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Use of CHROMagar Candida Medium for Isolation of Yeasts from Dental Samples

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A new differential medium, CHROMagar Candida, for the isolation of clinically important yeasts was investigated to determine its usefulness in facilitating the study of oral yeasts. The recovery of yeasts on the medium was not significantly different from the recovery on Sabouraud dextrose agar. The identities of 450 green colonies on CHROMagar Candida, presumptively identified as *Candida albicans* on the basis of the manufacturer's instructions, were confirmed by testing for β-*N*-acetylgalactosaminidase. *Candida tropicalis* also formed distinctive colonies, and other yeasts including *Candida* (*Torulopsis*) *glabrata*, *Candida parapsilosis*, *Candida magnoliae*, *Candida lusitaniae*, *Candida famata*, *Candida kefir*, and *Saccharomyces cerevisiae* were readily distinguished from *C. albicans* and *C. tropicalis* isolates. CHROMagar Candida is a very useful medium, and its use will facilitate the study of yeasts associated with dental diseases.

Yeasts, especially *Candida* spp., are members of the normal oral flora, and their isolation from the mouth can be used to investigate reduced salivary flow rate (9), excessive consumption of fermentable carbohydrates (12), dental caries risk and denture-wearing status (1), and oral candidiasis. Their isolation can also be used in the diagnosis of denture stomatitis (2, 4, 5). In the majority of clinical investigations yeasts are routinely cultured on Sabouraud dextrose agar (SDA) or malt extract agar. These media are reliable and permit the isolation of several different genera, but overall, the colonies on these media are very similar in appearance and their subsequent identification requires considerable investigative time in the laboratory. These difficulties may explain the apparent discrepancies between clinical studies. Budtz-Jörgensen et al. (2) and Kreher et al. (5) reported mixed yeast populations, with more than one yeast species being isolated from a significant proportion of patients with denture stomatitis, while Cumming et al. (4) reported that individual patients harbored only single species. However, most studies report only the total recovery of yeasts determined by using SDA and use these total counts in analyses, especially in relation to the assessment of the risk of caries and of oral candidiasis.

Candida albicans is the most frequently isolated yeast from clinical samples, and selective media which rely on the formation of colored colonies in which the coloration is species specific have been developed (3, 6, 8). Perry and Miller (10) demonstrated that *C. albicans* produces β-*N*-acetylgalactosaminidase, which enabled chromogenic or fluorogenic hexosaminidase substrates to be incorporated directly into the growth medium and *C. albicans* isolates to be identified directly on primary isolation (11). The direct identification of yeasts from clinical samples was taken a step further by Odds and Bernaerts (7), who reported the use of a differential and selective medium (CHROMagar Candida [CA]) that permitted the presumptive identification of *C. albicans*, other *Candida* spp., and isolates of related genera. The usefulness of CHROMagar Candida in diagnostic oral microbiology is not clear because only oral swabs from patients whose oral health status was not reported were studied by Odds and Bernaerts (7).

In the study described in this report we used the selective medium CA (CHROMagar, Paris, France) to study the yeast populations in microbiological samples from different groups of dental patients and compared the recovery of yeasts on CA with that on SDA. Oral rinses were obtained from patients by asking them to rinse their mouths with 10 ml of sterile phosphate-buffered saline for 10 to 15 s and to expectorate the rinse into a sterile container. The palate swabs were obtained by swabbing the palate with alginate swabs and placing these into 2 ml of transport medium (Fastidious Anaerobe Broth [FAB]; LabM, Bury, England) supplemented with 1% sodium hexametaphosphate to facilitate dissolution of the swabs and to improve the recovery of the oral microflora. The samples of root caries lesions taken with a sterile dental excavator were placed into 1 ml of FAB. In order to disperse the clinical samples, sterile glass beads (3.5 to 4.5 mm in diameter; BDH Limited, Poole, England) were added and the sample was rapidly agitated for 15 s on a vortex mixer. Oral rinses were taken from full denture wearers ($n = 70$), human immunodeficiency virus-positive patients with clinical candidiasis ($n = 20$), patients with clinical candidiasis attending a dry mouth clinic ($n = 32$), and patients attending a drop-in center for homeless people and drug users ($n = 14$). Palate swabs from full denture wearers ($n = 71$) with various degrees of denture stomatitis and samples of infected dentine were taken from individual root caries lesions requiring restoration ($n = 82$).

Each sample was diluted 1:10 with FAB. To estimate the number of yeasts in each sample, a 100-μl aliquot of the undiluted sample and the 1:10 dilution were plated onto SDA (Oxoid, Basingstoke, England) and CA. All plates were incubated for 2 days at 37°C in air. Preliminary studies indicated

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TABLE 1. Recovery of yeasts and their frequency of isolation from clinical samples[a] obtained from different groups of clinical dental patients

Yeasts species isolated	No. (%) positive					
	Denture wearers (n = 70)	Drop-in clinic (n = 14)	HIV-positive patients (n = 20)[b]	Dry-mouth patients (n = 32)	Palate swabs (n = 71)	Root caries lesions (n = 82)
Nil	20 (28.6)	4 (28.6)	2 (10.0)	3 (9.4)	28 (39.4)	34 (41.5)
Candida albicans	12 (17.1)	7 (50.0)	15 (75.0)	15 (46.9)	13 (18.3)	32 (39.0)
Candida glabrata	5 (7.1)	0	0	2 (6.3)	2 (2.8)	3 (3.7)
Candida tropicalis	1 (1.4)	0	0	0	1 (1.4)	0
Candida parapsilosis	2 (2.8)	0	0	1 (3.1)	0	0
Candida albicans, *Candida glabrata*	13 (18.6)	0	0	6 (18.8)	12 (16.9)	10 (12.2)
Candida albicans, *Candida tropicalis*	3 (4.2)	0	1 (5.0)	0	2 (2.8)	2 (2.4)
Candida glabrata, *Candida parapsilosis*	4 (5.6)	0	0	0	5 (7.0)	0
Candida glabrata, *Candida parapsilosis*	3 (4.2)	0	0	0	0	0
Candida glabrata, *Candida lusitaniae*	0	0	0	0	1 (1.4)	0
Candida albicans, *Saccharomyces cerevisiae*	1 (1.4)	2 (14.3)	2 (10.0)	3 (9.4)	1 (1.4)	0
Candida tropicalis, *Candida parapsilosis*	0	0	0	0	1 (1.4)	0
Candida albicans, *Candida glabrata*, *Candida tropicalis*	3 (4.2)	0	0	0	3 (4.2)	0
Candida albicans, *Candida tropicalis*, *Candida parapsilosis*	1 (1.4)	0	0	0	0	0
Candida glabrata, *Candida tropicalis*, *Candida magnoliae*	1 (1.4)	0	0	0	0	0
Candida glabrata, *Candida parapsilosis*, *Candida lusitaniae*	1 (1.4)	0	0	0	0	0
Candida albicans, *Candida glabrata*, *Candida parapsilosis*	0	0	0	0	2 (2.8)	1 (1.2)
Candida albicans, *Candida glabrata*, *Candida famata*	0	1 (7.1)	0	0	0	0
Candida albicans, *Candida glabrata*, *Saccharomyces cerevisiae*	0	0	0	1 (3.1)	0	0
Candida kehir	0	0	0	1 (3.1)	0	0

[a] Clinical samples were oral rinses unless stated otherwise.
[b] HIV, human immunodeficiency virus.

that CA could not be used satisfactorily if it was stored at 4°C for more than 14 days, because the coloration of the colonies was no longer specific or uniform for individual species. In particular, *C. albicans* colonies grew such that they presented on the same CA plate with a variety of green colors ranging from light green to dark green, often with the periphery of the colonies having a color distinctly different from that of the rest of the colony. These changes and variations in coloration reduced the usefulness of the agar. Extensive additional testing was required to identify many of the different colonial color variants associated with a single species producing several different colonial variants from each sample. All media were therefore used within 2 weeks of preparation.

The total number of yeast colonies present on the SDA plates was counted. The identities of these colonies were not determined, because previous studies have indicated that the recovery of different species on SDA and CA plates was not significantly different (7). The total number of pigmented colonies isolated on CA was counted, and these colonies were also differentially counted on the basis of colonial coloration and morphology when they were examined with a 60-W tungsten filament laboratory lamp. There was no significant difference between the numbers of yeast colonies cultured on the two media (Mann-Whitney test for paired samples; $P > 0.1$). The discordance in the rates of recovery of yeasts between the two media was not significantly different; with both media yeasts were recovered from seven samples cultured on one medium and not on the other; in all but one case this was due to fewer than five colonies being present on the culture-positive medium (χ^2 test; $P > 0.5$).

Colonies producing a green coloration were presumptively identified as *C. albicans* on the basis of the manufacturer's instructions, and up to 10 of these from each sample were subjected to testing for β-*N*-acetylgalactosaminidase activity (10). These colonies either were tested directly from the CA plates or were first subcultured onto SDA plates. Individual colonies were picked directly from the media and were suspended in 50 μl of 50 mM *N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer (pH 7.5; Sigma Chemical Company, Poole, England) containing 100 μg of 4-methylumbelliferyl-β-*N*-acetylgalactosaminide (Sigma) per ml in nonsterile flat-bottom microtiter trays. The trays were incubated at 37°C for 3 h, and substrate hydrolysis was determined by measuring the increase in fluorescence at excitation and emission wavelengths of 380 and 460 nm, respectively, with a Perkin-Elmer fluorimeter. In all, 450 green colonies isolated on CA plates were tested for β-*N*-acetylgalactosaminidase activity, and all were found to be positive, confirming that the CA plates identified *C. albicans* correctly on the basis of colonial coloration.

According to the manufacturer's instructions, *Candida tropicalis* may be presumptively identified on the basis of its characteristic dark gray colony color and brown-purple halo formation in the medium. In the present study we found that all colonies (n = 20) with a brown-purple halo tested were identified as *C. tropicalis* by the API 20 C AUX system (BioMerieux, Basingstoke, England). The color of individual colonies did, however, vary from purple to dark blue. Colonies identified as *Candida* (*Torulopsis*) *glabrata* varied in color from purple to pale pink after 48 h of incubation, and this may lead to a degree of confusion with colonies subsequently identified as *Candida parapsilosis*. However, we found that colonies that

were not identified as *C. albicans* or *C. tropicalis* on the basis of colony coloration were readily distinguishable from *C. albicans* and *C. tropicalis*, and none was identified as either *C. albicans* or *C. tropicalis.*

The various combinations of yeasts present in the different patient groups studied are given in Table 1. It is apparent that the patients wearing full dentures harbored the most complex yeast flora, while all other patient groups harbored floras which were less varied. Overall, *C. albicans* was the most frequently isolated species, but it was often recovered in association with other yeasts. The presence of such mixtures and the presence of *C. albicans* were apparent upon visual examination of the primary isolation plates.

In conclusion, the use of CA plates, and perhaps of the other recently described differential media (11), in clinical dental microbiology would greatly facilitate the study of the oral yeast flora, in particular, by increasing the level of discrimination between *C. albicans* and other yeast species and enabling the presence of mixed yeast populations to be readily recognized and their clinical significance to be assessed.

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