Use of an Oxidation-Fermentation Medium in the Identification of Yeasts

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FOR MANY YEARS, differentiating *Candida albicans* and *Cryptococcus neoformans* from other yeasts has been considered adequate characterization of clinical specimens. It has become increasingly obvious in recent years, however, that other species of yeasts are also implicated in pathological conditions and that a more definitive characterization of yeasts as etiological agents is necessary.

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The identification of yeasts requires consideration of both morphological and physiological characteristics. The physiological characterization is achieved by differentiating the organisms according to the activity of urease, the assimilation of nitrate, and the fermentations and assimilations of carbohydrates.

The assimilation of carbohydrates is determined by the classic Wickerham broth procedure (*1*) or the agar auxanogram procedures. It is generally conceded that the Wickerham procedure is more sensitive than the comparable agar auxanogram techniques.

Preliminary studies in the laboratory of the Proficiency Testing Section have revealed that the differential carbohydrate assimilation patterns achieved by the Wickerham broth technique can be reproduced identically in an oxidation-fermentation (O-F) medium incubated under aerobic conditions at 25°C. In contrast to the classic assimilation tests, growth from the pure culture of yeasts was stabbed into the semisolid O-F medium with a straight wire without the requirement for starving the inoculum or tedious dilution and adjustment procedures. Inoculation of the carbohydrate-free basal medium failed to show that acid production from a carryover of a carbohydrate substrate in the inoculum was a problem.

Materials and Methods

The O-F medium used in our study was devised originally for characterization of bacteria (*2*) by the late Elizabeth O. King, of the Center for Disease Control,

and contains the following constituents:

Bacto casitone, 0.2 gm.
Bacto agar, 0.3 gm.
Phenol red, 0.003 percent (0.2 ml. of 1.5 percent solution)
Water, 100 ml.

The medium is prepared by dissolving the peptone in water with the aid of heat and by careful adjustment of the pH to 7.4. Three-tenths gram of agar is added and dissolved by heat. The medium is autoclaved at 15 pounds pressure (121°C.) for 15 minutes. To aliquots of the sterile melted basal medium are added filter-sterilized carbohydrates to a final concentration of 1.0 percent.

In these studies, carbohydrate mediums containing glucose, maltose, sucrose, lactose, galactose, melibiose, cellobiose, inositol, xylose, raffinose, trehalose, and dulcitol were prepared in tubes. This battery of carbohydrates is the same one used routinely in this laboratory in performing the Wickerham carbohydrate assimilation tests. The medium is placed in 5-ml. amounts in 15- by 125-mm. tubes that are then plugged with cotton.

During this study, the O-F medium described was found to be preferable to the commercially available O-F medium of Hugh and Liefson (*3*). The Hugh-Liefson medium contains the pH indicator bromthymol blue, which requires a larger amount of acid

Table 1. Comparison of Wickerham assimilation patterns with oxidation-fermentation (O-F) patterns in *Candida* species

Organism and test procedure [1]	Number of strains	Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol
C. albicans:													
Assimilation	15	+	+	+	−	+	−	−	−	+	−	+	−
O–F		+	+	+	−	+	−	−	−	+	−	+	−
Assimilation	1	+	+	+	−	+	−	−	−	−	−	+	−
O–F		+	+	+	−	+	−	−	−	[2] +	−	+	−
Assimilation	1	+	+	+	−	+	−	−	−	−	−	+	−
O–F		+	+	+	−	+	−	−	−	−	−	+	−
C. tropicalis:													
Assimilation	8	+	+	+	−	+	−	−	−	+	−	+	−
O–F		+	+	+	−	+	−	−	−	+	−	+	−
Assimilation	3	+	+	+	−	+	−	+	−	+	−	+	−
O–F		+	+	+	−	+	−	+	−	+	−	+	−
C. krusei:													
Assimilation	7	+	−	−	−	−	−	−	−	−	−	−	−
O–F		+	−	−	−	−	−	−	−	−	−	−	−
C. parapsilosis:													
Assimilation	6	+	+	+	−	+	−	−	−	+	−	+	−
O–F		+	+	+	−	+	−	−	−	+	−	+	−
C. guillermondii:													
Assimilation	5	+	+	+	−	+	+	+	−	+	+	+	+
O–F		+	+	+	−	+	+	+	−	+	+	+	+
C. stellatoidea:													
Assimilation	2	+	+	−	−	+	−	−	−	+	−	+	−
O–F		+	+	−	−	+	−	−	−	+	−	+	−
C. pseudotropicalis:													
Assimilation	2	+	−	+	+	+	−	+	−	+	+	−	−
O–F		+	−	+	+	+	−	+	−	+	+	−	−
C. vini:													
Assimilation	3	+	−	−	−	−	−	−	−	−	−	−	−
O–F		+	−	−	−	−	−	−	−	−	−	−	−

[1] Assimilation +; growth density greater than or equal to 1+ turbidity by the Wickerham card method. Assimilation −; growth density less than 1+ turbidity by the Wickerham card method. O–F +; oxidative production of acid. O–F −; no acid production.

[2] O–F, xylose oxidized; assimilation, xylose not utilized.

than phenol red to register a change in pH. The Hugh-Liefson medium worked well with most species of *Candida* since they produce large amounts of acid; however, we found that cryptococci produced acid at a slower rate and were unable to alter the color of the indicator without prolonged periods of incubation.

Results

Comparative studies with the Wickerham assimilation broth and the Center for Disease Control O-F medium were performed with reference strains and recent clinical isolates that had been identified and confirmed by the classic assimilation and fermentation procedures (tables 1–3). Complete correlation of carbohydrate assimilation and carbohydrate oxidation was achieved with the exception of one strain of *C. albicans*. This organism, which failed to assimilate xylose in 21 days, oxidized the carbohydrate when the O-F medium was held for 14 days. The pattern of carbohydrate utilization for the various genera and species is also seen in the tables.

A critical comparison of the two procedures showed that rapid, luxuriant growth in the assimilation medium was accompanied by rapid and intense production of acid in the comparable O-F medium, with development of a bright yellow color. Similarly, scant growth of the isolate in the assimilation medium was paralleled by weak production of acid in the O-F medium, demonstrated by the development of intermediate hues of orange and yellow. Weak utilization of the carbohydrates was definitely easier to interpret in the O-F medium, where most patterns of carbohydrate utilization were completed in 5 days. Because a few strains exhibited delayed reactions, however, we recommend holding the O-F medium for 10 days.

Discussion

In this study, we directed our attention to the oxidative assimilation of carbohydrates by yeasts. While the results of the carbohydrate fermentation studies are not presented in the text, we feel that the concept of fermentation of carbohydrates by yeasts should be clarified. The fermentative capabilities of yeasts are determined in a conventional carbohydrate-peptone medium containing an inverted Durham tube. Gen-

Table 2. Comparison of Wickerham assimilation patterns with oxidation-fermentation (O-F) patterns in *Cryptococcus* species

Organism and test procedure [1]	Number of strains	Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol
C. neoformans:													
Assimilation	15	+	+	+	−	+	−	+	+	+	+	+	+
O–F		+	+	+	−	+	−	+	+	+	+	+	+
C. albidus var. *albidus:*													
Assimilation	10	+	+	+	+	+	−	+	+	+	+	+	−
O–F		+	+	+	+	+	−	+	+	+	+	+	−
C. albidus var. *diffluens:*													
Assimilation	2	+	+	+	−	−	−	+	+	+	+	+	−
O–F		+	+	+	−	−	−	+	+	+	+	+	−
C. uniguttulatus:													
Assimilation	1	+	+	+	−	−	−	+	+	+	+	+	−
O–F		+	+	+	−	−	−	+	+	+	+	+	−
C. terreus:													
Assimilation	1	+	−	−	+	+	−	+	+	+	−	+	+
O–F		+	−	−	+	+	−	+	+	+	−	+	+
Assimilation	1	+	+	−	+	+	−	+	+	+	−	+	+
O–F		+	+	−	+	+	−	+	+	+	−	+	+
C. luteolus:													
Assimilation	2	+	+	+	−	+	+	+	+	+	+	+	+
O–F		+	+	+	−	−	+	+	+	+	+	+	+

[1] Assimilation +; growth density greater than or equal to 1 + turbidity by the Wickerham card method.
Assimilation −; growth density less than 1 + turbidity by the Wickerham card method. O–F +; oxidative production of acid. O–F −; no acid production.

Table 3. Comparison of assimilation and oxidation-fermentation (O-F) patterns in other yeasts encountered in the clinical laboratory

Organism and test procedure [1]	Number of strains	Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol
Torulopsis glabrata:													
Assimilation	9	+	−	−	−	−	−	−	−	−	−	+	−
O–F		+	−	−	−	−	−	−	−	−	−	+	−
Saccharomyces cerevisiae:													
Assimilation	2	+	+	+	−	+	−	−	−	−	+	+	−
O–F		+	+	+	−	+	−	−	−	−	+	+	−
Prototheca wickerhamii:													
Assimilation	3	+	−	−	−	+	−	−	−	−	−	+	−
O–F		+	−	−	−	+	−	−	−	−	−	+	−
Trichosporon cutaneum:													
Assimilation	8	+	+	+	+	+	+	+	+	+	+	+	+
O–F		+	+	+	+	+	+	+	+	+	+	+	+
Trichosporon inkin:													
Assimilation	3	+	+	+	+	+	−	+	−	+	−	+	−
O–F		+	+	+	+	+	−	+	−	+	−	+	−
Rhodotorula rubrum:													
Assimilation	2	+	+	+	−	+	−	−	−	+	+	+	−
O–F		+	+	+	−	+	−	−	−	+	+	+	−
Geotrichum candidum:													
Assimilation	1	+	−	−	−	+	−	−	−	+	−	−	−
O–F		+	−	−	−	+	−	−	−	+	−	−	−
Trichosporon capitatum:													
Assimilation	1	+	−	−	−	+	−	−	−	−	−	−	−
O–F		+	−	−	−	+	−	−	−	−	−	−	−
Trichosporon pullulans:													
Assimilation	1	+	+	+	+	+	−	+	−	+	−	+	−
O–F		+	+	+	+	+	−	+	−	+	−	+	−
Trichosporon penicillatum:													
Assimilation	2	+	−	−	−	+	−	−	−	+	−	−	−
O–F		+	−	−	−	+	−	−	−	+	−	−	−

[1] Assimilation +; growth density greater than or equal to 1 + turbidity by the Wickerham card method.
Assimilation −; growth density less than 1 + turbidity by the Wickerham card method. O–F +; oxidative production of acid. O–F −; no acid production.

eration of gas during alcoholic fermentation is an important taxonomic criterion. Fermentations that do not produce gas (for example, acidic fermentations) are not considered in the classification of yeasts.

In determinations of carbohydrate assimilations by yeasts, use of the classic Wickerham assimilation technique is cumbersome for the average laboratorian. Preliminary starvation of the yeast inoculum is required or, at the very least, preparation of a diluted yeast suspension adjusted to a prescribed density. To further complicate matters, growth resulting from the presence of contaminating sugars in some carbohydrates (for example, free glucose in maltose) can confuse interpretation of the assimilation tests. Some growth of *Torulopsis glabrata* in maltose assimilation broth has been observed on occasion in this laboratory.

Parallel studies with the O-F medium have failed to reveal that the presence of contaminating sugars is a problem. If some growth occurred in the conventional maltose assimilation broth, the same sugar failed to give rise to discernible acid production in the O-F medium. The amount of acid produced from the contaminating sugar was insufficient to produce a color change in the O-F medium.

We find these results encouraging enough to warrant the attention of laboratorians who routinely perform assimilations of carbohydrates. Stabbing the O-F

medium directly from a pure yeast culture is not as time consuming as either starving the yeasts or making a dilute yeast suspension and adding 0.1 ml. of the suspension to the assimilation broths. The development of yellow in the O-F medium as compared with the degree of turbidity in a Wickerham assimilation broth is easier to interpret visually for the utilization of a carbohydrate.

Another advantage of the O-F medium benefits the laboratorian who performs both bacteriological and mycological investigations. The O-F medium that we have reported has been used in bacteriological laboratories for several years. The O-F medium of Hugh and Liefson (*3*) was found to be unsatisfactory for characterization of numerous strains of oxidative bacteria that produced low levels of acid. The O-F medium of Elizabeth King (*2*) was developed to characterize these weak producers of acid.

In this study we observed a comparable situation in the heterogeneity of acid production in yeasts, and King's O-F medium was adopted. In 117 strains of yeasts studied, no false positive biochemical reactions were observed. The O-F medium in our hands offered advantages over the Wickerham technique in method of inoculation and ease of interpretation.

REFERENCES

(*1*) Wickerham, L. J.: Taxonomy of yeasts. Technical Bulletin No. 1029. U.S. Department of Agriculture, Washington, D.C., 1951.

(*2*) Vera, H., and Dumoff, M.: Culture media: O–F basal medium base. *In* Manual of clinical microbiology, edited by J. E. Blair, E. H. Lennette, and J. P. Truant. American Society of Microbiology, Bethesda, Md., 1970.

(*3*) Hugh, R., and Liefson, E.: The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J Bact 66: 24–26 (1953).

HALL CHARLES T. (Center for Disease Control), WEBB, C. DOUGLAS, Jr., and PAPAGEORGE, CALOMIRA: ***Use of an oxidation-fermentation medium in the identification of yeasts. HSMHA Health Reports, Vol. 87, February 1972, pp. 172–176.***

An oxidation-fermentation (O-F) medium, originally developed for use in bacteriology, was adopted to determine the carbohydrate utilization patterns of yeasts. Using the O-F medium, 12 different carbohydrates were inoculated with growth from a pure culture of yeast.

With 117 yeast isolates, the O-F medium produced carbohydrate utilization patterns comparable to the classic Wickerham broth assimilation test. With the exception of one yeast strain, there was complete correlation of carbohydrate assimilation and carbohydrate oxidation patterns.

The results of this study warrant the attention of the laboratorian who performs both bacteriological and mycological investigations, since a single medium could be used for both disciplines. As a measure of carbohydrate utilization, the production of acid in the O-F medium is easier to interpret than the development of turbidity in a Wickerham assimilation broth.