academicJournals

Vol. 8(50), pp. 3918-3922, 10 December, 2014
DOI: 10.5897/AJMR2014.7202
Article Number: E307D3A49526
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Chrome agar *Candida* for species level identification of isolates of *Candida* sp. from oral cavity

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Received 17 October, 2014; Accepted 1 December, 2014

Recently, *Candida* has become an important nosocomial pathogen. They are normal flora of skin, mouth, gut and vagina of healthy humans. They become opportunistic with immunocompromised and immunosuppresed individual. Since it is not possible to identify the species directly on Sabouraud's dextrose agar (SDA), CHROM agar for candida is used for easy recognition of species by colour of the colonies. This study was conducted in 38 patients with symptoms of oral candidiasis aged 25 to 75 years. Oral swabs were taken from oral cavity and were cultured on Sabouraud's dextrose agar and CHROM agar for candida. Gram staining and germ tube test were done with the samples. Four different species of *Candida* were isolated from the samples using CHROM agar, that is, *Candida albicans, Candida tropicalis, Candida glabrata* and *Candida krusei*. It was observed that, of the 38 isolates, *C. albicans* was obtained in higher rate (58%) followed by *C. tropicalis* (24%), *C. krusei* (16%) and *C. glabrata* (2%). *C. albicans* produced light green colonies, *C. tropicalis* produced dark blue with purple diffusion colonies, *C. glabrata* showed pink with a darker mauve center colonies, *C. krusei* produced pink with pale borders colonies. Thus, CHROM agar candida medium was found to be helpful in direct and easy identification of multiple yeast species simultaneously.

Key words: *Candida albicans*, oral swabs, CHROM agar, differential medium.

INTRODUCTION

Yeasts, especially *Candida albicans* is a member of the native born microbial flora of the skin, mucous membranes of the gut, mouth and vagina in healthy human. Although, *C. albicans* rarely causes infections in healthy human without predisposing factors, immune-suppressed patients can suffer from mucosal, cutaneous or systemic candidiasis. Oropharyngeal candidiasis is the most common opportunistic infection. Oral thrush is a common form of the oropharyngeal candidiasis and its clinical features include white patches appearing as discrete lesions on the buccal mucosa, throat, tongue and gum linings that develop into confluent pseudo-membranes resembling milk curds (Marsh and Martin, 2009). The incidence of candidiasis has increased markedly with the advent of diseases like AIDS and the development of immune suppressive therapy (Smitha and Shashanka, 2011). Among the various species of *Candida*, *C. albicans* was the most frequently isolated species (72.7%)

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(Back-Brito et al., 2009). Although *C. albicans* remains the major species isolated, non-albicans such as *C. glabrata, C. krusei* and *C. tropicalis* also involved in the incidence of candidiasis. In a study conducted by Vijaya et al. (2011), non *C. albicans* was isolated at a higher rate (55.8%) than *C. albicans*. Isolation and prompt identification of infecting microorganism from the mixed yeast population are required for early antifungal therapy.

Traditional method of identification of *Candida* species is germ tube formation by the fungi in serum (Mackenzie, 1962). In most clinical investigations, fungal pathogens are routinely cultured on Sabouraud's Dextrose Agar (SDA) (Baveja, 2010). The drawback with these media is that, the colonies on these media are very similar in appearance and their subsequent identification requires considerable investigative time (Zarei Mahmoudabadi et al., 2000; Beighton et al., 1995).

CHROM agar for *Candida* is a differential culture medium which facilitates the species level identification of *Candida* isolates of various clinical specimens. These chromogenic media provide different colours of colonies secondary to chromogenic substances that react with enzymes secreted by the organisms (Murray et al., 2005; Yucesoy et al., 2001). A major advantage of these media is that identifications of species can be done in shorter duration within 48 h with great accuracy (Pfaller et al., 1996). In addition, mixed yeast infections are seen in the oral cavity frequently in immunocompromised patients, CHROM agar is useful because differences in the colour of the colony make the identification simple and selective (Odds and Bernaerts, 1994; Pfaller et al., 1996). Therefore, the present study was conducted to evaluate the performance of CHROM agar for the isolation, direct presumptive identification and species differentiation of *Candida* from oral specimens.

MATERIALS AND METHODS

Preparation of CHROM agar *Candida*

CHROM agar *Candida* (Himedia India) was prepared according to the manufacturer's instructions. CHROM agar *Candida* is composed of (per litre): peptone (10 g), glucose (20 g), agar (15 g), chloramphenicol (0.5 g) and Chromogenic mix (2 g). Twelve grams of CHROM agar *Candida* powder (one vial) was added to 250 ml of sterile distilled water in a sterile Erlenmeyer. The suspension was completely dissolved by boiling (<100°C) and mixing. The medium does not require sterilization by autoclave, therefore after cooling in a water bath to 45°C, the agar was poured into sterile Petri dishes (Odds and Bernaerts, 1994). After being allowed to cool, the plates were stored at 4°C prior to use. CHROM agar was prepared as per the instruction manual. *Candida* species isolate were inoculated on CHROM agar and incubated at 37°C for 48 h.

Collection of samples

A total of 38 clinical samples were obtained from patients attending tertiary care Hospital, Coimbatore, TamilNadu, India, with symptoms of oral candidiasis. Oral swabs were collected with all aseptic precautions using sterile swabs from tongue and buccal mucosa by gently rubbing a sterile cotton swab over the lesional tissue (18) (Ax'ell et al., 1985). The swabs were then dispensed in a test tube containing sterile SDA broth.

Processing of samples

The samples were inoculated on HiCHROM *Candida* differential agar and Sabouraud's Dextrose agar and incubated at 37°C for 48 h. From that Gram staining was done. Germ tube test was done which is the standard laboratory method to differentiate the *C. albicans* from other *Candida* species. The test involved the induction of hyphal outgrowths (germ tubes) when sub cultured in serum at 37°C for 2 - 4 h (Williams and Lewis, 2000). Growth on the CHROM agar was observed with in 24 h in most of the cases. For few isolates, the plate had to be incubated for up 48 h to appreciate the growth. Colour of the colonies was noted and the species was identified.

RESULTS AND DISCUSSION

A total of 38 species isolated from oral specimen were studied for morphological characteristics by Gram staining and cultural characteristics by growth on SDA. Gram positive budding yeast cells were observed in Gram staining. On SDA (Figure 1a) creamy white colored, smooth, pasty convex colonies were observed. After 48 h incubation at 37°C, positive cultures produced colonies of 1 to 5 mm in diameter. On CHROM agar appearance of *Candida* species were as follows: *C. albicans* - Green (Figure 1b), *C. tropicalis* - metallic blue (Figure 1c), *C. krusei* - pink (Figure 1d) and *C. glabrata* - Mauve (Table 2).

The germ tube test (Figure 1e) was used for the confirmation of *C. albicans*. *C. albicans* alone gave positive result for germ tube test (Table 2). A distribution of *Candida* species isolated is shown in Table 1. Of the 38 isolates obtained, predominantly isolated *Candida* species was *C. albicans* (58%) and then *C. tropicalis* (24%), *C. krusei* (16%) and *C. glabrata* (2%). Distribution of specimen between different age group is shown in Table 3.

Among the 38 *Candida* species isolated from the oral cavity, *C. albicans* was found to be predominant with 58%. This observation correlated with the previous studies. Manjunath et al. (2012) found that, *C. albicans* was the most common isolate from both HIV and non-HIV infected patients. This observation was also reported (Odds and Bernaerts, 1994; Al-Dwairi et al., 2014). According to Back-Brito et al. (2009) and Williams and Lewis (2000), the majority of yeast isolates from oral swabs were *C. albicans*, but it was often recovered in association with other yeasts. This was followed by *C. tropicalis* 24%, *C. krusei* 16% and *C. glabrata 2*% (Table 1). In our study, the isolation rates of *Candida* species is high in ages ranging from 36-70 years old. This observation is more or less similar with the results shown by Zaremba et al. (2006) and Pinho Resende et al. (2002).

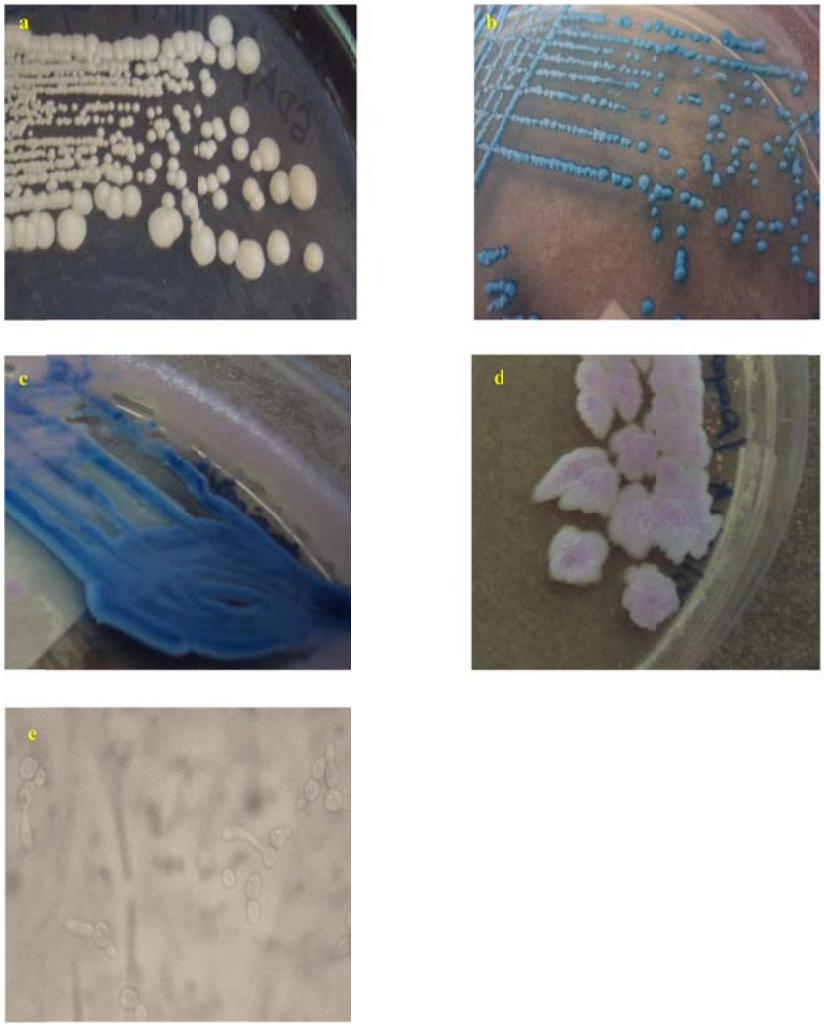

Figure 1. Growth of *Candida* sp. on SDA (a), *Candida albicans* on CHROM agar (b), *Candida tropicalis* on CHROM agar (c), *Candida krusei* on CHROM agar (d) and Germ tube formation by *Candida albicans* (e).

Table 1. Distribution of different species of *Candida* isolated from oral cavity

Species	No. of isolates	Percentage of isolates
C. albicans	22	58%
C. tropicalis	9	24%
C. krusei	6	16%
C. glabrata	1	2%

Table 2. Growth characteristics of *Candida* species isolated from oral cavity.

Species	Growth on CHROM agar	Germ tube test
C. albicans	Green	Positive
C. tropicalis	Metallic blue	Negative
C, krusei	Pink	Negative
C. glabrata	Mauve	Negative

Table 3. Age distribution between the collected isolates.

Age (years)	No. of isolates	Percentage of isolates
21-35	9	24%
36-50	17	45%
51-70	12	31%
Total	38	100%

It has generally been assumed that old age represent a predisposing condition for increased candidal colonization. Lockhart et al. (1999) found that frequency and intensity of carriage of candidal colonization increased as a function of age. According to Sumitra and Megha (2014), sensitivity and specificity of CHROM agar for *C. albicans* were 100 and 96%, *C. tropicalis* were 100% and 100%, *C. krusei* were 100% and 100% and *C. glabrata* 75% and 100%, respectively. Germ tube test has been the gold stranded method for species differentiation of *Candida* yeast. But it may lead to false positive and false negative results. Though SDA has been used for routine culturing of yeast cultures, precise identification by colony appearance is not possible with mixed cultures (Jean-Philippe et al., 1996). In our study, with in 48 h, candida species were differentiated based on colony colour and morphology.

Hence, the identification of *Candida* species is technically simple, rapid and cost effective as compared to technically demanding time consuming and expensive conventional method.

In recent years, other differential media have been developed that allow identification of certain *Candida* species based on colony appearance and colour following primary culture (Houang et al., 1997). The advantage of such media is that the presence of multiple *Candida* species in a single infection can be determined which can be important in selecting subsequent treatment options (Odds and Bernaerts, 1994). CHROM agar *Candida* is a new Chromogenic differential culture medium that is used for the isolation and identification of some of the most clinically important yeast pathogens on the basis of colony colour. CHROM agar *Candida* has previously been shown to be an effective and selective medium for the direct identification of *Candida* species from clinical materials (Odds and Bernaerts, 1994; Pfaller et al., 1996). This medium has previously also been used for the isolation and identification of yeasts from dental samples (Beighton et al., 1995) and from swabs of soft tissues in oral cavity (Odds and Bernaerts, 1994). A major advantage of CHROM agar is the ability to detect mixed cultures of yeasts in clinical specimens.

Conclusion

CHROM agar *Candida* medium was found to be helpful, allowing direct and presumptive identification of *C. albicans* and the easy recognition of association of multiple yeast species. Thus, CHROM agar for *Candida* was proved to be easy to use, time saving and appears to be well suited for routine use in the clinical mycology laboratories.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank the Principal and the Management of PSG College of Arts and Science, Coimbatore, India for providing facilities to carry out the work. They also thank the Principal and the Management of Karpagam University, Coimbatore, India for their consistent support and help during this study.

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