	+	+	−	−
T. papillosum	CBS 347.55	−	−	−	−
T. quinckeanum	CBS 572.75	+	+	+	−
A. benhamiae (MT −)	CBS 807.72	+	+	+	+
A. benhamiae (MT+)	CBS 808.72	+	+	+	+
A. simii (MT −)	CBS 417.65	+	−	−	−
A. simii (MT+)	CBS 448.65	+	+	+	+

+, positive; −, negative.

inoculation ($P < 0.001$, Fig. 3, Table 4a). Our results also revealed that both LJA and BHIA supported rich sporulation of micro- and macroconidia, and these two media might be used as routine mycological media like UPA (Figs. 4 and 5).

Kane *et al*. [8] reported that *T. rubrum* strains with *fischeri*, *kanei, megninii*, and *raubitschekii* morphotypes had strong, restricted growth on 3–5% NaCl-supplemented SGA and produced no conidia. In our study, the growth in NaCl concentrations was found to be statistically significant for 3% NaCl ($P = 0.001$). It was observed that the salt tolerance test was not as effective in differentiating the two taxa, as well as the two related species and morphotypes ($P > 0.05$, Table 5).

In the present study, we critically evaluated the value of conventional diagnostic tests in the identification of dermatophyte species as summarized in Tables 2–4a,b, and 5. Several of the isolates included in our investigation, i.e., *T. fischeri* CBS 100081, *T. fluviomunionse* CBS 592.68, *T. kanei* CBS 289.86, *T. kuryangei* CBS 422.67, *T. kuryangei* CBS 517.63, *T. raubitschekii* CBS 287.86, *T. raubitschekii* CBS 100084, *T. rodhainii* CBS 376.49, *T. rubrum* CBS 392.58, *T. pervesii* CBS 303.38, *T. rubrum* var. *nigricans* CBS 100237 (ATU TR9) were evaluated by Gräser *et al*. [4]. The results in the latter for urea hydrolysis in urea broth, hair perforation, and the production of micro- and macroconidia on MEA are similar to those we found (Tables 2 and 3, and 4a).

Dr Julius Kane [30] proposed *T. fischeri* in 1977 based on studies of primary clinical isolates which readily formed micro- and macroconidia. Gräser *et al*. [4] investigated 2 isolates of the organism (CBS 288.86 and CBS 100081) after 23 years of continuous culturing and could not find macroconidial production in either strain. Our results with CBS 100081 correspond to those of Gräser *et al*. [4] (Table 4a,b). It is a well known fact that majority of dermatophyte species maintained under laboratory conditions for a longer period of time

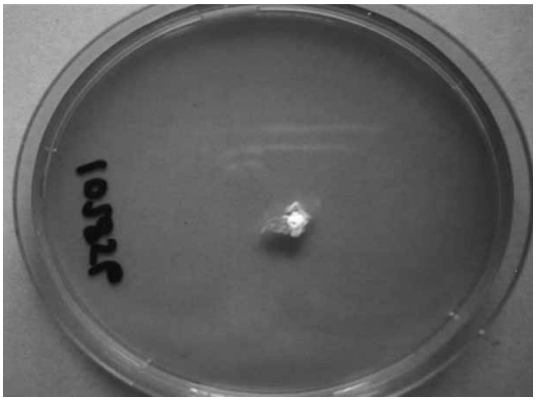

Fig. 1 Culture of *Trichophyton raubitschekii* CBS 102.856 at 28°C in urease Petri agar after 3 days.

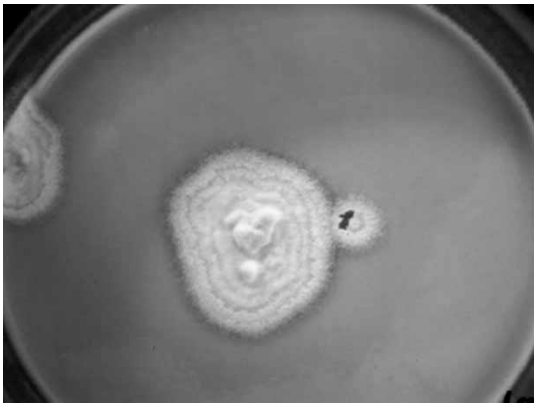

Fig. 3 *Trichophyton mentagrophytes* var. *mentagrophytes* CBS 110.65 at 28°C in Lowenstein-Jensen agar after 15 days.

loose their ability to form conidia, especially macroconidia. The mating types of *Arthroderma* spp. maintained under ideal laboratory conditions have been found to lose their mating abilities.

In another study by Gräser *et al*. [3], isolates of *T. interdigitale* CBS 558.66, *T. interdigitale* CBS 428.63, *T. mentagrophytes* CBS 318.56, *T. papillosum* CBS 347.55, *A. simii* (MT-) CBS 417.65, *A. simii* (MT+) CBS 448.65 were investigated for urea hydrolysis in Christensen's urea broth and hair perforation test. Their results are very similar to those noted in this study (Tables 2 and 3). To the best of our knowledge, our investigations are the first to discuss the morphological, physiological and biochemical characteristics of *Trichophyton kuryangei* CBS 518.63, *T. megninii* CBS 389.58, *T. raubitschekii*, CBS 202.88, *T. raubitschekii* CBS 102856, *T. rubrum* CBS 302.60, *T. rubrum* CBS 363.53, *T. rubrum* CBS 363.62, *T. asteroides* CBS 424.63, *T. langeronii* CBS 764.84, *T. mentagrophytes* var. *mentagrophytes* CBS 110.65, *T. mentagrophytes* var. *mentagrophytes* CBS 160.66, *T. quinckeanum* CBS 572.75, *A. benhamiae* (MT−) CBS 807.72, and *A. benhamiae* (MT+) CBS 808.72.

Summerbell *et al*. [18] obtained five *A. benhamiae* strains from Centers for Disease Control, Atlanta, Georgia and two *A. vanbreuseghemii* isolates from Department of Health, New York, which produced a strongly positive urea hydrolysis, perforated hair, and showed rapid and diffuse growth with a concomitant moderate-to-strong alkaline reaction visible within 7 days on BCP-MS-G. We obtained parallel results with these fungi as well as *A. simii* (Tables 2 and 3).

While some dermatophyte strains have been sequenced to allow for their accurate identification, most isolates of *T. mentagrophytes* sensu lato, i.e., in the CBS collection database, need to be sequenced to ensure that they have been appropriately identified. Hence, the classification is currently in a state of change and the identities of many isolates are not yet settled (R. C. Summerbell, pers. comm.). This suggests that more data from additional studies are required to enhance the validity of findings obtained from this study.

Finally, conventional procedures are still important for laboratory diagnosis. This study underlines the practical, simple and easy-to-perform tests, for species identification of dermatophyte strains that are conducted by a large number of laboratories. The results of this study suggests that; (i) optimum urea hydrolysis test results were detected on UPA incubated at 28°C ($P < 0.01$), (ii) better sporulation was provided by LJA and BHIA incubated for 15 days ($P < 0.001$), (iii) highest pigment formation was noted on CMDA ($P < 0.01$) and BCP-MS-G media, might yield reliable differentiation. We conclude that the media mentioned

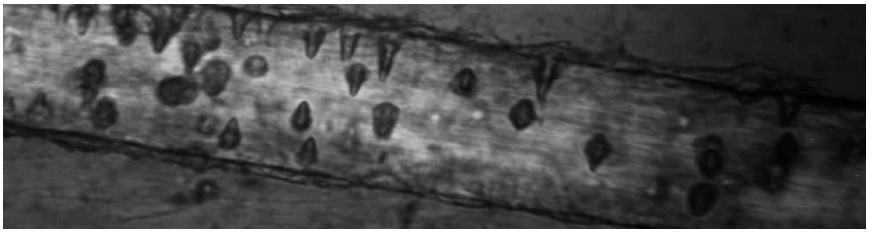

Fig. 2 Hair perforation (blond child) by *Trichophyton mentagrophytes* var. *mentagrophytes* CBS 110.65 at 28°C after 5 days.

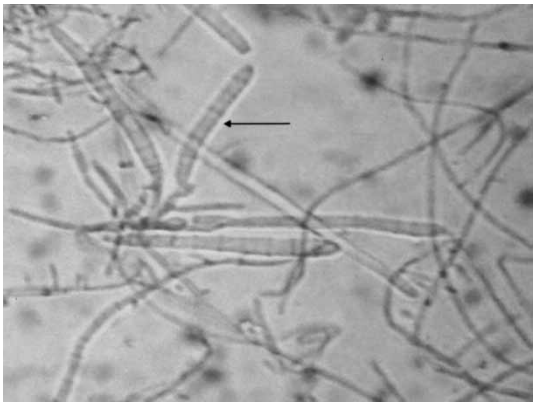

Fig. 4 Typical *Trichophyton*-macroconidium (arrow) formed by *Trichophyton rubrum* CBS 363.62 at 28°C in brain heart infusion agar after 15 days (Lactophenol cotton blue ×400).

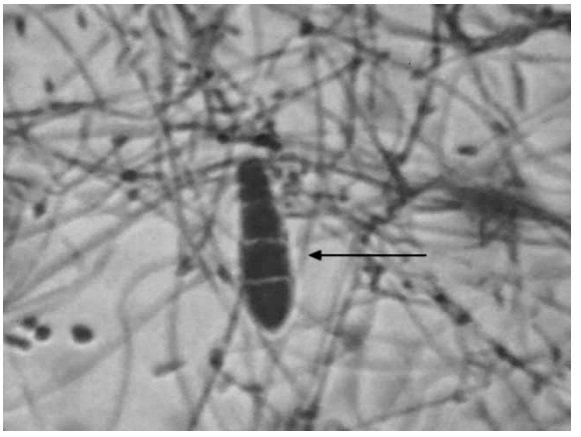

Fig. 5 Macroconidium (arrow) formed by *Arthroderma simii* CBS 417.65 (MT-) at 28°C in Lowenstein-Jensen agar after 15 days (Lactophenol cotton blue ×400).

above are highly efficacious in facilitating the identification of these two taxa based on the basis of their morphological and physiological characteristics.

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