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Assimilation of Protocatechuic Acid and *p*-Hydroxybenzoic Acid as an Aid to Laboratory Identification of *Candida parapsilosis* and Other Medically Important Yeasts

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Tests for the ability of yeasts isolated from clinical specimens to utilize protocatechuic acid and *p*-hydroxybenzoic acid were carried out by using techniques that are commonly employed to test assimilation of carbon sources. A total of 60 isolates of *Candida parapsilosis* and 5 isolates of *Candida humicola* readily assimilated these two phenolic acids, whereas other *Candida* species gave uniformly negative results. *Cryptococcus albidus*, *Cryptococcus terreus*, and some isolates of *Cryptococcus laurentii* also assimilated protocatechuate and *p*-hydroxybenzoate, whereas *Cryptococcus neoformans* did not. Results of these tests suggest that assimilation of protocatechuate and *p*-hydroxybenzoate may be a useful characteristic, when used in conjunction with traditional tests, for identifying *C. parapsilosis* and *C. albidus*.

The ability of yeasts and other fungi to metabolize phenolic compounds has been regarded as a unique characteristic of certain isolates that might be exploited for reducing the phenolic content of sewage and other waste materials (1-4, 7). However, to our knowledge, utilization of a particular phenolic compound as a carbon source has not been previously evaluated as a characteristic that could be used for differentiating yeast species.

Earlier, Kunze (5) identified nine phenolic compounds derived from the decomposition of lignin, including vanillin (4-hydroxy-3-methoxybenzaldehyde), vanillic acid (4-hydroxy-3-methoxybenzoic acid), *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid (PHBA), ferulic acid (4-hydroxy-3-methoxycinnamic acid), coumaric acid (*p*-hydroxycinnamic acid), protocatechuic acid (PCA; 3,4-dihydroxybenzoic acid), caffeic acid (3,4-dihydroxycinnamic acid), and gallic acid (3,4,5-trihydroxybenzoic acid). In a preliminary survey in which the ability of yeasts to assimilate these nine phenolic compounds was tested, it was observed that *Candida parapsilosis* and *Cryptococcus albidus* were capable of utilizing PCA and PHBA, whereas eight other species lacked this capability. These results suggested the hypothesis that assimilation of PCA and PHBA might be a characteristic that could be used to differentiate these two species from other yeasts. The following report is a description of a study that was undertaken to test this hypothesis.

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MATERIALS AND METHODS

Sources of isolates. A total of 451 isolates, including both recent clinical isolates and reference strains obtained from the American Type Culture Collection and other culture collections, were utilized in the study. The majority of the isolates were originally obtained from clinical material from hospitalized patients that was submitted to a clinical mycology laboratory for diagnostic purposes. The isolates mentioned above were selected on the basis of preliminary testing so that frequently isolated species such as *Candida albicans* would not dominate those tested. A few isolates from soil and other natural sources were also included, and at least one reference strain of each species tested, including the type strain of *C. parapsilosis* (kindly contributed by Sally A. Meyers), was evaluated along with the other isolates.

Preparation of media. Wickerham's yeast nitrogen base (YNB; Difco Laboratories) was used as the basal medium into which either PCA or PHBA was incorporated as the sole carbon source. For each test, a growth control tube containing YNB and 0.5% glucose as the carbon source and a negative control tube containing YNB only were inoculated along with the test media.

Because of the poor water solubility of PCA and PHBA, they were first dissolved in a small volume of 1 M sodium hydroxide and then adjusted to pH 7.5 with 1 M hydrochloric acid. Each solution was then mixed with 100 ml of 10× YNB in deionized water containing 0.1% bromothymol blue indicator. The pH of the resultant solution was adjusted to between 6.0 and 6.5 and was made up to 200 ml with deionized

water. This stock solution was sterilized by filtration and stored at 4°C until needed. The test media were prepared as agar slants by incorporating 1 ml of one of the stock solutions into 4.0 ml of 2% Noble agar (Difco) that had been sterilized by autoclaving at 15 lb (ca. 1.05 kg/cm^2) of pressure for 15 min and then cooled to 50°C in a water bath. The tubes of media were cooled in a slanted position and stored at 4°C until used. The final concentration of either PCA or PHBA in the test media was 0.01 M.

Cultural conditions. For inoculation, 0.1 ml of a slightly turbid suspension (+1, using a Wickerham card) of the yeast isolate being tested was added to a slant of each of the test media and to the control tubes as well. The inoculated tubes were incubated at 30°C and examined at 48-h intervals for evidence of growth. All tubes were held for a total of 14 days before being recorded as negative.

Incorporation of the indicator into the medium was done to increase the sensitivity of detecting growth. Growth on slants containing either of the phenolic acids caused a shift in pH to a more alkaline reaction, resulting in a change of the color of the indicator from yellow to green or blue.

RESULTS

C. parapsilosis and *Candida humicola* were the only representatives of the genus *Candida* that were capable of utilizing PCA or PHBA as sole carbon source. Both varieties of *C. albidus* (*C. albidus* and *Cryptococcus diffluens*) along with *Cryptococcus terreus*, as well as some isolates of *Cryptococcus laurentii*, were capable of assimilating PCA and PHBA. These results contrasted with the negative results obtained with isolates of *Cryptococcus neoformans*. The results obtained with *C. neoformans* required careful evaluation. PCA and PHBA reacted with phenol oxidase in the cell walls of individual *C. neoformans* cells to produce a brown coloration of the inoculum, increasing its visibility and suggesting growth of these isolates; however, when compared with the growth control tube, it was obvious that little if any increase in cell numbers had occurred.

Rhodotorula species yielded variable results; however, each isolate tested was capable of assimilating at least one of the two phenolic acids, and the data obtained thus far do not permit distinction of species within this genus by using assimilation of PCA and PHBA. *Trichosporon cutaneum* assimilated PCA and PHBA, whereas the few other *Trichosporon* isolates tested did not, and all *Saccharomyces* isolates tested yielded negative results.

Most of the isolates assimilating PCA and PHBA did so within 4 to 7 days from the time of inoculation, with only a rare positive later. In a few tests, duplicate sets of media were inoculated, and the extra set was incubated at 37°C. Isolates of *C. albidus* that were incapable of growing at 37°C yielded negative results when incubated at that temperature; however, the temperature of incubation had no detectable influence on the outcome of tests with isolates capable of growth at the higher temperature.

DISCUSSION

Conventional methods used for identifying yeasts (6) include tests for assimilation of carbohydrates, nitrogen sources, and in some instances, fatty acids or alkanes along with a variety of other tests. The data presented in this paper suggest that tests for assimilation of PCA and PHBA might also be used to advantage as identifying characteristics of certain species.

Of the 235 isolates of 14 *Candida* species tested, only *C. parapsilosis* and *C. humicola* were capable of utilizing PCA and PHBA as a sole carbon source. (Admittedly, only a few isolates of some of the more unusual *Candida* species were tested.) Of those *Candida* species that are commonly isolated from clinical materials, the ability to assimilate PCA and PHBA appeared to be a characteristic that is unique to *C. parapsilosis*, since in our experience, *C. humicola* is only rarely isolated from human patients.

Negative tests for assimilation of cellobiose and soluble starch in addition to negative tests for fermentation of maltose and sucrose are the principle biochemical characteristics that serve to distinguish *C. parapsilosis* from *C. albicans* and *Candida tropicalis* by conventional methodology. The data presented here, then, offer the promise that assimilation tests with PCA or PHBA or both could be used to shorten the time required for a definitive identification of *C. parapsilosis* to be made, since assimilation of PCA and PHBA could be observed in a shorter period of time than was necessary to be certain that negative assimilation and fermentation tests were truly negative.

The data presented in Table 1 also suggest that assimilation tests with PCA and PHBA might be a useful adjunct for differentiating *C. neoformans* from some other *Cryptococcus* species. The positive assimilation of these phenolic acids by *C. albidus*, *C. terreus*, and some isolates of *C. laurentii* appears to set these species apart from *C. neoformans*, which turned brown but did not grow. The variable results obtained with isolates of *C. laurentii* (11 of 24 were positive) cannot be readily explained at the present time. Too few isolates of *Trichosporon capitatum*, *Trichosporon pullulans*, and *Torulopsis anatomiae* were available for testing in this study to

TABLE 1. *Assimilation of PCA and PHBA by selected yeast isolates*

Species tested	PCA[a]	PHBA
Candida albicans	0/46	0/46
Candida guilliermondii	0/10	0/10
Candida humicola	5/5	5/5
Candida krusei	0/13	0/13
Candida parapsilosis	60/60	60/60
Candida pseudotropicalis	0/4	0/4
Candida stellatoidea	0/27	0/27
Candida tropicalis	0/58	0/58
Miscellaneous *Candida* spp.[b]	0/12	0/12
Cryptococcus albidus	55/56	55/56
Cryptococcus laurentii	11/24	11/24
Cryptococcus luteolus	0/1	0/1
Cryptococcus neoformans	0/29	0/29
Cryptococcus skinneri	0/1	0/1
Cryptococcus terreus	4/4	4/4
Cryptococcus uniguttulatus	0/2	0/2
Geotrichum	2/3	2/3
Rhodotorula glutinis	9/10	10/10
Rhodotorula graminis	1/1	1/1
Rhodotorula rubra	3/5	3/5
Saccharomyces cerevisiae	0/15	0/15
Torulopsis anatomiae	1/1	1/1
Torulopsis candida	0/5	0/5
Torulopsis glabrata	0/46	0/46
Trichosporon capitatum	0/2	0/2
Trichosporon cutaneum	9/10	10/10
Trichosporon pullulans	0/1	0/1

[a] Each value indicates number positive per total number tested.

[b] Includes *C. aaseri*, *C. lipolytica*, *C. maltosa*, *C. rugosa*, *C. utilis*, and *C. zeylanoides*.

permit any definite conclusions to be drawn regarding the potential usefulness of these tests for identifying species of *Trichosporon* and *Torulopsis*.

The data presented in this paper suggest the possibility of using assimilation tests with PCA and PHBA in conjunction with conventional tests for identifying isolates of *C. parapsilosis*, some *Cryptococcus* species, and, perhaps, other yeasts as well. Although the phenolic assimilation media are somewhat more complex to prepare than carbohydrate assimilation media, they can be prepared without undue difficulty if the procedure outlined earlier in this paper is followed. These tests are similar to conventional assimilation tests and do not require unusual equipment or extraordinary expertise to perform. Two isolates assimilated PHBA which did not also assimilate PCA (Table 1), and growth of several isolates was noted to be more rapid on the former compound than on the latter. For that reason, routine testing using only PHBA would provide the same information for identification purposes as would testing the assimilation of both compounds.

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