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Effectiveness of FastFung agar in the isolation of *Malassezia furfur* from skin samples

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Abstract

Background: Lipophilic basidiomycetous yeasts of the *Malassezia* genus can cause various skin diseases, such as seborrheic dermatitis, pityriasis versicolor, folliculitis and atopic dermatitis, and even life-threatening fungemia in newborns and immunocompromised individuals. Routine mycological media used in clinical practice do not contain sufficient lipid ingredients required for the growth of *Malassezia* species. A recently developed medium, FastFung agar, is promising for culturing fastidious fungal species.

Methods: In this study, we compared FastFung agar and mDixon agar for culturing *Malassezia* species from nasolabial fold and retroauricular specimens of 83 healthy individuals and 187 and 57 patients with acne vulgaris and seborrheic dermatitis, respectively.

Results: *Malassezia* species were identified using conventional tests and matrix-assisted laser desorption/ionisation mass spectrometry. In total, 96 of 654 samples (14.6%) contained *Malassezia* species. The total isolation rate was significantly higher in patients with seborrheic dermatitis (40.4%) than in healthy volunteers (21.7%; $p < .05$), and the rate of *M. furfur* isolation was significantly higher for patients with acne vulgaris (13.9%) and seborrheic dermatitis (24.6%) than for healthy individuals (1.5%; $p < .05$). FastFung agar was superior to mDixon agar in *M. furfur* isolation ($p = .004$) but showed similar performance in the case of non-*M. furfur* species ($p > .05$). Among cultured *Malassezia* species, perfect agreement between mDixon agar and FastFung agar was found only for *M. globosa* ($\kappa = 0.90$).

Conclusion: Our results indicate that FastFung agar favours the growth of *Malassezia* species and should be useful in clinical mycology laboratories.

KEYWORDS
acne vulgaris, FastFung agar, fungal infection, *Malassezia* spp., seborrheic dermatitis

1 | INTRODUCTION

Lipophilic basidiomycetous *Malassezia* yeast is the most eukaryotic component of the skin microbiome of the scalp, face, chest and upper back, which are mostly on sebaceous sites.[1] Most species of *Malassezia* are associated with different clinical manifestations, such as pityriasis versicolor, seborrheic dermatitis, atopic dermatitis, folliculitis, dandruff, psoriasis and rarely onychomycosis.[2] However,

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under certain conditions, they can cause life-threatening systemic infections, including fungemia in newborns and immunocompromised individuals.[3,4]

Because the growth of *Malassezia* strains depends on lipids, Sabouraud glucose agar is not appropriate for routine isolation of these pathogens in clinical microbiology laboratories, which perform the identification of infectious fungal species for therapeutic purposes. A wide range of agar-based media have been used to culture *Malassezia* species from clinical samples, such as CHROMagar *Malassezia*™, potato dextrose agar with olive oil, mDixon agar, and modified Leeming and Notman agar.[5,6] Because *Malassezia* strains vary in their lipid requirements, they tend to exhibit dissimilar growth patterns in the same medium. Despite advances in internal transcribed spacer and metagenomic studies, the results obtained with culture are insufficient.[1] Variations in culture methods enable different species to be obtained more rapidly in elucidating the relationship between yeast and several diseases. The distribution of *Malassezia* species differs depending on the ethnic background, sex and skin site of the host. Global epidemiological studies indicate that *Malassezia globosa*, *M. sympodialis*, *M. restricta* and *M. furfur* are frequently isolated from patients with chronic inflammation and those with acute infection episodes.[5,7,8] Therefore, the development of selective media to culture these fungal pathogens would facilitate the detection and differentiation among the most common species, which should aid in the improvement of treatment practice.

Malassezia furfur is often isolated from different hosts and body parts, most commonly from skin lesions due to pityriasis versicolor, but other locations, such as scalp, hair, nasal cavity, dandruff, urine, blood, nails and eyes, have also been observed; furthermore, it has been detected in the environment, such as hospital floors.[9] Along with *M. pachydermatis*, *M. furfur* is the most prevalent species causing chronic infections in hospitalised patients and immunocompromised individuals.[10,11] *Malassezia furfur* has been identified as the species most frequently colonising the skin of newborns, who, if hospitalised, are prone to develop fungemia.[10] It has also been reported that *M. furfur* strains isolated from nonhealthy skin have increased resistance to antifungal agents.[11]

Standardisation of the methods for skin sample collection is important for culture-based approaches, particularly those using agar plates.[5,6] FastFung medium has been recently reported to be promising for the cultivation of fungal pathogens, including some *Malassezia* spp.[6,12] The aim of this study was to compare FastFung medium with mDixon agar in terms of culturing *Malassezia* isolates from human skin. We report that FastFung agar is superior to mDixon agar for the growth of *M. furfur* isolates from clinical samples.

2 | MATERIALS AND METHODS

2.1 | Patients

Overall, 327 volunteers, including healthy individuals ($n = 83$) and patients with acne vulgaris ($n = 187$) and seborrheic dermatitis ($n = 57$), who had been admitted to Başakşehir Çam ve Sakura City Hospital Dermatology outpatient clinics (Istanbul) from January to July 2021 were recruited for the study. Patients who had been taking systemic or local antifungal treatment in the 15 days preceding the study and those with serious/recurrent immunological problems and hormonal disorders were excluded. The patients did not use daily cream, and no cleaning was performed before sampling. All samples were taken from the same site for standardisation, regardless of disease involvement area.

2.2 | Clinical samples, media and growth conditions

Two samples (from nasolabial folds and retroauricular areas) were collected from each participant by rubbing with saline-soacked non-flocking rayon swabs for 10–15 s. Samples were inoculated onto customised mDixon agar (3.6% malt extract, 1% mycological peptone, 2% ox bile, 1% Tween 40, 0.2% glycerol, 0.2% oleic acid, 1.5% agar; pH 6.0) and Fast Fung agar (4.3% Schædler agar, 2% peptone, 1% glucose, 1% malt extract, 0.5% ox bile, 0.5% Tween 60, 0.2% oleic acid, 0.25 glycerol; pH 6.0).[6,8] Chloramphenicol (0.4%) was added to both media to inhibit flora bacteria. The plates were incubated in aerobic conditions at 32°C in a humid environment for 2 weeks and monitored on a daily basis. Colonies with yeast-like morphology were carefully collected and used for identification.

2.3 | Identification

The morphological features and physiological characteristics of the isolates were examined, including colony size and shape, gram staining, failure to grow on Sabouraud glucose agar (except *M. pachydermatidis*), reaction of CHROMagar™ *Malassezia* (CHROMagar, Paris, France), growth at different temperatures (32°, 37 and 40°C), catalase and β-glucosidase activity, and the ability to utilise Tween 20, 40, 60 and 80 and Cremophor-EL.[9,13] Selectively isolated *M. furfur* strains were confirmed by matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-TOF MS) using a referral library (courtesy of Prof Dr Ramazan Gümral, Gulhane Training and Research Hospital, University of Health Sciences, Ankara, Turkey).

In both agar media, semiquantative evaluation of all positive *Malassezia* cultures following 2 weeks of incubation (graded as +, if one macroscopic colony was formed after 10 days; ++, if 2–5 macroscopic colonies; and +++, if more than five macroscopic colonies).[14,15]

2.4 | Statistical analysis

Data analysis was performed using weighted Cohen κ tests in the R package 'psych' (R-Ver 2.1.9; Rewelle W, IL, USA), McNemar's test (SPSS ver. 17.0, Chicago, IL, USA) and Wilcoxon signed-rank test. $p < .05$ was considered to indicate statistical significance.

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TABLE 1 *Malassezia* spp. isolation rates in healthy individuals ($n = 83$) and patients with acne vulgaris ($n = 187$) and seborrheic dermatitis ($n = 57$)

	Healthy volunteers *n* (%)	Acne vulgaris *n* (%)	Seborrheic dermatitis *n* (%)
M. furfur	4 (4.8)	26 (13.9)	14 (24.6)
M. globosa	6 (7.2)	7 (3.7)	3 (5.3)
M. restricta	5 (6.0)	4 (2.1)	4 (7.0)
M. sympodialis	3 (3.6)	1 (0.5)	2 (3.5)
M. sloffiae	–	1 (0.5)	–
Total	18 (21.7)	39 (20.9)	23 (40.4)

3 | RESULTS

Overall, 654 specimens from 327 volunteers were analysed in the study, and 96 (14.6%) tested positive for *Malassezia* spp. Among the positive samples, 21.7%, 20.9% and 40.4% were identified in healthy individuals and patients with acne and seborrheic dermatitis, respectively, and the difference between the healthy group and seborrheic dermatitis group was significant ($p < .05$; Table 1). Furthermore, a significantly higher rate of *M. furfur* isolation was detected for patients with acne vulgaris and seborrheic dermatitis compared with healthy volunteers (13.9% and 24.6%, respectively, vs. 1.5%; $p < .05$).

In most cultures, at least one *Malassezia* macroscopic colony, that is grade (+) growth, was observed: 69.0% and 72.7% on mDixon agar and FastFung agar, respectively ($p < .05$; Table 2). For each medium, the median culture time was 4 days, and there was no significant difference ($p > .05$).

Among the cultured *Malassezia* species, perfect agreement between mDixon agar and FastFung agar was observed only for *M. globosa* ($\kappa = 0.90$) according to the Landis and Koch scale,[16] whereas different levels of agreement were detected for *Malassezia resticta*, *M. sympodialis* and *M. furfur* ($\kappa = 0.74$, 0.71 and 0.67, respectively; Table 3). There was no difference between the two media in the variety of the isolated species (Wilcoxon test; Table 3). The results of the McNemar test revealed a higher isolation effectiveness of FastFung agar for *M. furfur* ($p = .004$), whereas both media showed the same performance regarding the isolation of non-*M. furfur* species (Table 3).

TABLE 2 Comparison of the mDixon agar and FastFung agar media via the frequency of isolation and semiquantitative assessment results

	mDixon agar	FastFung agar
Only mDixon agar positive	8	–
Only Fast Fung agar positive	–	25
Both positive	63	
Semiquantative results; *n* (%)		
(+)	49 (69.0)	64 (72.7)
(++)	16 (22.5)	19 (21.6)
(+++)	6 (8.5)	5 (5.7)
Total	71 (100.0)	88 (100.0)

4 | DISCUSSION

Malassezia species may not be detected in standard clinical microbiology laboratories because of the lack of appropriate lipophilic media. Currently, mDixon agar is the preferred medium for *Malassezia* species; however, FastFung agar has also been reported to be useful for the cultivation of these species.[5,6] Our findings clearly demonstrate the superior performance of FastFung agar in the isolation of *M. furfur* from clinical skin samples (Table 3). The maximum agreement between mDixon and FastFung agar media was observed for *M. globosa* isolates ($\kappa = 0.90$). FastFung agar could better support the growth of *M. furfur* than mDixon agar, whereas both media were similar regarding the growth of other *Malassezia* species, and there was no difference between the two media in the spectrum of the isolated species (Table 3).

Different media were used for the culture of *Malassezia* spp. Usually, Tween 40 (contained in mDixon agar) or Tween 60 (contained in Leeming–Notman agar) provides sufficient lipophilic reproductive support for the growth and maintenance of different species. Tween 60 is thought to be more effective in *Malassezia* cultures, which FastFung includes.[6,9] Another component of FastFung agar, ox bile, supports the growth of yeasts for *Malassezia* and allows it to be joined into routine use of FastFung medium.[6,12,17]

Malassezia furfur was predominantly identified in patients with acne vulgaris and seborrheic dermatitis rather than in the healthy group ($p < .05$), which is in contrast to a previous report, indicating that *M. globosa* is the species most frequently isolated from patients with various skin diseases;[4] however, in this study, 33% of *M. globosa* isolates were detected in healthy individuals. It has been reported that among *Malassezia* species associated with various pathological conditions, *M. furfur* and a few other species are involved in colonisation.[7,18] The incidence of infectious diseases due to dermal colonisation is dramatically increasing worldwide, emphasising the need for the development of special media to isolate pathogenic *Malassezia* species.

Recent findings regarding *M. furfur* diagnosis are consistent with those described approximately 150 years ago. Frequently encountered systemic infections such as pityriasis versicolor, atopic dermatitis, folliculitis, seborrheic dermatitis and psoriasis, which are predominantly observed in hosts with compromised immunity, might be disguised as recalcitrant acne.[19] Furthermore, in patients

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TABLE 3 Contrasted values of mDixon agar and FastFung agar media with 327 coupled (totally $n = 654$ culture) samples

Species	mD	mD %	FF	FF %	Cohen κ	Wilcoxon *p*	McNemar *p*
M. furfur ($n = 53$)	44	6.72	53	8.10	0.67	.28	.004
M. globosa ($n = 18$)	9	1.37	14	2.14	0.90	.15	.62
M. restricta ($n = 16$)	10	1.52	13	1.98	0.74	.16	.25
M. sympodialis ($n = 8$)	7	1.07	7	1.07	0.71	–	–
M. sloffiae ($n = 1$)	1	0.15	1	0.15	–	–	–

Abbreviations: FF, FastFung agar; mD, modified Dixon agar.

receiving long-term ototopical antibiotic therapy, *M. furfur* may cause subsidiary infections, affecting the flora in the outer ear canal.[20] Particular attention should be given to *M. furfur* strains exhibiting strong resistance to antifungal drugs.[11] In this respect, FastFung agar would be helpful, as it demonstrated better results in *M. furfur* isolation than mDixon agar, although there was no difference in the variety of isolated species between the two media (Wilcoxon $p = .28$, McNemar $p = .004$; Table 3).

Although comparisons have been made on clinical samples, the most important limitation is the lack of data on the performance of rare strains in human primary culture isolation. In addition, *Malassezia* in deeper folliculitis was not cultured, and culture rates do not indicate the aetiology of deep folliculitis.[2,21] Apart from the routine clinical settings of the medium as a limitation of study, the growth ability of FastFung agar performance for newer animal species, especially those requiring different environmental conditions, such as *M. vespertilionis* and *M. cuniculi*, should also be tested for animal mycology.[22]

FastFung agar has been recognised as a convenient isolation medium in medical mycology.[12] Further studies are needed to confirm the superior performance of FastFung agar in culturing *Malassezia* isolates from patients with skin diseases.

AUTHOR CONTRIBUTIONS

NA, ÇE and Mİ contributed to conceptualization. ÇE and Mİ involved in data curation. Mİ involved in involved in formal analysis and validation. NA involved in involved in funding acquisition. NA, ZT and NC involved in investigation. NA, ÇE and NC involved in methodology. NA and ZT involved in project administration. NA and AD involved in resources. ÇE, ZT and AD involved in supervision. ÇE involved in visualisation. NA and ÇE wrote the original article. All coauthors involved in writing—review and editing.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest. The authors alone are responsible for the content and writing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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