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Evaluation of the API 20C Yeast Identification System for the Differentiation of Some Dematiaceous Fungi

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Ninety-seven isolates of *Cladosporium* spp., *Exophiala* spp., *Fonsecaea* spp., *Lecythophora hoffmannii*, *Phaeoannellomyces werneckii*, *Phialophora* spp., *Wangiella dermatitidis*, and *Xylohypha bantiana* were used to evaluate the API 20C Yeast Identification System for the differentiation of dematiaceous fungi. Using the API 20C system, we were able to distinguish most species of *Phialophora* and *Cladosporium* and to separate *L. hoffmannii* from the species of *Phialophora* tested; *X. bantiana* from *C. carrionii*, *C. resinae*, and *C. sphaerospermum*; and *W. dermatitidis* from *Exophiala jeanselmei* and *Exophiala spinifera*. Ninety-two (60.1%) of 153 possible species-pair combinations were separated.

Dematiaceous fungi represent a large group of microorganisms that normally occur as saprobes in soil and vegetative material but may cause opportunistic infections in animals and humans. Mycoses caused by the dematiaceous fungi include chromoblastomycosis, mycetoma, and phaeohyphomycosis. Infections may be superficial, cutaneous, subcutaneous, or systemic, depending on the etiologic agent and the host.

The identification of dematiaceous fungi is based primarily upon morphological characteristics, with other tests such as thermotolerance, protease production, and physiological characteristics (4, 9, 16) being useful in the separation of some morphologically similar taxa. The microscopic evaluation of clinical specimens obtained from pathological processes is useful at times in recognizing the presence of these fungi. Several serological tests, including exoantigen tests for the in vitro identification of some dematiaceous fungi, have been developed (3, 5, 6, 8, 10, 11). Unfortunately, the exoantigen test is limited, because there is a lack of commercially available specific antisera.

The evaluation of the conidiogenesis and morphogenesis of microscopic structures used for identification requires expertise and at times can be confusing. In addition, some of the dematiaceous fungi of medical interest are polymorphic; i.e., an isolate may form more than one anamorph. The physiological characteristics of these fungi have limited usefulness as an identification tool because they are not very reliable, their determination is time-consuming, and they may aid in the differentiation of only a few taxa (4). Owing to these types of problems, we evaluated the API 20C Yeast Identification System (Analytab Products, Plainview, N.Y.) as an easy, commercially available tool which could simplify the identification of some morphologically similar species of both saprobic and pathogenic isolates of dematiaceous fungi.

MATERIALS AND METHODS

Cultures. Ninety-seven isolates of pathogenic and saprobic dematiaceous fungi were used in this study (Table 1). The isolates were obtained from the culture collections of the Medical College of Virginia, Virginia Commonwealth University, Richmond; University of Texas Medical Branch, Galveston; University of Alberta, Edmonton, Canada; and State University of New York, Syracuse, where they were maintained for various periods. The purity and identity of the isolates were confirmed, after which they were maintained at 25°C in sterile water. Serial transfers on modified Sabouraud glucose agar (Difco Laboratories, Detroit, Mich.) incubated at 30°C were performed when the cultures were needed for testing.

API 20C Yeast Identification System. The API 20C Yeast Identification System consists of 20 cupules containing 19 different carbon source substrates and a negative control. The system was used according to the manufacturer's instructions, except for modifications made to accommodate the slower, more filamentous growth of some of the dematiaceous fungi. Modifications included an extended culture incubation time of 8 to 10 days, in contrast to the 2 to 3 days required for yeasts, and an indirect (versus direct for yeasts) inoculum preparation technique, as described below. However, only the 3-day readings were used for the final analysis.

Procedure. Mature (8- to 10-day) cultures grown at 30°C on modified Sabouraud glucose agar were used to obtain inoculum suspensions. The fungal colonies were covered with approximately 1 ml of sterile distilled water. Suspensions were made by gently probing the colony with the tip of a Pasteur pipette. The resulting mixture was withdrawn and transferred to a sterile tube. The heavy portions of the suspension were allowed to settle, and the upper homogeneous suspension was used as the inoculum (approximately 10^7 CFU/ml as demonstrated by quantitative plating on modified Sabouraud glucose agar). The molten basal medium supplied by the manufacturer was held at 50°C and seeded with the mycelial-conidial suspensions and then adjusted to a density slightly less than 1+ using a Wickerham card. The API 20C strips were inoculated with a sterile Pasteur pipette by filling each cupule with 0.2 ml of the seeded medium, as indicated by the manufacturer. The inoculated strips were wrapped in plastic bags to increase humidity and incubated at 30°C for 8 to 10 days. Cultures of *Cryptococcus laurentii* and *Torulopsis glabrata* were used as controls for each batch of strips, as recommended by the manufacturer. Reactions (growth) were read daily for 3 days and on days 8 and 10.

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TABLE 1. Isolates studied

Fungus (no. of isolates)	Isolate no.[a]
Cladosporium carrionii (6)	MCV 53.18, 53.35; UTMB 779, 781, 791, 1451
C. cladosporioides (6)	UTMB 122; UAMH 399, 4146, 5063; SUNY P-55, P-118
C. elatum (3)	MCV 53.39, 53.21; ATCC 11280
C. herbarum (6)	UAMH 470, 1303, 1812; SUNY D-122, D-139, D-152
C. resinae (4)	MCV 53.22; SUNY ED-66, P-137, ED-199
C. sphaerospermum (5)	MCV 19.7, 53.16, 53.23; UTMB 1402, 1199
Exophiala jeanselmei (5)	MCV 29.26, 29.19, 29.28; UTMB 123, 135
E. spinifera (6)	MCV 29.39, 29.49; UTMB 152, 2511, 1361; UAMH 3695
Fonsecaea compacta (6)	UTMB 10, 684, 904, 1000, 1271; UAMH 144
F. pedrosoi (6)	UTMB 11, 707, 925, 1475, 1490; UAMH 145
Lecythophora hoffmannii (4)	UTMB 19, 1039, 2777; UAMH 4383
Phaeoannellomyces werneckii (6)	UTMB 75, 765; UAMH 3683, 3696, 4981, 4984
Phialophora parasitica (6)	UTMB 134; UAMH 4034, 5024, 5059; SUNY P-710, P-754
P. repens (5)	MCV 29.16; UTMB 227, 184, 2724; UAMH 4377
P. richardsiae (6)	MCV 29.25; UTMB 490, 491, 728, 1231, 1359
P. verrucosa (5)	MCV 29.14, 29.15; UTMB 1060, 2509, 2989
Wangiella dermatitidis (6)	MCV 29.27, 29.31, 29.32; UTMB 941, 1070, 1223
Xylohypha bantiana (6)	MCV 53.20, 53.29, 53.34; UTMB 113, 115, 121

[a] Abbreviations: MCV, Medical College of Virginia, Virginia Commonwealth University; UTMB, University of Texas Medical Branch; UAMH, University of Alberta Microfungus Collection and Herbarium; SUNY, State University of New York.

Cupules showing turbidity (carbohydrate assimilation) significantly heavier than that of the negative control cupule were read as positive. Positive reactions were compared with that of the glucose cupule, which served as the positive control. Weak-to-strong reactions were all considered positive. Questionable reactions and absence of growth were considered negative.

Analysis of the data. A biotype profile number constructed from the results of each isolate was obtained by following the instructions for yeast profiles. The percentage of positive reactions was also evaluated. The carbohydrate assimilation reactions of each of the 18 species were then evaluated (by comparison of species pairs) against the other 17 species.

A numerical analysis was performed to evaluate the separation value (SV) and $\%SV_{max}$ for each of the carbohydrates tested. This same analysis (SV and $\%SV_{max}$ calculations) was performed on the API 20C yeast percentage positive chart provided by the manufacturer. Because the system is very reliable for the identification of clinically important yeasts (1), these calculations provided a reliable method for the comparison of our data. Percent positive reactions were converted to "positive" (100%), "negative" (0%), or "variable" (17 to 83%); and a matrix of these converted results was constructed. The number of positive reactions was multiplied by the number of negative reactions for each test to calculate its SV. All variable results were excluded from this calculation. The maximum obtainable SV (SV_{max}) for 18 taxa tested is an even distribution of positive and negative reactions, i.e., $9 \times 9 = 81$. SVs for each test were divided by 81 (SV_{max}) and multiplied by 100 to calculate $\%SV_{max}$.

RESULTS

A total of 153 species pairs were evaluated, and 92 pairs (60.1%) were separated by one or more tests. The remaining 61 pairs (39.9%) showed variable or identical assimilation patterns. The percentages of positive reactions were also evaluated. Of the 19 carbohydrates tested, 10 were most useful for separation of these species. A summary of these results is shown in Table 2. Separation of each species from the remaining 17 species ranged from 52.9 to 94.1% for 13 species, as shown in Table 3. The remaining 5 taxa, which are not included in this table, showed less than 52% separation from the other 17 species tested.

The most useful tests included the assimilation of glycerol (GLY) and 2-keto-D-gluconate (2KG), with $\%SV_{max}$ of 37%; and inositol (INO), with $\%SV_{max}$ of 30%. Seven other tests, sucrose (SAC), melezitose (MLZ), sorbitol (SOR), raffinose (RAF), lactose (LAC), galactose (GAL), and xylitol (XLT), demonstrated potential for the differentiation of these 18 species of dematiaceous fungi (Table 4). The $\%SV_{max}$ values calculated from the API 20C yeast percentage positive chart ranged between 0 and 65%, with a mean of 30%. If criteria for positive and negative reaction assignments were less restrictive (90 to 100% and 0 to 10%, respectively), the $\%SV_{max}$ values ranged between 18 and 93%, with a mean of 60%.

Among the species of *Cladosporium*, we were able to differentiate by their assimilation patterns *C. carrionii* from *C. cladosporioides* (INO), *C. resinae* (GLY, 2KG), and *C. sphaerospermum* (GLY); *C. cladosporioides from C. resinae* (2KG, INO, SOR, SAC, MLZ, RAF) and *C. sphaerospermum* (SOR); *C. elatum* from *C. resinae* (SAC, MLZ, RAF); and *C. resinae* from *C. sphaerospermum* (2KG, SAC, RAF). Carbohydrate reactions of *C. herbarum* were variable. We also differentiated *Xylohypha bantiana* from *C. carrionii* (INO), *C. resinae* (GLY, 2KG, INO, SOR, MLZ), and *C. sphaerospermum* (GLY, SOR). The carbohydrate assimilation reactions of *X. bantiana* and the remaining three species of *Cladosporium* tested gave either identical or variable patterns (Table 5).

Among the species of *Phialophora* tested, we were able to distinguish *P. parasitica* from *P. richardsiae* (2KG) and *P. verrucosa* (GLY); *P. repens* from *P. verrucosa* (LAC); and *P. richardsiae* from *P. verrucosa* (2KG, INO). There were no obvious assimilation differences between either *P. parasitica* and *P. repens* or the latter and *P. richardsiae*. Unique assimilation patterns were observed between the four species of *Phialophora* and *Lecythophora hoffmannii* (Table 5). *Fonsecaea compacta* and *F. pedrosoi* had identical assimilation patterns (Table 2).

Among the black yeasts, isolates of *Wangiella dermatitidis* could be separated from *Exophiala jeanselmei* and *E. spinifera* (MLZ), but no differences were seen between these two species of *Exophiala*. *Phaeoannellomyces werneckii* could not be differentiated from any of the other three species of black yeasts tested (Table 5).

TABLE 2. Carbohydrate assimilation profiles for some dematiaceous fungi tested with the API 20C Yeast Identification System

Taxon (no. of isolates)	% positive reactions for carbohydrate[a]:									
	GLY	2KG	XLT	GAL	INO	SOR	LAC	SAC	MLZ	RAF
Cladosporium carrionii (6)	100	100	33	67	0	17	0	83	83	17
C. cladosporioides (6)	17	100	17	100	100	100	67	100	100	100
C. elatum (3)	33	33	0	100	33	33	0	100	100	100
C. herbarum (6)	50	50	50	50	33	33	0	67	67	67
C. resinae (4)	0	0	0	75	0	0	0	0	0	0
C. sphaerospermum (5)	0	100	20	100	60	0	20	100	80	100
Exophiala jeanselmei (5)	100	60	60	80	0	60	0	100	100	20
E. spinifera (6)	100	100	100	33	0	0	0	100	100	67
Fonsecaea compacta (6)	100	100	0	100	0	100	67	100	100	17
F. pedrosoi (6)	100	100	0	100	0	100	50	100	100	0
Lecythophora hoffmannii (4)	100	0	0	100	75	50	0	0	100	25
Phaeoannellomyces werneckii (6)	100	83	17	50	33	17	0	83	33	50
Phialophora parasitica (6)	0	100	17	100	33	67	67	100	67	100
P. repens (5)	60	20	20	100	20	40	100	60	40	40
P. richardsiae (6)	33	0	0	100	100	83	17	100	100	33
P. verrucosa (5)	100	100	20	60	0	80	0	100	80	60
Wangiella dermatitidis (6)	100	100	17	0	0	33	0	50	0	0
Xylohypha bantiana (6)	100	100	0	100	100	100	0	83	100	67

[a] Figures indicate the percentage of positive reactions (72 h) only for tests with SVs of >0. Only the 10 most useful carbohydrates in the system are included: GLY, glycerol; 2KG, 2-keto-D-gluconate; XLT, xylitol; GAL, galactose; INO, inositol; SOR, sorbitol; LAC, lactose; SAC, sucrose; MLZ, melezitose; and RAF, raffinose.

DISCUSSION

The API 20C Yeast Identification System is a commercially available micromethod system used to identify most of the clinically significant yeasts and yeastlike fungi. This system contains 19 assimilation tests specifically selected because of their usefulness for the differentiation of yeast taxa. We evaluated these substrates as a means to identify representative species of clinically important and similar species of saprobic dematiaceous fungi. The dematiaceous fungi are difficult to identify because most of the species of medical interest are polymorphic and their microscopic structures are often difficult to evaluate. Their identification is based not only upon conidiogenesis and morphogenesis but upon which anatomical forms are present. Thus, their differentiation is often difficult and confusing.

Identification of the black yeasts is more perplexing than that of the dematiaceous moulds because they often initially grow as yeasts. Development of mycelial elements is frequently delayed and sometimes may not occur even after several passages on routine mycological agar. Their identification is primarily based on conidiogenesis. The presence or absence of blastoconidia and conidial structures, such as annellides and phialides, is the feature commonly used for their identification. Moreover, the observation of these structures may require significant expertise and skill in using light microscopy.

Physiological characteristics, such as urea hydrolysis, gelatin liquefaction, and decomposition of casein, xanthine, hypoxanthine, and tyrosine, have been proven to be of no diagnostic value (4, 8). The growth of dematiaceous fungi on Mycosel or mycobiotic agar may aid in the separation of some taxa of *Cladosporium* spp. and *E. jeanselmei* from other opportunistic dematiaceous pathogens (13). However, the use of cycloheximide in these media serves only as a limited screening test because some of the saprobic *Clado-*

TABLE 3. Taxa with >50% separation

Taxon[a]	No. of 17 remaining taxa not separated from	% Separation[b]
Cladosporium resinae	1	94.1
Lecythophora hoffmannii	2	88.2
C. sphaerospermum	4	76.5
Xylohypha bantiana	4	76.5
Phialophora richardsiae	4	76.5
Wangiella dermatitidis	4	76.5
P. parasitica	5	70.6
Exophiala spinifera	5	70.6
C. cladosporioides	6	64.7
P. repens	6	64.7
Fonsecaea pedrosoi	7	58.8
E. jeanselmei	8	52.9
F. compacta	8	52.9

[a] Taxa ranked by percent separation.
[b] % Separation = [(17 − number of taxa not separated)/17] × 100.

TABLE 4. Useful tests for differentiation of some dematiaceous fungi

Carbohydrate[a]	No. of reactions[b] for 18 species tested			SV[c]	%SV_{max}[d]
	+	−	V		
GLY	10	3	5	30	37
2KG	10	3	5	30	37
INO	3	8	7	24	30
SAC	10	2	6	20	25
MLZ	9	2	7	18	22
SOR	4	3	11	12	15
RAF	4	3	11	12	15
LAC	1	11	6	11	14
GAL	10	1	7	10	12
XLT	1	7	10	7	9

[a] GLY, Glycerol; 2KG, 2-keto-D-gluconate; INO, inositol; SAC, sucrose; MLZ, melezitose; SOR, sorbitol; RAF, raffinose; LAC, lactose; GAL, galactose; XLT, xylitol.
[b] +, 100% Positive; −, 0% positive; V, 17 to 83% positive for multiple isolates of the same species.
[c] SV = number + × number −. V was excluded from calculation.
[d] $\%SV_{max}$ = SV/81 × 100 (9 × 9, i.e., the maximum SV obtainable for 18 species).

TABLE 5. Separation among species of the same or related taxa

Fungus (no. tested)	Reaction[a]							
	GLY	2KG	INO	SOR	LAC	SAC	MLZ	RAF
Cladosporium carrionii (6)	+	+	−	V		V	V	V
C. cladosporioides (6)	V	+	+	+		+	+	+
C. elatum (3)	V	V	V	V		+	+	+
C. herbarum (6)	V	V	V	V		V	V	V
C. resinae (4)	−	−	−	−		−	−	−
C. sphaerospermum (5)	−	+	V	−		+	V	+
Xylohypha bantiana (6)	+	+	+	+		V	+	V
Phialophora parasitica (6)	−	+	V		V	+		
P. repens (5)	V	V	V		+	V		
P. richardsiae (6)	V	−	+		V	+		
P. verrucosa (5)	+	+	−		−	+		
Lecythophora hoffmannii (6)	+	−	V		−	−		
Black yeasts (23)								
Exophiala jeanselmei (5)							+	
E. spinifera (6)							+	
Phaeoannellomyces werneckii (6)							V	
Wangiella dermatitidis (6)							−	

[a] Only the carbohydrate reactions that separated these species are included: GLY, glycerol; 2KG, 2-keto-D-gluconate; INO, inositol; SOR, sorbitol; LAC, lactose; SAC, sucrose; MLZ, melezitose; and RAF, raffinose. +, Positive; −, negative; V, variable.

sporium spp. may grow on cycloheximide-containing media. Sodium chloride tolerance of dematiaceous fungi has been reported to provide some useful taxonomic information but only for the separation of specific species, such as *P. werneckii* (9).

Some attempts have been made to use carbohydrate profiles for the characterization of dematiaceous fungi. The first studies were based on carbohydrate fermentation reactions. Carrión and Silva (2) reported that isolates of *F. pedrosoi*, *P. verrucosa*, and *W. dermatitidis* could not ferment glucose, galactitol, galactose, glycerol, lactose, maltose, mannitol, or xylose. DeVries (G. A. DeVries, Thesis, Uitgeverij and Drukkerij, Baarn, The Netherlands, 1952) reported similar results for species of *Cladosporium*. These observations were expanded later to include isolates of *E. jeanselmei*, *E. spinifera*, *P. werneckii*, and *W. dermatitidis* (14). Montemayor (15) found that isolates of *E. jeanselmei*, *F. pedrosoi*, *F. compacta*, and *P. verrucosa* did not assimilate lactose, in contrast to isolates of saprobic *Cladosporium* spp. His work was a timely contribution toward demonstrating the usefulness of assimilation data for distinguishing some fungi.

Recently, Steadham et al. (16) conducted an extensive study of the assimilation of carbohydrates by dematiaceous fungi. Thirteen carbohydrates and numerous representative species of dematiaceous fungi were included in their study. Although they tested only one or two isolates of some species, they reported that lactose, inositol, and galactitol were important carbon sources for the separation of some of the species they tested. These investigators concluded that assimilation profiles can complement microscopic morphological features as a means to facilitate the identification of this group of fungi. In our study using the API 20C Yeast Identification System, we evaluated the usefulness of 19 carbohydrates. It was unfortunate that only a few authentic isolates of *C. elatum*, *C. resinae*, and *L. hoffmannii* were available for testing (Table 1). The evaluation of each of the 18 species against each of the other 17 species demonstrated that 13 species could be distinguished from 9 to 16 of the species tested (52.9 to 94.1%) (Table 3).

The results of the assimilation data obtained with the API 20C Yeast Identification System demonstrate its potential usefulness for distinguishing species of *Cladosporium* and *X. bantiana*. This is important because many *Cladosporium* species can morphologically resemble the medically important pathogens *C. carrionii* and *X. bantiana*, which in addition closely resemble each other. Morphological differentiation of *C. carrionii* and *X. bantiana* is based upon the length of conidial chains, shape and size of conidia, and the conidiophore shape (12). The thermotolerance test is a reliable means to differentiate these two species (12, 13). However, these tests are time-consuming and require great expertise. Moreover, it has been demonstrated that some isolates of saprobic *Cladosporium* spp. can also grow at 40°C, like isolates of *X. bantiana*, and that proteolytic and other physiological tests are unreliable for the differentiation of these fungi because the results are too variable (4).

Carbohydrate assimilations could be used to distinguish some species of *Phialophora* and also *L. hoffmannii* from the four species of *Phialophora* (Tables 2 and 5). This is valuable information that can be used in the identification of this difficult group of fungi which produce phialides and adelophialides (7). The results for the black yeasts are less encouraging. Of the 10 carbohydrates (Table 4) that were useful for differentiating the dematiaceous fungi studied, only melezitose provided a useful character to distinguish between *W. dermatitidis* and isolates of either *E. jeanselmei* or *E. spinifera*. Although this could be a very important aid to separate these two genera, assimilation profiles were too variable among the other three species of black yeasts tested (Tables 2 and 5).

In conclusion, the API 20C Yeast Identification System demonstrated a potential usefulness for the differentiation of some of the dematiaceous fungi. The system could be used to complement traditional morphological and thermotolerance studies. Testing additional isolates and taxa by using the API 20C system would provide more data that should allow less restrictive criteria for conversion of quantitative (percent) to qualitative (positive, negative, variable) results. An analysis of the API 20C yeast data base showed that all values of $\%SV_{max}$ increased and the mean value showed a twofold increase when conversion criteria were relaxed (positive, 90 to 100%; negative, 0 to 10%; variable, 11 to 89%). In fact, $\%SV_{max}$ for xylitol increased from 0 to 18%. It

seems probable that additional testing of dematiaceous fungi will enable greater separation with the 10 tests cited and may provide utility of the remaining 9 carbohydrates.

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