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Original Article

EXPLORING THE POTENTIAL OF CHROMOGENIC MEDIUM FOR THE IDENTIFICATION OF MEDICALLY IMPORTANT YEAST SPECIES OTHER THAN *CANDIDA*

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ABSTRACT

Objective: The incidence of yeast infections has increased in the recent decades, with *Candida albicans* still being the most common cause of infections. However, infections caused by *Non - Candida albicans* and other less-common *Non - Candida* yeasts have been widely reported. The identification of yeast pathogens with this increasing diversity by conventional methods may be difficult and sometimes inconclusive. Hence, a simple, reliable, and cost-effective identification method is required for the rapid identification of these human yeast pathogens. In the present study we have tried to explore the potential of chromogenic medium in identifying medically important yeast species beyond *Candia albicans*.

Methods: 138 yeast isolates including *C. albicans, C. parapsilosis, C. glabrata, C. tropicalis, C. lusitaniae, C. intermedia, C. dubliniensis, Kodamaea ohmeri, Malassezia spp., and Trichosporon* spp. were studied using chromogenic medium. The colors and colony features were observed at 24 and 48 hours and confirmation of identification was carried out using MINI – API.

Results: A total of 10 different yeast species were identified using the chromogenic medium. These included *C. albicans* (green), *C. parapsilosis* (cream), *C. intermedia* (dark purple), *C. tropicalis* (blue), *C. lusitaniae* (bluish purple), *C. glabrata* (dark pink), *C. dubliniensis* (dark bluish green), *K. ohmeri* (dark pink to blue), *Malassezia* (pale), *Trichosporon asahii* (white to lavender).

Conclusion: The medium allowed the differentiation of not only *Candida* species but also other unusual *Non - Candida* yeast species. In conclusion, the identification of clinically relevant yeasts by chromogenic medium is highly reliable and can be used as an accurate alternative to conventional identification methods.

Keywords: Chromogenic medium, *Candida, Kodamaea ohmeri, Malassezia, Trichosporon,* Yeast, Sabouraud's Dextrose Agar, Seborrheic dermatitis, Dandruff, MINI API.

INTRODUCTION

Fungal infections of the human skin are a common global problem as the human skin is colonized by a diverse micro biota, including bacteria and fungi that tend to be pathogenic under suitable conditions [1, 2, 3]. The epidemiology of fungal infections has changed during the past 25 years since the incidence has increased, and the populations at risk have expanded. The high prevalence of fungal infections shows that 20–25% of the world's population has skin mycoses, making these one of the most frequent forms of infection [4]. Furthermore, the aetiology of these infections has changed, since, apart from the yeasts (particularly *Candida albicans*) that were considered to be the major yeast pathogens, several other *Candida* species and other genera of yeasts are now considered medically important[5]. Fungal infections have emerged as a world-wide healthcare problem, owing to the extensive use of broad-spectrum antibiotics and immune-suppressive agents and the ever increasing population of terminally ill, debilitated and immune-compromised patients. Fungal infections are also considered to be associated with some very serious cosmetic problems such as Seborrheic dermatitis and Dandruff [5].

Considering the ever changing antifungal spectrums, identification of yeasts to the species level has now become essential, for efficient diagnosis and treatment. Identification of yeasts requires evaluation of microscopic morphologies and a whole range of biochemical studies [6]. Most widely used medium for the isolation of all yeasts from clinical specimens with a minimum incubation period of 48 hours is Sabouraud's Dextrose Agar (SDA). The yeast identification protocol starts with a Germ tube test in serum to differentiate *C. albicans* from other yeast species, followed by more time-consuming methods including microscopic identification based on morphology of growth on corn meal-tween 80 agar (CMA) and an array of biochemical tests. Automated techniques are being increasingly employed for yeast identification, but complete identification of yeasts up to the species level may take 72 hours or more [7]. In order to facilitate rapid identification, several chromogenic substrate containing culture media have been developed. These media yield microbial colonies with varying colors secondary to chromogenic substrates that react with enzymes secreted by microorganisms [6, 8]. HiCrome Candida Differential Agar (HiMedia, Mumbai, India) employs this methodology to differentiate several *Candida* yeasts by color and morphology [9]. It is a yeast differential and selective medium that allows the presumptive identification of C. *albicans* from other *Candida* spp. Yeast populations are differentiated by colony morphologies and colours which are generated by a chromophore in the agar [10]. C. *albicans* strains produce P-N-acetylgalactosaminidase, which interacts with the chromophore (chromogenic hexosaminidase substrate) incorporated into the agar, and with incubation for 48 hours produces green colonies, characteristic of all C. *albicans* isolates [11,12]. Other *Candida* spp. can also be differentiated, e.g., *C. tropicalis* as steel blue colonies, *C. glabrata* as pink colored colonies, *C. parapsilosis* as off-white to cream colored colonies [9].

In the present study yeasts from human skin and scalp Seborrheic dermatitis samples were isolated onto an appropriate medium and identified using chromogenic medium.

MATERIALS AND METHODS

Study Population

The study population comprised of 50 volunteers who had given consent to participate in the study. These included 15 healthy volunteers, 22 clinical suspected cases of scalp Seborrheic dermatitis, 6 clinical suspected cases of skin as well as scalp Seborrheic dermatitis and 7 clinical suspected cases of skin Seborrheic dermatitis, attending the Dermatology department of Rajiv Gandhi Medical College and Chhatrapati Shivaji Maharaj Hospital, Kalwa, Thane. Ethical approval was obtained from the

hospital ethics committee of Rajiv Gandhi Medical College and Chhatrapati Shivaji Maharaj Hospital, Kalwa, Thane.

Sample collection

Skin and scalp surface samples were collected using a sterile swab moistened in sterile saline. Sterile conditions were maintained during sample collection. Yeast isolates were subcultured and stored on Sabouraud's Dextrose Agar (SDA).

Sample processing

Samples were inoculated on Sabouraud's Dextrose Agar (SDA) and all the plates were incubated at 37 degree Celsius for a minimum of 48 – 72 hours and read for up to 7 days [13]. The yeast isolates obtained were further analyzed using macroscopic and microscopic observations, germ tube test and colony characteristics on chromogenic medium (HiCrome Candida Differential Agar, procured from HiMedia laboratories ltd., Mumbai, India). Confirmation of identification was carried out using MINI API [9, 14, 15]

Yeast isolates

A total of 379 yeast isolates were obtained which were screened using macroscopic and microscopic morphologies. Based on these observations 214 yeasts were screened out. Further these 214 isolates were examined using germ tube test. Finally a total of 138 yeast isolates were taken for identification up to species level using chromogenic media. Suspensions of sub cultured isolates were prepared in sterile saline. The turbidity was adjusted to match a 2 McFarland standard. One loopful of suspension was streaked on to the chromogenic medium. The plates were incubated at 37 degree Celsius for 24 to 48 hours and observed for colours and colony morphology up to 7 days.

RESULTS

A total of 10 different yeast species were identified from the human skin and scalp samples using the chromogenic medium. These 10 yeasts species included *Candida albicans*, six *non albicans Candida* (*Candida parapsilosis, Candida intermedia, Candida tropicalis, Candida lusitaniae, Candida glabrata* and *Candida dubliniensis*), and three other, non *Candida* yeast species (*Kodamaea ohmeri, Malassezia, Trichosporon asahii*). The medium supported growth of all clinical isolates. A wide variety of colony colours were observed which were found to be species specific. All the *C. albicans* isolates formed smooth, convex, green colonies after incubation for 48 hours (Fig. 1 (e)). Isolates of *C. dubliniensis* developed dark bluish green colonies (Fig. 1 (a) - F, (c) - E). Similar metallic bluish colonies were obtained for *C. tropicalis* creating confusion in the identification of the two isolates (Fig. 1 (a) – C). *C parapsilosis* produced cream color colonies (Fig 1 (a) – D) and *C. glabrata* produced small, convex, dark pink colored colonies after 48 hours of incubation (Fig. 1 (b) – E, (c) – A). Some colonies identified as *C. parapsilosis* varied in color from purple to pale pink which led to a degree of confusion in their identification (Fig. 2). C. *lusitaniae* showed the presence of smooth, convex, bluish purple colored colonies with a pale border (Fig. 1 (b) – C). Colonies of *C. intermedia* appeared as smooth, convex, dark purple coloured, similar to *C. lusitaniae* (Fig. 1 (b) – D).

A rare and emerging clinical yeast pathogen *Kodamaea ohmeri* was also isolated using the chromogenic media. *K. ohmeri* showed the presence of smooth, convex, pin point dark pink colonies upon 24 hours of incubation which increased in size after 48 hours and changed color to blue after 72 hours (Fig. 1 (d)). Skin commensal yeast belonging to the genus *Malassezia* was also isolated and showed the presence of pale, dry colonies with an irregular border (Fig. 1 (a) – A, (b) – B). Isolates of *Trichosporon asahii* produced smooth, flat, pale lavender colonies with a white rim (Fig. 1 (b) – F, (c) F).

All the isolates obtained were confirmed for identification using MINI API and were found to be in accordance with the results obtained using chromogenic medium [6,9, 13].

Table 1: Colony Characteristics and Identification of isolated yeast isolates

Yeast species	No. of isolates	SDA morphology	Microscopic morphology	Germ tube test	Chromogenic medium Colour
Candida albicans	4	Cream, smooth, Butyrous,	Oval cells	Positive	Light Green
Candida dubliniensis	2	Brown, smooth, Butyrous	Large oval cells with long hyphae	Positive	Dark bluish green
Candida glabrata	28	Cream, smooth, Butyrous	Small oval cells	Negative	Dark pink
Candida intermedia	1	Cream, smooth, Butyrous, wrinkled margin	Ellipsoidal cells	Negative	Dark purple
Candida lusitaniae	14	Brown, Flat with raised centre	Oval cells	Negative	Bluish purple
Candida parapsilosis	228	Cream, smooth, Butyrous. *	Oval and elongated cells	Negative	Cream to Pale pink
Candida tropicalis	34	Brown, smooth, Butyrous	Oval cells	Negative	Metallic blue
Kodamaea ohmeri	21	Cream, Butyrous, with irregular margins	Oval and elongated cells	Negative	24 – 48 hours incubation – Dark Pink 72 hours incubation – Dark blue with a green haze
Malassezia	46	Cream, Smooth, Butyrous. *	Ellipsoidal cells	Negative	Pale pink, wrinkled
Trichosporon asahii	1	Cream, Flat, Butyrous	Small oval cells	Negative	Pale Lavender with a white rim

*Some colonies showed wrinkled appearance

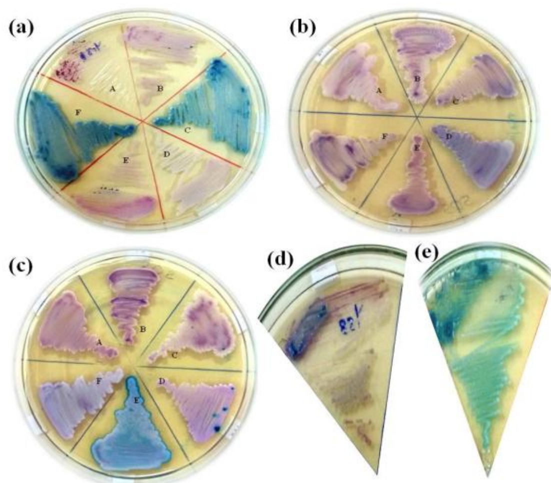

Fig. 1: Appearance of yeast colonies on Chromogenic medium

(a): A - *Malassezia*, B – *C. parapsilosis*, C – *C. tropicalis*, D – *C. parapsilosis*, E – *C. parapsilosis*, F – *C. dubliniensis*

(b): A – *C. parapsilosis*, B – *Malassezia*, C – *C. lusitaniae*, D – *C. intermedia*, E – *C. glabrata*, F – *Trichosporon asahii*

(c): A – *C. glabrata*, B – *C. lusitaniae*, C – *Malassezia*, D – *C. parapsilosis*, E – *C. dubliniensis*, F – *Trichosporon asahii*

(d): *K. ohmeri* ; (e) *C. albicans*

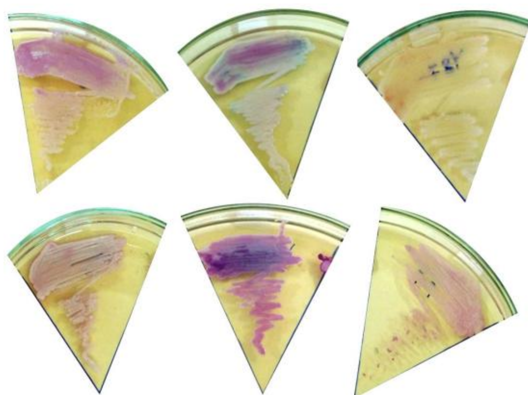

Fig. 2: Varied morphologies of *C. parapsilosis* observed on chromogenic medium

DISCUSSION

In spite of their world – wide presence, very few fungi are considered to be pathogenic, that give rise to infections in animals and human beings. Most of these fungi cause infections of the superficial layers of the integument. Recently there has been an increase in the incidence of fungal infections majorly due to the frequent usage of antibiotics, immunosuppressive drugs and various conditions like organ transplantations, lymphomas, leukaemia and human immunodeficiency virus (HIV) infections [16].

Fungal skin infections have become a significant health problem affecting children, adolescents and adults. A correct diagnosis is important to initiate appropriate treatment [16, 17]. An important challenge faced by hospital and reference laboratories is to select an approach for isolation and identification of yeasts that is not only accurate and cost effective but also time saving. Rapid identification of yeast species guides early appropriate antifungal therapy. Thus, it has become important to identify all yeast isolates up to the species level. Many new molecular techniques like real-time PCR, MALDI – TOF MS and multiplex-tandem PCR are being increasingly employed for the identification of yeast species [7, 10, 18, 19]. However, such methods are costly, and most of them cannot be afforded by diagnostic microbiology laboratory in Indian settings [7].

The present study was undertaken to isolate various fungi causing mycoses among the patients suffering from both skin and scalp Seborrheic dermatitis and to identify these isolates by an easy and cost effective method using chromogenic differential medium.

Here we report a broad-range analysis of the fungal micro biota in healthy and diseased scalp and skin lesions by culture dependent methodology. In the present study chromogenic medium helped in the rapid identification of *C. albicans, C. tropicalis, C. glabrata, C. intermedia, C. lusitaniae, C. dubliniensis and C. parapsilosis*, and the results obtained were comparable with other studies [7]. The chromogenic medium facilitated the presumptive identification of yeast isolates to the species level within 24 - 48 hours of incubation. Given the fact that many clinical microbiology laboratories do not perform identification beyond a Germ Tube Test, the use of chromogenic medium provides rapid and accurate identification of commonly isolated yeast up to species level.

Additionally some *Non Candida* yeast species were also successfully identified using the chromogenic medium. One of these *Non Candida* yeast species is *Kodamaea (Pichia) ohmeri*, ascosporogenous yeast and a teleomorph of *Candida guilliermondii var. membranaefaciens*. *K. ohmeri* is an emerging fungal pathogen, particularly in immunocompromised patients. To date, 12 cases of *K. ohmeri* infection have been reported, including nine cases presenting with fungemia plus two cases that occurred as a nosocomial cluster [20]. This is the first case of *K. ohmeri* isolated from the human skin and scalp samples in the Indian population. 24 hours after subculture on Sabouraud's dextrose agar, white rough colonies of *K. ohmeri* were observed. No germ tube formation was detected. On the chromogenic medium, the yeast exhibited colonies that changed colour from pink to blue within 72 hours; however, a full week was required for *K. ohmeri* to complete its blue color development with a green haze (Fig. 3) [21].

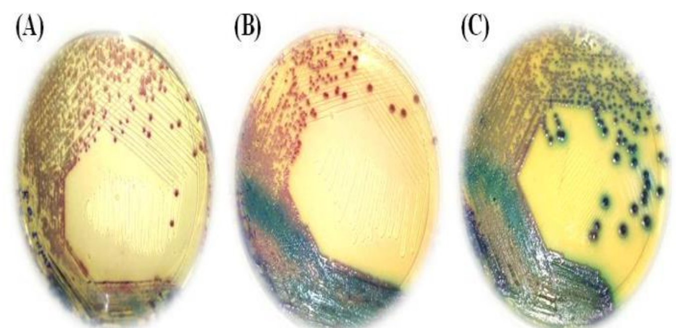

Fig. 3: Varied Morphologies of yeast colonies of *Kodamaea ohmeri* on Chromogenic medium

(A) Colony colour and morphology after 24 hours of incubation.

(B) Increase in colony size after 48 hours of incubation.

(C) Change in colony colour from pink to blue with a green haze and increase in colony size after 72 hours of incubation.

Another important skin commensal lipid dependent yeast *Malassezia* was successfully identified on the chromogenic medium. This genus has been described as part of the commensal skin micro

biota, but it also has been associated with pityriasis versicolor, Seborrheic dermatitis, atopic dermatitis, folliculitis, and psoriasis [1]. As members of the genus *Malassezia* share similar morphological and biochemical characteristics, it is thought that differentiating between them based on phenotypic features is difficult. While molecular biological techniques are the most reliable for the identification of *Malassezia*, they are not available in most clinical laboratories. Therefore, culture methods for the identification of *Malassezia* species are required. Some of these identification or differentiation methods have been reported previously. Guillot et al. have reported a lipid usage pattern, catalase reaction, growth temperature, and cell shape for the identification of *Malassezia* species [22]. Differentiation on the basis of precipitate production by some *Malassezia* strains on Dixon's agar has been reported by Hammer and Riley [22]. Mayser et al. have reported the use of esculin hydrolysation and polyethoxylated castor oil assimilation for the differentiation of some *Malassezia* species [22]. In our study it was found that, the chromogenic medium for Candida could be used for isolating and differentiating between *Malassezia* and *Candida* spp. simultaneously. However, we found that *Malassezia* species differentiation was not possible with chromogenic medium. Isolates of *Malassezia* appeared as small, pale pink colonies with a varied morphology ranging from smooth to rough to wrinkled. Since the identification of *Malassezia* species is not possible using the most reliable and widely used automated yeast identification systems, the results presented here indicate that chromogenic medium is a useful tool for simple, reliable, and cost-effective identification of clinically important *Malassezia* species.

In addition to the strains mentioned above, *Trichosporon* species were also obtained on the chromogenic medium. *Trichosporon* strains were readily differentiated from *C. albicans* by their colony morphology. *Trichosporon* species appeared as pale purple, smooth colonies with a white border.

In this study, chromogenic medium was successfully used to identify up to the species level, yeast isolates obtained from human skin and scalp samples. Results obtained in this study reveal that, the chromogenic agar medium can be explored for its potential as a chromogenic growth medium to assist in making an identification of not only *Candida* species but also other emerging medically important non *Candida* yeast species, based on the development of coloured colonies.

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