JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1987, p. 162–164
0095-1137/87/010162-03$02.00/0

Vol. 25, No. 1

Comparison of Sabouraud Dextrose and Pagano-Levin Agar Media for Detection and Isolation of Yeasts from Oral Samples

LAKSHMAN P. SAMARANAYAKE,* T. WALLACE MACFARLANE, AND MICHAEL I. WILLIAMSON

Oral Microbiology Unit, Department of Oral Medicine and Pathology, University of Glasgow Dental Hospital and School, Glasgow G2 3JZ, Scotland

Received 21 May 1986/Accepted 2 September 1986

The sensitivities of Sabouraud dextrose agar and modified Pagano-Levin agar for the primary isolation of yeasts and the recovery of multiple yeast species from single clinical samples were compared by using oral-rinse samples. Although there was a highly significant positive correlation between the numbers of yeasts recovered from both media, modified Pagano-Levin agar was far superior in detecting multiple yeast species in a single sample. Of 150 oral samples containing yeasts, 23 (15.3%) contained more than one yeast species. The most frequent combination of different yeasts was *Candida albicans* and *Torulopsis glabrata*.

Polymicrobial infections of either bacterial or fungal origin are becoming increasingly common, owing to the widespread use of antibiotic and immunosuppressive agents (1, 2, 4, 5).

An essential prerequisite for the laboratory detection of polymicrobial diseases is a suitable primary culture medium which facilitates the recovery and differentiation of phenotypically similar microorganisms. Although specialized media, such as MacConkey agar, are widely used in the differentiation of related bacterial species, no equivalent medium is used for the primary isolation or differentiation of *Candida* species. Sabouraud dextrose agar (SDA) is the medium most commonly used for the isolation of these opportunistic pathogens. However, it rarely permits the distinction of different yeast species within the same sample. In contrast, Pagano et al. (11) have described a culture medium which allows various yeast species to be distinguished through visual differences in coloration facilitated by a reduction of triphenyltetrazolium chloride incorporated into the medium.

Subsequent studies have confirmed that Pagano-Levin medium is useful for the retrieval of multiple yeast species from a single clinical specimen (7, 12, 13). Surprisingly, however, the sensitivity of Pagano-Levin medium and SDA in isolating yeasts from clinical specimens has never been compared. If Pagano-Levin medium is to be considered as a substitute for SDA, then the sensitivity of the former should equal or exceed that of the latter. Therefore, the main aim of this study was to compare the sensitivity of these media for the primary isolation of yeasts from clinical samples. The opportunity was also taken to assess, quantitatively and qualitatively, the presence of multiple yeast species in oral samples obtained from a group of diseased individuals.

A total of 200 patients attending the Oral Medicine Clinic of the University of Glasgow Dental Hospital were chosen for the study. Patient selection was based on clinical grounds; i.e., we selected patients who were thought to harbor yeasts intraorally because of reduced salivary gland function or nutritional deficiency.

SDA (GIBCO, Paisley, Scotland) was obtained commercially, and modified Pagano-Levin (MPL) agar, prepared by the method of Yamane and Saitoh (15), contained Pagano-Levin base (Difco Laboratories, Detroit, Mich.), 2,3,5-triphenyltetrazolium chloride (0.1 mg/ml; Difco), and gentamicin (50 μg/ml).

All samples were obtained by the oral-rinse technique described elsewhere (L. P. Samaranayake, T. W. MacFarlane, P.-J. Lamey, and M. M. Ferguson, J. Oral Pathol., in press). In brief, the technique requires instructing patients to rinse their mouths with 10 ml of phosphate-buffered saline (0.1 M, pH 7.2) for 60 s. The rinse is then expectorated back into a universal container and concentrated by centrifugation at 17,000 × *g* for 10 min. The supernatant is discarded, and the deposit is resuspended in 1 ml of phosphate-buffered saline to obtain a concentrated oral rinse which is inoculated onto SDA and MPL agar as described below.

A spiral plating technique (3) was used to inoculate the culture media; this technique replaced the conventional pour plate and spread plate techniques. The spiral plating system (Spiral Systems Marketing Limited, Cincinnati, Ohio) involves the mechanical inoculation of an adjustable volume of a liquid sample onto the surface of a rotating culture plate by a dispenser arm. A variable cam-activated syringe dispenses the fluid from the center to the edge of the plate in an Archimedean spiral, such that the volume of fluid deposited on any given area on the plate is known. This system is rapid, highly sensitive, and simpler and less expensive than either the pour plate or spread plate method of microbial quantitation (3, 14). In the present investigation, the spiral plating system was adjusted to deliver 25 μl of the concentrated oral rinse onto each SDA and MPL agar plate. The plates were then incubated at 37°C for 48 h. Finally, the number of CFU on each plate was estimated by using a colony counter (Gallenkamp, Leicestershire, England), and the numbers of CFU per milliliter of sample on each medium were quantified and compared.

All yeast isolates which demonstrated color variations and different colony morphologies were selected and identified by tests for germ tube production (9), sugar assimilation, and fermentation patterns (8).

Of the 200 patients investigated, 150 were found to be carriers of yeast species when the overall results obtained from both media were assessed. The sensitivity of MPL agar was slightly superior to that of SDA in detecting yeasts from oral-rinse samples, as shown by the low number of false-negative results obtained with MPL agar (Table 1).

Linear regression analysis revealed a highly significant positive correlation between the numbers of CFU recovered

* Corresponding author.

TABLE 1. Comparison of SDA and MPL agar for the detection of the oral carriage of yeast flora in a diseased population

Medium	No. (%) of specimens[a]			Sensitivity (%)
	Total positive	Total negative	False-negative	
SDA	139 (69)	61 (31)	11 (5.5)	93
MPL agar	142 (71)	58 (29)	8 (4)	95

[a] Positive or negative indicates presence or absence of yeast growth, respectively.

from SDA and MPL agar ($P < 0.001$). However, in 81 (54.3%) instances, SDA yielded higher counts; MPL agar yielded higher counts in 61 (40.3%) instances. Nevertheless, on 46 of the 81 occasions when SDA yielded higher counts, the difference between the counts on the two media was less than 20. On 8 (5.4%) occasions, the numbers of CFU obtained from both media were identical.

Different yeast species growing on MPL agar could be easily detected because of their color reactions; for instance, *Candida albicans* colonies appeared creamy to faintly pink, whereas *C. tropicalis* developed as dark, maroon-red colonies after 48 h of incubation. Of the 150 specimens containing yeasts, 23 (15.3%) contained more than one yeast species. The frequency of samples containing more than a single yeast species was significantly greater ($P < 0.001$; chi-square test) on MPL agar than on SDA. Multiple yeast species in a single sample could be detected in 6 of the 23 specimens on SDA and in all of the 23 specimens on MPL agar. Table 2 shows the number of specimens from which multiple yeast species were isolated and the frequency with which individual species were associated. The most frequent association was *C. albicans* and *Torulopsis glabrata*, seen in 6 of 22 specimens containing two yeast species (27%). In addition, three different yeast species, *C. albicans*, *T. glabrata*, and *C. tropicalis*, were simultaneously recovered from one specimen obtained from a leukemic patient.

There was no discernible difference in the speed with which the two media yielded positive cultures.

The common bacterial contaminants which were isolated from oral-rinse samples on MPL agar were gram-positive cocci, especially *Staphylococcus* species, and gram-negative bacilli, such as *Enterobacter* species. However, the characteristic color changes and colony morphology exhibited by these bacteria on MPL agar enabled their easy differentiation from yeasts, which grew into large, pigmented colonies.

TABLE 2. Numbers of oral specimens from which multiple yeast species were isolated and the frequency of their association

Associated yeast species	No. of specimens with indicated association on:	
	MPL agar	SDA
Combination of two species		
C. albicans + *T. glabrata*	6	0
C. albicans + *C. tropicalis*	4	1
C. albicans + *Saccharomyces cerevisiae*	3	0
C. albicans + *C. krusei*	2	2
C. albicans + *C. parapsilosis*	1	0
C. albicans + *C. guilliermondii*	1	1
C. tropicalis + *T. glabrata*	2	0
C. tropicalis + *C. krusei*	1	1
C. tropicalis + *S. cerevisiae*	1	0
Combination of three species: *C. albicans* + *T. glabrata* + *C. tropicalis*	2	0

Although MPL agar has been suggested as a substitute medium for the isolation of *Candida* species from clinical specimens, there are no reports in the literature comparing the sensitivity of the latter with SDA, which is universally used for the primary isolation of yeasts in clinical microbiology laboratories. The present study demonstrates that the sensitivity of MPL agar is at least equal to that of SDA in the primary isolation of yeasts. Additionally, a major advantage of MPL agar is its ability to allow various yeast species from a single specimen to be visually differentiated because of their color reactions. This advantage was clearly shown in our study by the recovery rates of multiple yeast species from a single specimen: 26% for SDA and 100% for MPL agar.

In clinical terms, this difference means that if SDA were used for yeast isolation, mixed-yeast infections would be missed in about 74% of cases. Nondetection of certain yeasts which may be resistant to antibiotics may have therapeutic and prognostic consequences if a patient is compromised or debilitated. Indeed, multiple-yeast infections are most commonly seen in such patient groups (6). Furthermore, the introduction of MPL agar has significantly raised the percentage of mixed-yeast infections of the oral cavity diagnosed in our laboratories. Despite these advantages, MPL agar is known to suppress the growth of some *C. albicans* isolates because of the presence of tetrazolium salts in the medium (13), although we did not observe this suppression in the present study. One reason for this discrepancy may be variations in the quality and concentration of the triphenyltetrazolium chloride used by different workers. However, from the present study it would appear that MPL agar could be used as a valuable adjunct to or as a substitute for SDA in isolating yeasts from clinical samples.

Our results also indicate that the oral carriage of more than one yeast species is not an uncommon phenomenon. No data comparable to ours are available in the literature, but the retrospective study of Yamane and Saitoh (15) recorded mixed yeast species in 9% of pharyngeal specimens, as opposed to the 16% reported in the present study. As in the present investigation, the most common yeast association observed in the Japanese study was *C. albicans* and *T. glabrata*. This is not surprising, as these organisms constitute the most common *Candida* species isolated from sites in the human body, including the oral cavity (10).

LITERATURE CITED

1. **Allison, R. T.** 1967. An evaluation of Pagano-Levin medium in a quantitative study of *Candida albicans*: preliminary communication. J. Med. Lab. Technol. **24**:199–202.
2. **Eschenbach, D. A., T. M. Buchanan, H. M. Pollock, P. S. Forsyth, E. R. Alexander, J. S. Lin, S. P. Wang, B. B. Wentworth, W. M. McCormack, and K. K. Holmes.** 1975. Polymicrobial aetiology of acute pelvic inflammatory disease. N. Engl. J. Med. **293**:166–171.
3. **Gilchrist, J. E., C. B. Donnelly, J. J. Peller, and J. E. Campbell.** 1977. Collaborative study comparing the spiral plate and aerobic plate count methods. J. Assoc. Off. Anal. Chem. **60**:807–812.
4. **Isselbacher, K. J., R. D. Adams, E. Braunwald, R. G. Petersdorf, and J. D. Wilson.** 1980. Principles of internal medicine, p. 363. McGraw Hill Book Co., London.
5. **Kiani, D., E. L. Quinn, K. H. Birch, T. Madhaven, L. D. Saravolatz, and T. R. Neblett.** 1979. The increasing importance of polymicrobial bacteraemia. J. Am. Med. Assoc. **242**: 1044–1047.
6. **Kostiala, I., A. A. I. Kostiala, A. Kahanapaa, and E. Elonan.** 1982. Acute fungal stomatitis in patients with haematologic

malignancies. J. Infect. Dis. **146:**101–102.
7. **Kutscher, A. H., L. Seguin, E. V. Zegarelli, R. M. Rankow, J. B. Campbell, and J. Mercadante.** 1959. Pagano-Levin culture medium for differentiation of *Candida albicans*: American Type Culture Collection studies. Antibiot. Chemother. (Washington D.C.) **9:**649–659.
8. **Lodder, A. (ed.).** 1970. The yeasts: a taxonomic study, 2nd ed. North-Holland Publishing Co., Amsterdam.
9. **MacKenzie, D. W. R.** 1962. Serum tube identification of *Candida albicans*. J. Clin. Pathol. **15:**563–565.
10. **Odds, F. C.** 1979. *Candida* and candidosis, p. 121. Leicester University Press, Leicester, United Kingdom.
11. **Pagano, J., J. D. Levin, and W. Trejo.** 1958. Diagnostic medium for differentiation of *Candida* species. Antibiot. Annu. **1957–1958:**137–143.
12. **Sinski, J. T.** 1960. The effectiveness of Pagano-Levin medium for the detection and identification of *Candida albicans* in clinical specimens. J. Invest. Dermatol. **35:**131–133.
13. **Stedham, M. A., D. C. Kelley, and E. H. Coles.** 1966. Modified Pagano Levin medium to isolate *Candida* species. Appl. Microbiol. **14:**525–528.
14. **Walsh, J. T., W. E. Venanzi, and D. M. Dixon** 1985. Quantification of medically important *Candida* species and *Torulopis glabrata* by a spiral inoculation system: correlation with pour plate and spread plate methods. J. Clin. Microbiol. **22:**745–747.
15. **Yamane, N., and Y. Saitoh.** 1985. Isolation and detection of multiple yeasts from a single clinical sample by use of Pagano-Levin agar medium. J. Clin. Microbiol. **21:**276–277.