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Comparison of the contamination rates of culture media used for isolation and identification of dermatophytes

Ramazan GÜMRAL[1], Aylin DÖĞEN[2], Mehmet Macit İLKİT[3,*]

[1]Department of Microbiology, Gülhane Military Medical Academy, Ankara, Turkey
[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Mersin University, Mersin, Turkey
[3]Division of Mycology, Department of Microbiology, Faculty of Medicine, Çukurova University, Adana, Turkey

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Background/aim: Mycological media that promote spore production are essential for the diagnosis of dermatophytosis. However, these culture media frequently become contaminated by multiple fungal or bacterial species during culture. The aim of this study was to compare the contamination rates of 6 culture media used for the isolation and identification of dermatophytes, including Borelli's lactritmel agar (BLA), brain-heart infusion agar (BHIA), Lowenstein–Jensen agar (LJA), malt extract agar (MEA), potato dextrose agar (PDA), and Sabouraud glucose agar (SGA).

Materials and methods: Agar plates were inoculated with 43 well-characterized dermatophyte strains, belonging to the genera *Arthroderma*, *Epidermophyton*, *Microsporum*, or *Trichophyton*. The agar plates were incubated at 26 °C and examined every 5 days for 1 month.

Results: By the end of the incubation period, 97 of the 258 plates (37.6%) were contaminated by fungi. No bacteria were detected. Overall, BLA demonstrated the lowest rate of contamination, followed by SGA, MEA, BHIA, PDA, and LJA. Sequencing of the internal transcribed spacer region rDNA of the contaminant fungi revealed that *Aspergillus* and *Penicillium* species were the most common contaminants.

Conclusion: These results suggest that nonenriched culture media types, such as BLA or SGA, reduced contamination during dermatophyte subculture.

Key words: *Aspergillus*, ITS sequencing, laboratory diagnosis, molds, *Penicillium*, *Trichoderma*

1. Introduction

Dermatophytes are medically important fungi with keratinophilic and keratinolytic properties, affecting the scalp, nails, and skin of mammals, including humans, and may have a tendency to promote a chronic or recurring condition (1,2). In the 21st century, the medical relevance of dermatophytes has grown due to increased urbanization, traveling, immigration, communal life (such as sports and fitness facilities), and the prevalence of obesity and an aging population (2). The clinical importance of proper isolation of dermatophytes needed for reliable diagnosis is well recognized by many physicians and microbiologists; however, proper isolation is not routinely utilized in most laboratories (3,4). Although fungal culture is more time-consuming than molecular diagnosis, the former is readily available throughout the world and is currently the "gold-standard" method of diagnosing fungal infections and/or carrier states and for the direct identification of dermatophytic fungi on primary isolation media (1,3–6).

Dermatophytes require unique culture conditions, such as 1 to 4 weeks of growth at 22–26 °C (i.e. less than 30 °C). A primary medium for the isolation of dermatophytes should select against bacteria and nondermatophytic molds. Sabouraud glucose agar (SGA, a glucose-peptone agar) containing cycloheximide (Actidione), chloramphenicol, and gentamicin is routinely used as a primary isolation medium (1–3,7). Some laboratories also use potato dextrose and/or potato flake agars containing cycloheximide and chloramphenicol for primary isolation (1,3). However, not surprisingly, these antibiotics do not ensure purity from keratinophilic fungi (8). The long incubation period required also increases the chance of contamination by bacteria or saprophytic molds that are known to inhibit the growth of dermatophytes isolated

* Correspondence: milkit@cu.edu.tr

from scalp, nails, or skin samples (1,5,8). Well-known culture contaminants include *Acremonium* spp. (which cause nail infection and/or eumycetoma), *Aspergillus* spp. (which cause invasive and/or superficial mycoses), *Penicillium* and *Talaromyces* spp. (which, except for *T.* [*Penicillium*] *marneffei*, do not cause any well-recognized human diseases), and *Trichophyton terrestre* (a soil fungus that lives saprophytically on animal keratin) (9). In addition, *Pseudomonas aeruginosa* restricts dermatophyte growth, particularly during dermatophyte isolation (10).

Fungal and bacterial contamination is an important and widespread problem when attempting to culture dermatophytes even in biosafety cabinets and with the plates kept in incubators. Contamination problems may cause misdiagnoses by either suppressing the growth of or masking the presence of the fungal pathogen, which may affect the cost of health care. The objective of this investigation was to compare the contamination rates of 6 different enriched and nonenriched media types that are commercially available or prepared in-house, which are used to isolate and identify dermatophytic fungi.

2. Materials and methods

2.1. Dermatophyte strains

The 43 dermatophyte strains used in this study were as follows: *Arthroderma* spp. (n = 4), *Epidermophyton floccosum* (n = 1), *Microsporum audouinii* (n = 3), *M. canis* (n = 1), *M. ferrugineum* (n = 2), *M. gypseum* (n = 1), *M. praecox* (n = 1), the *T. mentagrophytes* complex (n = 12), the *T. rubrum* complex (n = 14), *T. tonsurans* (n = 2), and *T. verrucosum* (n = 2). Most of these strains were previously identified by DNA sequencing and primarily obtained from the culture collection service Centraalbureau voor Schimmelcultures (CBS)-KNAW at the Fungal Biodiversity Centre, Utrecht, the Netherlands. One *E. floccosum* isolate obtained from the Refik Saydam Hygiene Center Presidency (RSHCP) and 3 *T. interdigitale* strains and 1 *M. canis* strain obtained from the working collection of Macit İlkit (MI) were also included in the study (Table 1).

2.2. Culture media

We used 6 mycological media, some of which were prepared in-house and some of which are commercially available. All strains were subcultured on the following media: Borelli's lactritmel (lac, milk; trit, wheat flour; and mel, honey) agar (BLA) (11); brain-heart infusion agar (BHIA; Merck, Darmstadt, Germany); Lowenstein–Jensen agar (LJA) (12); malt extract agar (MEA; Oxoid, Basingstoke, UK); potato dextrose agar (PDA; Merck); and SGA (Merck) containing 100 µg/mL cycloheximide (Sigma, Steinheim, Germany), 100 µg/mL chloramphenicol (Sigma), and 50 µg/mL gentamicin (Sigma; SGA+CCG). The LJA and BLA were prepared in our laboratory using media recipes and growth conditions that have been previously described (11–14). Antibiotics were added to only the SGA and BLA media. A 1 × 1 cm fragment from the edge of a 10-day-old colony of each strain was inoculated onto each medium. The plates were not wrapped in Parafilm or protective bags. All of the plates were incubated at 26 °C and examined for contamination every 5 days for a 30-day period. Two plates of each medium that were not inoculated with the dermatophyte strains but were incubated under the same conditions were used as negative controls.

2.3. DNA extraction, PCR, and molecular analysis

DNA extraction and PCR amplification were performed as described previously (15). The rDNA sequences spanning the internal transcribed spacer (ITS) region were amplified on an ABI PRISM 3130XL genetic analyzer at Refgen Biotechnology (Ankara, Turkey) using the universal fungal primers ITS1 and ITS4. The CAP contig assembly software, included in BioEdit Sequence Alignment Editor 7.0.9.0, was used to edit the sequences (16). Assembled DNA sequences were examined using the BLAST (nucleotide-nucleotide) program from the National Center of Biotechnology Information (National Institutes of Health, Bethesda, MD, USA).

2.4. Statistical analysis

Fisher's exact test and the chi-square test were used to determine the differences in contamination rates between the culture media. Statistical analysis was carried out using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Fungal contamination rates on media

Fungal contamination was observed more frequently on enriched media (LJA and BHIA) than nonenriched media (BLA and MEA) (Figure). Bacterial contamination was not detected on any of the analyzed media. The highest incidence of contamination was detected on LJA (53.5%), while the lowest incidence of contamination was on BLA (20.9%). BLA also displayed the greatest difference in contamination rates, and this difference was significant ($P < 0.05$). PDA was the second most commonly contaminated media, with a rate of 46.5%. *Aspergillus* and *Penicillium* species contaminated all of the media types, while other species contaminated only a few types of the tested media (Table 2). Only a few of the tested organisms, such as *Trichoderma atroviride*, did not grow on BLA or SGA+CCG.

3.2. Fungal contamination according to days

Table 2 shows the mold contamination rates at the 5-day intervals of the 30-day incubation period. At 5 days after

Table 1. Dermatophyte strains used in this study.

Species	CBS no.	MI no.	Clinical source	Geographic location
Arthroderma spp.				
A. simii	448.65	-	Poultry	India
A. simii	132352	19658	Tinea inguinalis	Mersin, Turkey
A. vanbreuseghemii	117724	-	Human	France
A. vanbreuseghemii	132253	19654	Human, groin carrier	Adana, Turkey
Epidermophyton spp.				
E. floccosum [a]	-	03018	Unknown	Unknown
Microsporum spp.				
M. audouinii	732.88	-	Hair and skin	Egypt
M. audouinii	545.93	-	Head hair	The Netherlands
M. audouinii	102894	-	Head hair and skin	The Netherlands
M. canis	-	19561	Tinea cruris	İzmir, Turkey
M. ferrugineum	457.80	-	Head	Kenya
M. ferrugineum	118548	-	Hair	China
M. gypseum	130948	-	Human, tinea pedis	Rasht, Iran
M. praecox	128067	-	Human, skin lesion	Graz, Austria
T. mentagrophytes complex				
T. asteroides	424.63	-	Human, arm	Haarlem, the Netherlands
T. erinacei	344.79	-	Human, skin (arm)	The Netherlands
T. erinacei	511.73	-	*Erinaceus europaeus* (hedgehog)	New Zealand
T. erinacei	677.86	-	Human, nail	Germany
T. interdigitale	-	19670	Tinea pedis	Adana, Turkey
T. interdigitale	-	19671	Tina unguium	Adana, Turkey
T. interdigitale	-	19672	Tinea pedis	Adana, Turkey
T. interdigitale	132350	19655	Trunk carriage	Mersin, Turkey
T. interdigitale	132351	19656	Tinea inguinalis	Mersin, Turkey
T. interdigitale	132353	19657	Tinea inguinalis	Mersin, Turkey
T. m. var. *mentagrophytes*	110.65	-	Human, pubic hair	The Netherlands
T. quinckeanum	572.75	-	Human, skin (leg)	Germany
T. rubrum complex				
T. fischeri	100081	-	Contaminant	-
T. raubitschekii	202.88	-	Human, tinea pedis	Toronto, Canada
T. raubitschekii	100084	-	Human, skin	Canada
T. raubitschekii	287.86	-	Human, skin	Toronto, Canada
T. raubitschekii	102856	-	Human, thumbnail	Cameroon, Italy
T. rubrum	392.58	-	Human, tinea pedis	Rotterdam, the Netherlands
T. rubrum	132251	19651	Tinea unguium	Adana, Turkey
T. rubrum	132249	19652	Human, groin carrier	Adana, Turkey
T. rubrum	132250	19653	Human, groin carrier	Adana, Turkey
T. soudanense	436.63	-	Skin, head	Africa (country unknown)
T. violaceum	253.88	-	Skin, head	The Netherlands
T. violaceum	119446	-	Tinea capitis	Gabon
T. violaceum	120322	-	Tinea capitis	Switzerland
T. yaoundei [b]	677.82	-	Scalp	The Netherlands (patient from Morocco)
Trichophyton spp.				
T. tonsurans	132348	19341	Scalp carriage	Mersin, Turkey
T. tonsurans	132349	19342	Scalp carriage	Mersin, Turkey
T. verrucosum	134.66	-	Hair	The Netherlands
T. verrucosum	282.82	-	Hair, skin	Norway

MI, Macit İlkit working collection; a, this strain was obtained from the RSHCP; b, this *T. yaoundei* isolate was identified as *T. violaceum* in the CBS depository until January 2001.

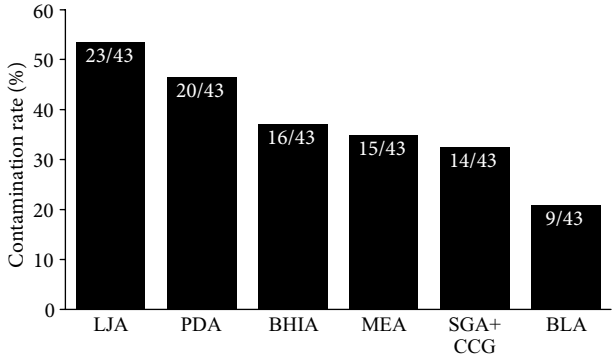

Figure. Comparison of the total contamination rates in the culture media types inoculated with dermatophytes. LJA, Lowenstein–Jensen agar; PDA, potato dextrose agar; BHIA, brain-heart infusion agar; MEA, malt extract agar; BLA, Borelli's lactritmel agar; SGA+CCG, Sabouraud- glucose agar inoculated with cycloheximide, chloramphenicol, and gentamicin.

Table 2. Incidence of contamination at 5-day time intervals on six media inoculated with 43 reference dermatophyte strains.

Species (total number)	LJA (n = 43)	PDA (n = 43)	BHIA (n = 43)	MEA (n = 43)	SGA+CCG (n = 43)	BLA (n = 43)
	5/10/15/20/25/30	5/10/15/20/25/30	5/10/15/20/25/30	5/10/15/20/25/30	5/10/15/20/25/30	5/10/15/20/25/30
Aspergillus calidoustus (n = 3)	1 / - / - / - / 1 / -	- / - / - / - / - / -	- / - / - / - / - / -	- / - / - / - / - / -	- / - / - / 1 / - / -	- / - / - / - / - / -
Aspergillus flavus (n = 17)	2 / - / 1 / 1 / 1 / -	- / - / 2 / - / - / 1	- / 2 / - / - / 1 / -	1 / - / - / - / 1 / -	- / - / 1 / 1 / 1 / -	- / - / - / - / 1 / -
Aspergillus fumigatus (n = 18)	1 / 2 / 1 / 1 / 1 / 1	- / - / 1 / 1 / 1 / -	- / - / 1 / 2 / - / -	1 / 1 / - / - / - / -	- / - / - / - / 1 / -	- / - / 1 / 1 / - / -
Aspergillus oryzae (n = 15)	1 / - / - / 1 / - / -	- / - / - / - / 1 / 1	- / - / - / - / 1 / 1	- / - / - / - / 3 / 1	- / - / - / 1 / 1 / 1	- / - / - / - / 1 / 1
Aspergillus pseudodeflectus (n = 3)	- / - / - / - / - / 1	- / - / - / - / 1 / -	- / - / - / - / - / -	- / - / - / 1 / - / -	- / - / - / - / - / -	- / - / - / - / - / -
Aspergillus versicolor (n = 9)	- / 1 / - / - / 1 / -	- / - / - / 1 / 1 / -	- / - / 1 / - / - / -	- / 1 / 1 / - / - / -	- / - / - / - / 1 / -	- / - / - / 1 / - / -
Cryptococcus victoriae (n = 2)	- / - / - / - / - / 1	- / - / 1 / - / - / -	- / - / - / - / - / -	- / - / - / - / - / -	- / - / - / - / - / -	- / - / - / - / - / -
Eurotium cristatum (n = 3)	- / - / - / - / - / -	- / 2 / - / - / - / -	- / - / - / - / - / -	- / - / - / - / - / 1	- / - / - / - / - / -	- / - / - / - / - / -
Hypocrea lixii (n = 1)	- / - / - / - / - / 1	- / - / - / - / - / -	- / - / - / - / - / -	- / - / - / - / - / -	- / - / - / - / - / -	- / - / - / - / - / -
Penicillium commune (n = 15)	1 / - / 1 / - / - / -	- / - / 1 / 1 / - / -	- / - / - / 1 / 1 / 2	- / - / 2 / - / - / -	- / - / 1 / - / 1 / 1	- / - / - / - / 1 / 1
Penicillium polonicum (n = 8)	- / - / - / - / - / -	- / - / - / - / 1 / -	- / - / - / 1 / 1 / 1	- / - / - / 1 / - / -	- / - / - / - / 1 / 1	- / - / - / - / - / 1
Trichoderma atroviride (n = 2)	- / - / - / - / - / -	- / 1 / 1 / - / - / -	- / - / - / - / - / -	- / - / - / - / - / -	- / - / - / - / - / -	- / - / - / - / - / -
Total (n = 97)	6 / 3 / 3 / 3 / 4 / 4	- / 3 / 6 / 3 / 6 / 2	- / 2 / 2 / 4 / 4 / 4	2 / 2 / 3 / 2 / 4 / 2	- / - / 2 / 3 / 6 / 3	- / - / 1 / 2 / 3 / 3

LJA, Lowenstein–Jensen agar; PDA, potato dextrose agar; BHIA, brain-heart infusion agar; MEA, malt extract agar; BLA, Borelli's lactritmel agar; SGA+CCG, Sabouraud-glucose agar inoculated with cycloheximide, chloramphenicol, and gentamicin.

inoculation, contamination was detected on both the LJA and MEA plates, but the contamination incidence was higher on the LJA plates. Approximately two-thirds of the contamination detected occurred within 15 days after inoculation. Unlike the other agars, contamination of LJA was more frequent during the first 15 days of incubation compared with the last 15 days. In addition, nearly one-third of the tested media were contaminated within 30 days of inoculation. No growth was observed on the 2 plates of each media used as negative controls.

4. Discussion

We evaluated 6 different mycological culture media types for their contamination rates when culturing dermatophytes. Plates were incubated at optimal growth conditions for dermatophytes and then examined at specific time intervals for the presence of bacterial or fungal contamination. We observed only fungal contamination on 37.6% of the plates within 30 days of incubation. Notably, we did not detect bacterial contamination on any of the media types, even though only 2 media types (BLA and

SGA+CCG) were supplemented with chloramphenicol and gentamicin. We also demonstrated that enriched media (LJA and BHIA) were more prone to contamination than nonenriched media (BLA and MEA) or media that contained antibacterial antibiotics and/or cycloheximide (SGA+CCG). However, PDA, a nonenriched medium used for primary isolation or conidiation of dermatophytes, was the most frequently contaminated medium, followed by LJA (Figure).

Only 1 BLA plate and 2 SGA+CCG plates were contaminated within 15 days of inoculation, which is a sufficient length of time to isolate the most common dermatophytes, such as *M. canis*, the *T. mentagrophytes* complex, the *T. rubrum* complex, and *T. tonsurans*. Therefore, our results suggest the use of these 2 media types to prevent contamination while subculturing or maintaining dermatophytes. Although some saprophytic molds were identified, human pathogenic molds were the dominant contaminants within 30 days. In particular, *Aspergillus* species constituted 67% of the 97 total contaminants (Table 2). In Mersin, Turkey, where this study was conducted, the most common isolates from patients with invasive fungal infections are *A. niger*, *A. flavus*, and *A. fumigatus* (17). *Trichoderma* spp. are distributed worldwide but rarely infect humans. When human infections do occur, however, the effects range from localized infection to fatal disseminated disease (18). Notably, adding cycloheximide to SGA did not prevent the growth of contaminant molds.

Fischer and Kane (8) detected bacterial contamination in 43 of 1031 (4.2%) *T. mentagrophytes* and *T. rubrum* isolates cultured on BHIA and bromocresol purple-milk solids-glucose medium (BCP-MS-G). The contamination in these studies was from conventional medical sources, namely skin and nail specimens. The most commonly identified contaminants in that study were *Pseudomonas* spp., *Staphylococcus* spp., and *Micrococcus* spp. The authors also observed a yellowing of the medium (acidic reaction), the absence of microconidia, and the presence of short aerial hyphae on Sabouraud cycloheximide chloramphenicol (SCC) agar, all of which are indicators of fungal or bacterial contamination of *T. rubrum* cultures (8). While these results may have been false-positives due to the use of BCP-MS-G agar, the findings were later verified by Summerbell et al. (19). In addition, Summerbell et al. (19) noted that reliable identification of dermatophytes is possible regardless of whether isolates are contaminated with bacteria or yeast. Notably, the efficacy of novel, broad-spectrum antibacterial antibiotics in mycological isolation media remains unknown.

BLA has been successfully used for over 50 years for the primary isolation and identification of dermatophytes. Previous studies have shown that using BLA for primary isolation promotes conidiation and pigment production (11,14,20–22). Recently, it was reported that 2 enriched media types (LJA and BHIA) are effective in promoting macroconidia production by several dermatophyte species (22,23). A dermatophyte test medium (DTM) (24,25) and a dermatophyte identification medium (DIM) (26,27) have been suggested as alternatives to SGA for the isolation of dermatophytes. However, these media types have marked limitations, including the generation of both false-positive and false-negative results (24–27). Li et al. (28) developed a selective medium, "DBM", which was reported to be a more rapid, accurate, and inexpensive alternative to DTM. Rapid diagnoses of dermatophytes in patients with tinea pedis, either by collecting samples from each toe-web by hand (29) or by using specific media that minimize the carryover effect of recent antifungal medications, have been noted to successfully improve the diagnosis of these infections (30).

Furthermore, we do not think that laboratory contamination occurred as a result of poor sterilization, poor storage, or inappropriate handling of the strains or media. In addition, the incubator used did not contain a fan, which could circulate contaminants. The contamination that we detected may have been due to dust spores. Despite this possibility, we conclude that contamination is a significant problem, not only during the primary isolation of dermatophytes but also during the subculturing of these fungi (as 37.6% of plates with pure cultures became contaminated within 30 days). Because this comparison was conducted in only one laboratory at a single time point, it is possible that the contamination is laboratory-specific or a seasonal problem. Therefore, care should be taken to eliminate sources of contamination by exercising proper quality control and good laboratory practices. Seeding tubes instead of plates may help reduce contamination.

We did not use clinical samples, which excluded the possibility of biomedical contamination due to the presence of normal skin organisms. However, the pretreatment of skin and nails using 70% alcohol before withdrawal of samples is crucial. The most unexpected problem is the contamination of nails by colonizing but nonpathogenic fungi from the environment.

In conclusion, culture media differed with regards to the specific 5-day intervals in which they became contaminated. Nonenriched media types (e.g., BLA, SGA+CCG, and MEA), with the exception of PDA, were more suitable for subculturing dermatophytes with a reduced contamination rate (Figure). The diagnosis of infections caused by dermatophytes, including large numbers of clinical samples using nonenriched media, requires further work.

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