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MODIFIED SABOURAUD DEXTROSE AGAR FOR ISOLATION AND IDENTIFICATION OF DERMATOPHYTES

ABSTRACT: The most common causative agents of dermatomycoses are fungi belonging to genders *Trichophyton, Microsporum* and *Epidermophyton*. Media mainly used for isolation of dermatophytes are mycobiotic agar, dermatophyte test medium, Sabouraud agar (original formula or modification by Emmons) with or without antibiotics and cycloheximide.

Peptones are the most important components of the media, which enable adequate reproductivity in identification of dermatophytes. Standard medium for isolation of dermatophytes is not produced in our country. The aim of the study was to create an optimal, easily accessible and economic medium which enables isolation and identification of dermatophytes according to criteria for morphological diagnosis provided by identification guides.

We examined 57 strains of *Trichophyton*, 24 of *Microsporum* and 5 of *Epidermophyton floccosum* (*E. floccosum*). Each strain was seeded on Sabouraud dextrose agar (Torlak, Serbia and Montenegro), Sabouraud maltose agar (Torlak), two experimental modified Sabouraud dextrose agar media marked as SA-2 and SA-3 (Torlak), Sabouraud-Chloramphenicole agar (Biomerieux, France), Sabouraud-Chloramphenicole agar (Himedia, India), Glucose-peptone agar (Himedia, India) and Sabouraud Emmons dextrose Agar with Chloramphenicole and Cycloheximide (Biolife, Italy).

Colony morphology of *Trichophyton mentagrophytes* (*T. mentagrophytes*) was uniform on all the media, while morphology of *Trichophyton rubrum* (*T. rubrum*) and *Microsporum canis* (*M. canis*) depended more on the media type. Colonies of *E. floccosum* were typical and uniform on all the media, as were the control species of *Trichophyton schoenleinii* (*T. schoenleinii*) and *Trichophyton soudanense* (*T. soudanense*).

Experimental modified Sabouraud dextrose agar (Torlak) marked as SA-3 demonstrated the best results in identification of dermatophytes in this study.

KEY WORDS: dermatophytes, identification, isolation, media

INTRODUCTION

Dermatomycoses are diseases widespread throughout the world. Diagnosis of dermatomycoses is based on a mycological examination of skin and/or skin adnexa scrapings and finding of mycelial filaments in the material, as well as on isolation of a causative agent from the material. The most common cause of dermatomycoses are fungi belonging to the genera *Trychophyton, Microsporum* and *Epidermophyton*. Media mainly used for isolation of dermatophytes are mycobiotic agar and dermatophyte test agar (7, 9), as well as Sabouraud agar with original formula (glucose 4%, peptone 1%) or Emmons' modification of Sabouraud medium (glucose 2%, peptone 1%). These media can be made in house or purchased as ready-made.

Bacteria and saprophytic moulds which colonize the skin and/or it's adnexa (hair and nails) could interfere with isolation of dermatophytes since dermatophytes grow more slowly. Therefore, media for isolation of dermatophytes are supplemented with antibiotics (chloramphenicole, gentamycine) and antimycotic cycloheximide (actidione), which prevents growing of saprophytic moulds. The number of onychomycoses caused by saprophytic moulds like *Aspergillus, Scopulariopsis* etc. is increasing (5, 12), therefore nail scrapings should be seeded both on media with actidione and media without it.

Standard medium for the isolation of dermatophytes is not manufactured in our country. The aim of this study was to create an optimal, easily accessible and economic medium which would enable isolation and identification of dermatophytes according to criteria for morfological diagnosis provided by identification guides (3, 6, 8).

MATERIALS AND METHODS

We examined a total of 86 strains of dermatophytes: 57 strains belonging to genus *Trichophyton* (*T. mentagrophytes* 36, *T. rubrum* 16, *T. megninii* 1, *T. equinum* 1, *T. soudanense* 1, *T. schoenleinii* 1 and *T. tonsurans* 1), 24 strains belonging to genus *Microsporum* (*M. canis* 20, *M. audouinii* 1, *M. gypseum* 1, *M. persicolor* 1, *M. species* 1) and 5 strains of *Epidermophyton floccosum* (*E. floccosum*). Control strains of dermatophytes that had been tested on mycobiotic agar (Mycosel, Oxoid, UK) (*T. schoenleinii, T. soudanense, T. equinum, T. megninii, T. tonsurans, M. audouinii* and *M. persocolor*) were obtained thanks to Dr. Mary Moore (Department for Mycology, St. John's Institute for Dermatology, St. Thomas Hospital, London, UK).

Each strain was seeded on Sabouraud dextrose agar (SDA) (Torlak); Sabouraud maltose agar (SMA) (Torlak); two experimental media made by modification of composition of standard Sabouraud dextrose agar, termed SA-2 and SA-3 (Torlak); Sabouraud-Chloramphenicole agar (Biomerieux, France); Sabouraud-Chloramphenicole agar (Himedia, India); glucose-peptone agar (GPA) (medium made in our laboratory with peptone manufactured by Himedia, India) and Sabouraud Emmons Dextrose Medium with Chloramphenicole and Cycloheximide (BL) (Biolife, Italy). The experimental media SA-2 and SA-3

were modified Sabouraud dextrose media: SA-2 with changed peptone composition and SA-3 with changed peptone composition and concetration of glucose (2%). Media were poured in Petri dishes of 19 cm in diameter, about 5 mm thick. Fungal innocula of pinhead size were seeded using a needle, on three spots. Cultures were incubated in the dark at 27°C. Growth has been observed for 3 weeks, and examination of colonies was done after 7,14 and 21 days. Examination of colonies included: measuring of colony diameter depending on the duration of incubation, morphology of colony surface (with detailed record of the following parameters: uniformity, typical morfology regarding identification keys, and pigment), pigment on the reverse of the colony and microscopic findings.

The criteria for evaluation of the tested media were based on common morphology of dermatophytes on mycobiotic agar (Mycosel, Oxoid, UK), and on the identification keys (3, 6, 8).

RESULTS

Results of examination of *Trichophyton mentagrophytes* strains are shown in Table 1.

Table 1. *Trichophyton mentagrophytes* (n = 36)

Parameters	Details	Colony morphology
colony diameter (7 days)	without AB* with AB	21—48 mm 14—33 mm
colony morphology	uniformity typical appearance colour	stable within species not depending on the medium after 7 days center folded or bulged
pigment on the reverse of the colony	colour most intense	white cream (2 species: yellow and greyish) brownish red (varies from yellow to brown) BL, SA-3, SA-2
microscopic examination	optimal identification	SA-3 best (macroconidia, microconidia and spiral hyphae), on SA-2 no macroconidia, on BL chlamydospores dominate

* AB — antibiotic (chloramphenicole and/or actidione)

Notes:

1. After the second week colony diameter equalizes, independent on the presence of antibiotics in the media.
2. No connection was found between the shape of the center and the type of medium.
3. Diagnosis of *T. mentagrophytes* can be made in one week.

Results of examination of *Trichophyton rubrum* strains are shown in Table 2.

Table 2. *Trichophyton rubrum* n = 16

Parameters	Details	Colony morphology
colony diameter (7 days)	without AB with AB	10—28 mm 5—20 mm
colony morphology	uniformity	partial: all species are the same on the same medium; morphology depends on the medium not on the species
	typical appearance colour	SA-2, SA-3, SDA
pigment on the reverse of the colony	colour most intense	white (9 species: yellow, greyish or reddish after 2 weeks) brownish red (varies from yellow to brown after 2 weeks) SA-2, SA-3; best after 7 days
microscopic examination	optimal identification	SA-3 the best (macro i microconida, spirals), on SA-2 no macroconidia, SDA, on BL chlamydospores dominate

Note: Diagnosis of *T. rubrum* can be made after two weeks.

Results of examination of *Microsporum species* strains are shown in Table 3.

Tabela 3. *Microsporum species* (*canis, audouinii and species*) n = 23

Parameters	details	colony morphology
colony diameter (7 days)	with and without AB note	10—46 mm great variation, does not depend on AB in the medium
colony morphology**	uniformity	no*
	typical appearance	partial, species within gender difficult to differentiate
	colour	white-orange
pigment on the reverse of the colony	colour most intense	orange variable SA-3, SA-2
	note	intensity of pigment depends on a species
microscopic examination	after 2 to 3 weeks	SDA for 7 days macroconidia, a few days later also on SA-2, SA-3

* Four types of colonies could be differentiated: aberant, with fine mycelium grown in the medium, orange white colonies and white with aboundant mycelium (sterile).
** Morphology depended on the type of medium, not on the species. Typical morphology was present on SA-2 and SA-3, on SMA not satisfactory. On BL medium 5 of 11 species had satisfactory morphology.

Note: *M. audouinii* had most typical colonies on SMA and BL.

On all the media the appearance of *Epidermophyton floccosum* was uniform and typical. Colony diameter was 6 to 11 mm after 7 days on media with antibiotics, and 11 to 15 mm on media without antibiotics. Microscopic identification was possible after 7 to 14 days.

Comparative analyses of the control strains on the mycobiotic agar (Mycosel, Oxoid, UK) and our tested media

M. persicolor

The diameter of a colony after 7 days on mycobiotic agar was 25 mm, and on our tested media 22—25 mm without antibiotics, and 16—22 mm with antibiotics. On all the media colonies granulated surface and cream white colour with bulging center, except on SMA where the colour was rose. Pigment on the reverse of the colony was brown on mycobiotic agar, rose on SMA, and various intensity of yellow-brown on the other media, the strongest on SA-3. Microscopic examination of colonies grown on the mycobiotic agar revealed characteristic microconidia, macroconidia and spiral hyphae. Macroconidia and microconidia were found in colonies from SA-3, SDA and GPA, and only microconidia in colonies grown on the rest of the tested media.

T. equinum

The diameter of a colony after 7 days on mycobiotic agar was 30 mm, and on our tested media 25 mm without antibiotics and 18—20 mm with antibiotics. On all the media colonies were almost identical: flat, disheveled, with edges immersed in the media, coloured white to yellow. Pigment on the back of the colony was brown in the center and yellow on the circumference. The findings on SA-3 matched the most with the findings on the mycobiotic agar. Characteristic microconidia were found by microscopic examination of a colony from mycobiotic agar, while macroconidia in addition to microconidia of a colony from SA-3.

T. megninii

The diameter of a colony after 7 days on mycobiotic agar was 12 mm, and on our tested media 10—16 mm not depending on the presence of antibiotics. On mycobiotic agar the colony was flat, white, velvety, developing a rose pigment only after the second week. On our tested media colonies were uniform, white coloured with bulging center and the formation of radial grooves after the third week of incubation. Pigment on the reverse of the colony was characteristic light red only on the mycobiotic agar, and on our tested media no pigment was produced. Beside findings on the mycobiotic agar, microscopic examination was characteristic on SMA (macroconidia and microconidia), and only microconidia on SDA, SA-3 and BL.

T. tonsurans

The diameters of colonies after 7 days were about the same on all the media, including mycobiotic agar. Colonies were powdery, cream to brown

coloured, also identical on all the media. The pigment on the reverse of colonies was characteristic — yellow-brown — on the mycobiotic agar. On our tested media the production of pigment was satisfactory on SA-3, SDA and BL. Besides typical microscopic findings on mycobiotic agar (microconidia, baloon forms and chlamydospores), the same morphology was found on SA-2 and SA-3.

T. schoenleinii and *T. soudanense* were of the same morfology on all the tested media, including the mycobiotic agar.

Comparing the parameters of examination we found that the presence of antibiotics in a medium, no matter whether they were antibacterial (Chloramphenicol) or antimycotic (Actidione), had influence on growth of the majority of dermatophytes in the first week of incubation, reflecting on diameters of colonies, with exception of *T. tonsurans, T. megninii* and genus *Microsporum.*

Morphology of all the strains of *T. mentagrophytes* was uniform on all the media, in contrast to *T. rubrum* where morphology was more depending on the type of the medium. Therefore, usage of a non-standard medium in practice could lead to mistakes in diagnosis of *T. rubrum.*

Examination of 20 strains of *M. canis* established that colonies were distinguished as members of the genus *Microsporum*, but variations in appearance were present. We noted that the difference in morphology was due to the type of medium, and not to strain type.

Colonies of *Epidermophyton floccosum* were typical and uniform on all the media, as were control strains of *T. schoenleinii* and *T. soudanense*. Other control strains of *M. persicolor, T. tonsurans* and *T. equinum* were of almost identical morphology on all the media, including mycobiotic agar (Mycosel, Oxoid, UK), in contrast to *T. megninii* which showed somewhat different morphology on mycobiotic agar than on the other media.

The colour of colonies of all the tested strains corresponded to the description in identification guides. The control strain of *M. persicolor* was rose coloured on SMA, but cream on all the other media. For that reason SMA could be used for differential diagnosis of *T. mentagrophytes* and *M. persicolor.*

All tested strains of dermatophytes, except *T. megninii* and *T. schoenleinii*, produced pigment of various intensity depending on the type of medium or on the strain, which diffused into the media. SA-2 and SA-3 were most stimulative for the pigment production in *T. mentagrophytes, T. rubrum* and *Microsporum species.* Besides, SA-2 and SA-3 media performed best results in the production of characteristic structures for asexual reproduction of dermatophytes, which contributed to clarity of microscopic morphology of all the strains of *Trychophyton*, except *T. megninii.* The control strain of *T. megninii* developed more complete motphology on SMA. Microscopic finding of *Microsporum* strains was better on SDA, where typical macroconidia developed in 7 days, though they did develop on SA-2 and SA-3 in the second week of incubation. The control strain of *M. audoinii* demonstrated the best morphology on SMA.

DISCUSSION

The number of dermatophyte species is large, but only most commonly isolated in our country were included in this study (2).

Specialized laboratories in the world mostly use mycobiotic agar for isolation of dermatophytes (7). DTM (Dermatophyte Test Medium) and a new DIM (Dermatophyte Identification Medium) are used for quick diagnosis of dermatophytes (11). Diagnostic principle in these media is based on production of alcaline metabolic products during growth — a feature that distinguishes dermatophytes from saprophytic moulds. That leads to change in colour of a medium (indicator is phenol-red in DTM, and brom-cresol-purple in DIM). These media are mostly used in field research or in non-specialized laboratories.

Dermatophytes are identified on the basis of colony morphology, growth rate, pigment production, microscopic findings and physiological features. Most strains of dermatophytes can be identified basing on their macroscopic and microscopic features in primary culture. For further identification Lactrimel-agar and Trichophytone agars 1—7 (Difco) could be used, as well as a number of physiological tests (production of urease, hair perforation test etc.).

In addition to mentioned media, which are special for dermatophytes, standard Sabouraud agar can also be used. Standard Sabouraud agar is a medium consisting of 1% peptone, 1.5—2% agar, and 4% glucose, with final pH 5.6 (1). In practice, composition of Sabouraud media made by different manufacturers quite varies (1). Differences in media composition lead to differences in morphology and lack of reproducibility in diagnosis of colonies (1, 10). The most important component of the medium, one that morphology and especially pigment production depends on, is peptone (4). Peptones present in commercial Sabouraud media can be restrictively divided according to: enzyme used for peptone hydrolyses (pancreatic, peptic, papaic), protein source (casein, soya, meat), and according to purpose (mycological). These media are also different in final pH (5.6—6.8). Mycobiotic agar has similar composition as Sabouraud agar, but it contains plant source peptone (phytone peptone) (1%), dextrose (1%), actidione (0,04%) and chloramphenicole (0,005%) (7).

In our country only a few laboratories were able to perform isolation of dermatophytes on mycobiotic agar. One of the authors (N. B.) spent most of her long-standing experience working with Sabouraud dextrose agar supplemented with penicillin, streptomycin and cycloheximide for isolation of dermatophytes. Differential diagnosis of dermatophytes on that medium was not simple. Therefore, in this study, modifications of present Sabouraud dextrose agar (Torlak) were undertaken, which were supposed to enable faster and more accurate diagnosis of dermatophytes on an easily accessible and economic medium.

By changing the peptone type and dextrose concentration in Sabouraud dextrose agar (Torlak) we obtained a new medium SA-3, which enabled isolation and identification of majority of species of dermatophytes in our region according to identification guides.

Conclusion: Experimental medium SA-3 (Torlak) demonstrated the best results in identification of dermatophytes in this study. Macroscopic and microscopic morphology of dermatophytes on this medium corresponds the most with morphology of these fungi on standard media that contain mycological peptone as a main component. Sabouraud dextrose agar (Torlak), which was used in our country for primary isolation of dermatophytes, can be used for identification of *M. canis* because it supports production of macroconidia already in the first week of incubation. On all the media *T. mentagrophytes* can be diagnosed after 7 days, while for the identification of other species of dermatophytes more than 14 days is necessary.

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МОДИФИКОВАНИ САБУРО ДЕКСТРОЗНИ АГАР ЗА ИЗОЛАЦИЈУ И ИДЕНТИФИКАЦИЈУ ДЕРМАТОФИТА

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Резиме

Дерматомикозе су широко распрострањена обољења свугде у свету. Најчешћи узрочници дерматомикоза су гљиве из родова *Trichophyton, Microsporum* и *Epidermophyton*. За изолацију дерматофита углавном се користе микобиотски агар, дерматофит-тест агар, Сабуро агар са оригиналном формулом или Емонсова модификација Сабуро агара са и без антибиотика и циклохексимида. Пептони су најзначајнија компонента подлога која омогућава адекватну репродуктивност у идентификацији дерматофита. У нашој земљи се не производи стандардна подлога за изолацију дерматофита. Циљ овог рада је био да се креира оптимална, лако доступна и економична подлога, која омогућава изолацију и идентификацију дерматофита према критеријумима за морфолошку дијагностику предвиђеним водичима за идентификацију.

Прегледано је 57 сојева рода *Trichophyton*, 24 сојева рода *Microsporum* и 5 сојева *Epidermophyton floccosum* (*E. floccosum*). Сваки сој је засејан на Сабуро декстрозни агар (Торлак), Сабуро малтозни агар (Торлак), две експерименталне подлоге настале модификовањем састава стандардног Сабуро декстрозног агара под радним називом SA-2 и SA-3 (Торлак), Сабуро-хлорамфеникол агар (Biomerieux, Француска), Сабуро-хлорамфеникол агар (Himedia, Индија), глукозо пептонски агар (са пептоном Himedia, Индија) и Сабуро Емонс декстрозни агар са хлорамфениколом и циклохексимидом (Biolife, Италија). Критеријуми за оцену испитиваних подлога установљени су на основу познате морфологије коју сојеви дерматофита показују на микобиотском агару (Mycosel, Oxoid, Велика Британија) и на основу кључа за идентификацију гљива.

Морфологија колонија *Trichophyton mentagrophytes* (*T. mentagrophytes*) била је униформна на свим подлогама, док је морфологија *Trichophyton rubrum* (*T. rubrum*) и *Microsporum canis* (*M. canis*) више зависила од врсте подлоге него од соја. Колоније *E. floccosum* су биле типичне и униформне на свим испитиваним подлогама, као и контролни сојеви *Trichophyton schoenleinii* (*T. schoenleinii*) и *Trichophyton soudanense* (*T. soudanense*). Експериментална подлога SA-3 показала је најбоље резултате у идентификацији дерматофита у овој студији. Макроскопска и микроскопска морфологија дерматофита на овој подлози највише одговара морфологији ових гљива на стандардним подлогама у којима се као главна компонента налази миколошки пептон. Сабуро декстрозни агар (Торлак), који се досада у нашој земљи користио за примарну изолацију дерматофита, може се користити у идентификацији *M. canis* јер потпомаже продукцију макроконидија већ у првој недељи инкубације. На свим подлогама *T. mentagrophytes* се може дијагностиковати након 7 дана, док је за идентификацију осталих врста дерматофита потребно више од 14 дана.