THE EFFECTIVENESS OF PAGANO-LEVIN MEDIUM FOR THE DETECTION AND IDENTIFICATION OF *CANDIDA ALBICANS* IN CLINICAL SPECIMENS*

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Rapid and accurate identification of *Candida albicans* continues to be a problem in the diagnostic laboratory. Recently Pagano-Levin (P. L.) agar medium has been developed which utilizes the production of variously pigmented colonies to isolate and identify the more commonly occurring species of *Candida*. Kutscher *et al* (1, 2) have studied the development of pigment and other cultural characteristics of 100 strains of *C. albicans*, obtained from various clinics and culture collections, as well as 111 different yeasts and fungi, 94 of which were subcultures of the American Type Culture Collection. The results obtained from these surveys indicated that P. L. medium was a rapid, relatively accurate method for identification of *C. albicans* particularly well suited for clinical use. The present study is an attempt to evaluate the usefulness of this new medium using clinical specimens of unknown etiology rather than identified stock cultures. The accuracy of the identification of the unknown organisms on P. L. medium is checked by comparison with the more conventional method of determining ability to form chlamydospores (4), with subsequent determination of sugar fermentation reactions (3) in the event of failure to form chlamydospores.

MATERIALS AND METHODS

Some of the clinical specimens were streaked directly onto plates of Mycocel‡ and Littman's oxgall agar§ and growth from these media was used for testing as described below. Handling of other of the clinical material differed only in that P. L. medium was inoculated first, and growth from this medium was transferred to Mycocel and to Littman's oxgall agar to purify the predominant organism for other studies. Each primary isolate accepted for the study showed a substantial number of colonies, indicating the isolate to be the probable agent of the infection rather than a contaminant.

Three criteria for the identification of *C. albicans* were utilized, namely, (1) color changes on P. L. medium, (2) formation of chlamydospores in micro-culture using Nickerson-Mankowski agar‖, and (3) the ability to ferment glucose, maltose, sucrose and lactose.

The cultures were grown at room temperature and read 48 hours after inoculating following the original directions supplied with the P. L. medium. According to these directions, *C. albicans* is supposed to produce creamy white to faintly pink colonies under these conditions whereas other species, with the exception of *C. parakrusei* and *C. Krusei*, appear as red colonies. *Candida Krusei* is described as producing a white, flat, dry wrinkled appearance. Verbal description of *C. parakrusei* is not given, but a color photograph included among the original directions shows this organism producing a light pink colored colony on P. L. medium.

Micro-cultures for the production of chlamydospores also were read after 48 hours. Those with chlamydospores were recorded as *C. albicans*. If the micro-cultures of a strain were negative after three trials, the strain was recorded as not being *C. albicans* by the criterion of this particular method. However, chlamydospore-negative cultures were further tested for the ability to ferment glucose, maltose, sucrose and lactose. Cultures having the characteristic fermentation reactions, despite the failure to form chlamydospores, were recorded as *C. albicans* by this criterion.

RESULTS AND DISCUSSION

Ascospores were not observed in any of the 23 isolates tested. As shown in Table 1, of the 23 isolates tested, 8 isolates produced both chlamydospores in micro-culture using Nickerson-Mankowski agar and creamy white to pink colonies on P. L. medium; 8 were considered not *C. albicans* due to absence of chlamydospores in Nick-

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TABLE I

Classification of organisms isolated from cases of suspected candidiasis, based on tests using Pagano-Levin medium, chlamydospore production in microculture and fermentation reactions

	Strains						
Cultural criteria for identification	3865			3491			
	3756			3558			
	3758			3766			
	3848			3847			
	3866	3586		3813			
	3880	3720		3890			
	3902	3752	3765	3985			
	4034	3906	3972	4046	None	3715	None
Reaction on Pagano-Levin medium	+	+	+	−	−	−	−
Chlamydospore production in micro-culture	+	−	−	−	−	+	+
Fermentation reactions	Not done	−	+	−	+	+	−
Totals	8	4	2	8	0	1	0

+ = positive test for *C. albicans*.
− = negative test for *C. albicans*.

erson-Mankowski agar, presence of red pigment on P. L. medium, and failure to show fermentation reactions characteristic of *C. albicans*. Thus, the results obtained with 16 of the isolates were in complete agreement. However, conflicting data were obtained with 7 of the isolates. In 4 instances (cultures #3586, 3720, 3752 and 3906) developed white to light pink, creamy growth on P. L. medium, supposedly characteristic of *C. albicans*, but could not be identified as this organism by the other two criteria. Had the color reaction been read at 72 hours as suggested by Kutscher *et al* (1) and by a new set of directions now available from the manufacturers, it is conceivable these results may have been different. Nevertheless, this finding of false positive reactions is confirmed by the observations of both Taschdjian (5) and of Kustcher *et al* (2).

In two instances (isolates 3765 and 3972) the P. L. medium proved more accurate than the criterion of chlamydospore formation, inasmuch as two chlamydospore-negative strains behaved like typical *C. albicans* on P. L. medium and this behavior was confirmed by sugar fermentation reactions characteristic of *C. albicans*.

Finally, the P. L. medium completely "missed" one isolate (3715) which repeatedly formed red colonies after 2 days incubation on P. L. medium, indicative that the isolate in question was not *C. albicans*. However, this strain was subsequently identified as *C. albicans* by both the formation of chlamydospores in micro-culture and the characteristic fermentation of sugars. This failure of isolate 3715 to react properly on P. L. medium was confirmed by Dr. Pagano and is to the author's knowledge the first report of a "false negative" reaction on this medium.

SUMMARY AND CONCLUSIONS

Twenty-three recent, unidentified yeast isolates from clinical candidiasis were cultured on Nickerson-Mankowski agar, for the production of chlamydospores, and on Pagano-Levin medium. Isolates that gave negative tests for *C. albicans* on either of both media were further tested for fermentation of glucose, maltose, sucrose and lactose.

Divergent results were obtained with seven of the 23 isolates. In five instances, the results obtained on the P. L. medium were considered inaccurate. Of these, four were falsely positive for *C. albicans* inasmuch as the findings could not be confirmed either by production of chlamydospores or by fermentation reactions. Yet another isolate was falsely negative on P. L. medium in that chlamydospores were produced in microculture and fermentation reactions typical of *C. albicans* occurred. However, two isolates that failed to produce chlamydospores in micro-culture gave positive results on P. L. medium and these were confirmed by fermentation reactions.

None of the isolates produced ascospores.

Pagano-Levin medium should prove useful for screening for *C. albicans* although false reactions

both positive and negative do occur. The fact that most of the false reactions are positive indicates the method to be overly sensitive and, therefore, particularly well suited for screening. However, in laboratories when absolute identification is desirable the other standard criteria should also be used.

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