FastFung: a novel medium for the culture and isolation of fastidious fungal species from clinical samples

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Abstract

We developed a novel culture medium, referred to FastFung medium as suitable for the culture of clinical fungi, including fastidious ones, for both research and diagnostic studies. It is based on Schædler agar supplemented with many essential components for the growth of fastidious fungi. It also contains selective antibacterial agents for the inhibition of contaminant bacteria growth. In this preliminary study, the FastFung medium was compared to the gold standard Sabouraud medium for 98 fungal and 20 bacterial strains.

The fungal strain positive culture rate was 100% *vs*. 95% and the bacterial strain inhibition was 100% *vs*. 20%, for the FastFung and Sabouraud media, respectively. When compared to the Sabouraud medium on 120 clinical samples, the FastFung medium displayed both a higher fungal colonies count, and a lower culture contamination rate. Storage at 4°C for 4 weeks did not alter the FastFung culture medium performances for the six isolates of *Candida, Cryptococcus*, and *Penicillium* tested.

These encouraging results suggest future development of using the FastFung medium in clinical mycology and in mycobiome characterization. Further prospective evaluation aiming at assessing whether implementing the FastFung medium in the routine workflow simplifies and strengthen fungal isolation capacities in the clinical laboratory is warranted.

Keywords: culture media; clinical laboratory techniques; mycology; mycoses; Malassezia; Candida; Cryptococcus; mycobiome.

INTRODUCTION

Fungal species are ubiquitous environmental organisms. They can be found in food, water, soil and also isolated from humans and animals samples (1). They are often associated with some human infectious diseases (2;3). Fungal microorganisms of clinical interest are mainly grouped into three classes: Ascomycota, Basidiomycota and Zygomycota (4). Fungal infections are the current object of many studies (1;2;5). The predominant clinical species is *Candida albicans* that is involved in the majority of opportunistic invasive fungal diseases (6). Indeed, recent studies have pointed out *C. albicans* as a major cause of morbidity and mortality in critical care patients, as well as its involvement in other oropharyngeal diseases of the mouth, and digestive and vaginal tract in immunocompromised and antibiotic treated individuals (7). The genera *Cryptococcus* and *Aspergillus* also include many fungi involved in human infections (2;8). Many fungal species are opportunistic pathogens; *i.e*. they are commensals when the host is healthy, and they become pathogenic when the host is immunocompromised and/or critically ill (7). Thus, the population of immunocompromised patients has the highest risk of opportunistic fungal infections (2;3;9). Moreover, recent increases in emerging fungal infections call for an effective culture medium capable of timely isolating fastidious fungi from any biological sample at the clinical mycology laboratory.

While Sabouraud dextrose agar is the most frequently used medium for fungal isolation (10;11), other media are commonly used for culture of yeasts and filamentous fungi, including Potatoe Dextrose Agar (12), Czapek (13) and Dixon agar (14). These media are often used in combination in clinical laboratories. These, and several other culture media, are used as reference for the isolation or the identification of specific fungal taxa (15). This study aimed at developing a novel culture medium, which is suitable for the culture of clinical fungi, including fastidious ones, for both research and diagnostic studies. The FastFung medium is based on Schædler agar (Sigma-Aldrich) supplemented with many components needed for the growth of fungal species.

It also contains selective antibacterial agents for the inhibition of contaminant bacteria. In this preliminary study, the FastFung medium was evaluated on 98 fungal and 20 bacterial strains.

MATERIALS AND METHODS

Culture Media

In this study the performance of fungal culture on FastFung and Sabouraud dextrose agar (Oxoid, Dardilly, France) media were compared. The Sabouraud medium is supplemented with chloramphenicol and gentamycin. In addition to bacteriological agar, this culture medium contains glucose and peptone allowing the growth of many fungal species. Sabouraud medium was considered as the gold-standard fungal medium in this study. The FastFung medium proposed in this study is composed per liter of 43 g of Schædler Agar (Sigma-Aldrich, Saint-Quentin Fallavier, France), 20 g of peptone and 10 g of glucose as basic components, with the addition of 5 g of ox-bile 10 g of malt extract, 2 ml of oleic acid, 5 ml of Tween 60 and 2.5 ml of glycerol. Each component was autoclaved at 121°C for 30 min; except for Tween 60, glycerol and oleic acid, which were added after autoclaving. After cooling to 50°C, the FastFung medium was supplemented with a mixture of three antibiotics at 30 mg/l, including colistin, vancomycin and imipenem (Sigma Aldrich, St Louis, France). The final product was then dispensed into 90 mm sterile Petri dishes.

Bacterial and fungal strains

A collection of 98 fungal strains (Table 1) and 20 bacterial strains (Table 2) that were isolated in the laboratory by culturomics methods (16;17) were used to assess the FastFung medium. The identification of each strain was confirmed by the sequence analysis of the partial 16S rRNA gene for bacteria or the ITS rRNA region for fungi, as previously described (16;18). The fungal

89 strains used included *Candida* spp. (n = 50), *Cryptococcus* spp. (n = 2), *Aspergillus* spp. (n = 7),
90 *Penicillium* spp. (n = 3), *Malassezia* spp. (n = 2) and 34 other fungal strains (Table 1).
91 Appropriate negative and positive controls were used in each experiment.

92 **Evaluation of FastFung medium and Sabouraud agar on fungal strains**

93 All the strains used in this study have been preserved at - 80°C in sterile tubes with glycerol. The
94 fungal strains have been cultured onto Sabouraud, Dixon agar or Potatoes Dextrose agar and the
95 bacterial strains on 5% sheep blood-enriched Colombia agar (BioMérieux, France) to obtain
96 fresh and pure cultures. Each isolate was suspended in 1 ml of saline (0.85%) at a concentration
97 of approximately $1.5x10^5$ CFU/ml for fungi and $1.5x10^8$ CFU/ml for bacteria (0.5 McFarland
98 standard) using a densitometer (DEN-1 McFarland Densitometer, BioSan, Riga, Latvia). Serial
99 five-fold inoculum dilutions were then prepared as follows: 100 µl of each solution was diluted
100 in 900 µl of water following by four other serial dilutions. A 1 µl aliquot of each dilution was
101 inoculated onto the culture plate and spread with a sterile inoculation loop. All plates were
102 incubated at 28°C and 32°C for fungi and 37°C for bacterial strains under aerobic conditions.
103 The bacterial strains were inoculated in the same way as fungal strains, but no dilution was done.
104 **Assessment of the FastFung medium on routine clinical samples**
105 The effectiveness of the FastFung medium was further assessed on 120 clinical samples,
106 prospectively received at the Timone University Hospital (Marseille, France) microbiology
107 laboratory for routine analyses for a 6 week period in which a fungus was isolated. These
108 samples were obtained from various body sites, mostly urine and vagina, as detailed in Table 3.
109 An aliquot (50 µl) of each clinical sample was inoculated onto the Sabouraud and the FastFung
110 media in parallel. The plates were incubated under aerobic conditions from 24 to 48 hours at
111 28°C.

FastFung medium stability assessment

Forty FastFung medium plates were prepared and stored at 4°C for 4 weeks. Six fungal strains including *Candida* spp. (n = 2), *Cryptococcus* spp. (n = 2) and *Penicillium* spp. (n = 2), were used to test the stability of the culture medium. The same procedures described above were applied to inoculate fungal strains onto the FastFung medium. Inoculated plates were then incubated under aerobic conditions at 28°C and examined daily for 7 days.

RESULTS

FastFung medium assessment on fungal strains

The positive culture rate of the fungal strains after 6 days 98/98 (100%) with the FastFung medium. Moreover, each inoculum dilution yielded a positive culture with the FastFung medium. Among each fungal strain assayed on the FastFung medium, growth was visualized in 57/98 (58%) of the cultures after 24 hour of incubation and in 67/98 (68%) after 48 h of incubation. In contrast, the Sabouraud medium allowed the growth of 93/98 (95%) of the fungal strains, and in particular, *Candida zeylanoides*, *Cryptococcus diffluens*, *Cryptococcus uniguttulatus*, *Malassezia furfur* and *Torulaspora pretoriensis* did not grow (Table 1). Among bacterial strains inoculated onto the FastFung medium plates, 20/20 (100%) bacterial strains were effectively inhibited and did not grew on the FastFung medium (Table 2). In contrast, only 4/20 (20%) of the bacterial strains were inhibited and did not grow on the Sabouraud medium (Table 2).

FastFung medium assessment on prospective routine clinical samples

There was no discrepancy between the two media regarding the positive culture rate. All the tested were culture positive samples grew on both Sabouraud and FastFung culture media (Table

3). *Candida albicans*, *Candida tropicalis* and *Candida parapsilosis* were the most frequently isolated fungal species. *Candida albicans* was the most frequent isolated species, regardless of the clinical sample type and the culture medium. *Candida albicans* and *Candida tropicalis* were relatively more frequently isolated from urinary samples. *Candida tropicalis* and *Candida parapsilosis* were relatively more frequently isolated from bronchial aspiration samples. After only 24 hours of incubation the number of fungal CFU was higher on the FastFung medium than on the Sabouraud medium (Figure 1). Moreover, several filamentous fungi contaminations occurred on Sabouraud but not on FastFung medium (data not shown).

FastFung medium stability

Neither the quality of growth of the six fungal strains nor the inhibition bacterial strains and filamentous fungi was altered when the culture medium had been stored at 4°C for 4 weeks (data not shown).

DISCUSSION

Overall the FastFung medium proved more efficient than the Sabouraud medium for the rapid isolation of fungi and inhibition of bacteria and filamentous fungi contaminants from the clinical samples tested. Each of the 98 fungal strains tested grew on the FastFung medium, whereas only 67 grew on Sabouraud. A number of fastidious fungi, such as the lipid dependent *Malassezia* spp. yeast, require the specific culture media for their growth (14;15). The addition of specific components, such as oleic acid, considerably extended the spectra of culturable fungi as described previously (20). In addition, and although both two culture media contain antibacterials, the FastFung medium more efficiently inhibited bacterial growth than the Sabouraud medium.

Studies comparing the efficiency of different fungal culture media are scarce. To our knowledge, we report the first study that compares a new fungal culture medium with a reference culture medium commonly used for the isolation and culture of fungal species. One strengths of this study is the large number of bacterial and fungal strains assessed, which were all identified by MALDI TOF mass spectrometry and DNA sequence analysis (19).

When compared to the Sabouraud medium on routine clinical samples, FastFung medium displayed a higher fungal colonies count after 48 hours of culture (Figure1) a lower number of commonly encountered culture contamination (10;21). The FastFung medium proved stable for 4 weeks. Using this versatile culture medium in the clinical laboratory would advantageously avoid using various fungal culture media for the isolation of fastidious fungi and offers great opportunities in clinical microbiology and culturomics studies when associated with MALDI TOF mass spectrometry identification based on comprehensive fungal reference spectra databases.

In conclusion, implementing the FastFung medium in the routine clinical mycology laboratory workflow would advantageously reduce the time to fungal growth and makes unnecessary the use of Sabouraud and other complementary culture media for fungal isolation. Further prospective evaluation of the use of the FastFung medium for fungal isolation in the clinical laboratory is warranted.

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Ethical Approval

Not required

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Figure 1. The FastFung medium displayed higher fungal CFU counts compared to the Sabouraud medium. Here we show the isolation after 48 hours of incubation of three yeast species (*Candida albicans*, *C. parapsilosis*, and *C. tropicalis*) from routine clinical samples that were inoculated in parallel onto both culture media.

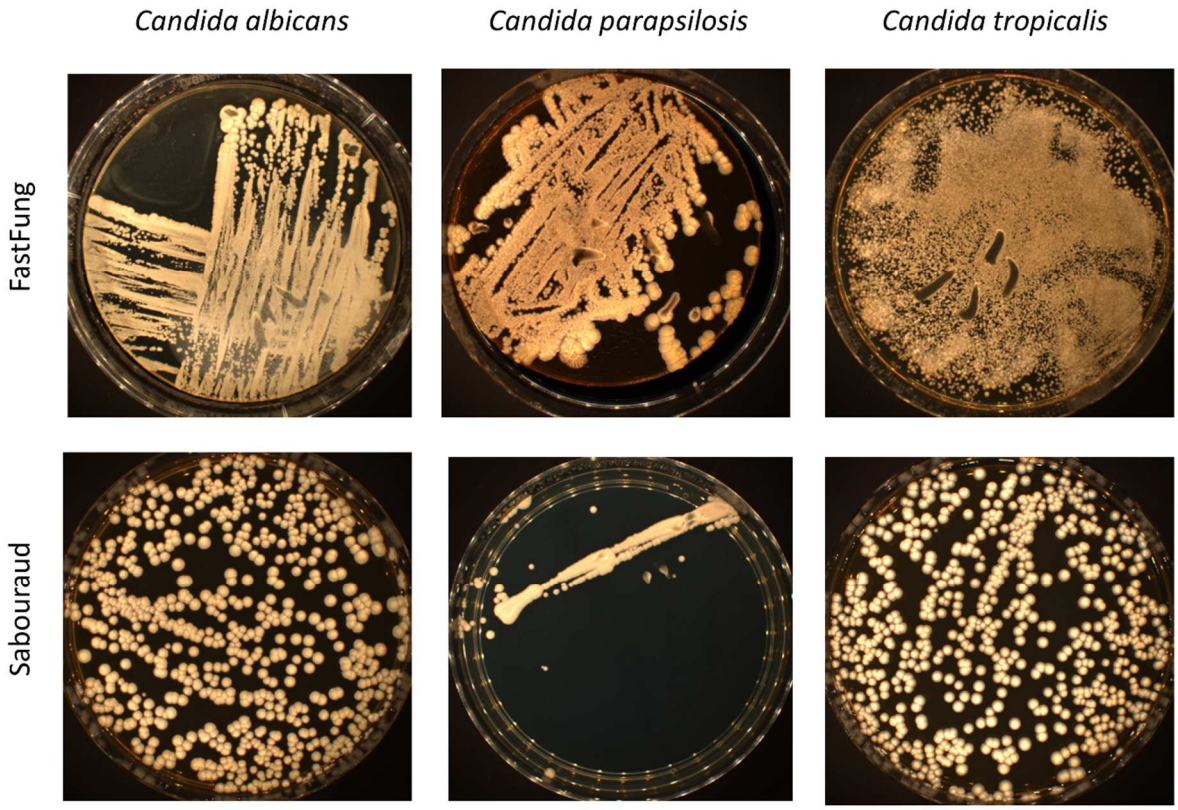

Table 1: Culture success rates of 98 pure culture clinical fungal isolates inoculated onto Sabouraud and FastFung media.

Fungal species	N	Sabouraud	FastFung
Acremonium kiliense	2	100%	100%
Aspergillus flavus	1	100%	100%
Aspergillus niger	5	100%	100%
Aspergillus sydowii	1	100%	100%
Beauveria bassiana	2	100%	100%
Candida albicans	10	100%	100%
Candida glabrata	6	100%	100%
Candida guilliermondii	5	100%	100%
Candida kefyr	4	100%	100%
Candida lusitaniae	3	100%	100%
Candida orthopsilosis	6	100%	100%
Candida parapsilosis	6	100%	100%
Candida tropicalis	6	100%	100%
Candida zeylanoides	1	0%	100%
Candida krusei	3	100%	100%
Clavispora lusitaniae	2	100%	100%
Cryptococcus diffluens	1	0%	100%
Cryptococcus unigutulatus	1	0%	100%
Debaryomyces hansenii	2	100%	100%
Galactomyces geotrichum	1	100%	100%
Isaria farinosa	1	100%	100%
Malassezia furfur	1	0%	100%
Malassezia pachydermatis	1	100%	100%
Mucor circinelloides	1	100%	100%
Mucor velutinosus	1	100%	100%
Penicillium chrysogenum	1	100%	100%
Penicillium glandicola	1	100%	100%
Penicillium dipodomyicola	1	100%	100%
Pichia caribbica	3	100%	100%
Pichia manshurica	1	100%	100%
Rhodotorula mucilaginosa	6	100%	100%
Saccharomyces cerevisiae	6	100%	100%
Trichosporon asahii	4	100%	100%
Preussia minima	1	100%	100%
Torulaspora pretoriensis	1	0%	100%
Total	**98**	**95%**	**100%**

Table 2: Isolation rates of pure bacterial strains inoculated onto Sabouraud and FastFung media.

Bacterial strains	**N**	**Sabouraud**	**FastFung**
Acinetobacter baumanii	2	100%	0%
Enterobacter cloacae	2	100%	0%
Enterococcus faecalis	2	100%	0%
Escherichia coli	2	100%	0%
Gardernella vaginalis	2	100%	0%
Haemophilus influenzae	2	100%	0%
Klebsiella oxytoca	2	100%	0%
Klebsiella pneumoniae	2	100%	0%
Proteus mirabilis	1	0%	0%
Pseudomonas aeruginosa	1	0%	0%
Rhizobium radiobacter	1	0%	0%
Staphylococcus aureus	1	0%	0%
Total	**20**	**80%**	**0%**

Table 3: Fungi cultivation rates from various routine clinical samples inoculated onto Sabouraud and FastFung media. .

Samples	**Species**	**N**	**Sabouraud**	**FastFung**
Vaginal	*Candida albicans*	52	100%	100%
Urines	*Candida albicans*	70	100%	100%
Urines	*Candida tropicalis*	15	100%	100%
Swab	*Candida albicans*	4	100%	100%
Nasopharyngeal aspiration	*Candida albicans*	4	100%	100%
Bronchial aspiration	*Candida tropicalis*	2	100%	100%
Bronchial aspiration	*Candida parapsilosis*	3	100%	100%
Sputum	*Candida albicans*	6	100%	100%
Pulmonary aspiration	*Candida albicans*	2	100%	100%
Aortic valve	*Candida albicans*	2	100%	100%
Biopsy	*Candida albicans*	1	100%	100%
Total		**161**	**100%**	**100%**