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Characterization and Differentiation of Filamentous Fungi Based on Fatty Acid Composition†

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Cellular fatty acid composition of 100 different filamentous fungi, including oomycetes, zygomycetes, ascomycetes, basidiomycetes, and sterile mycelia, was analyzed to determine if they can be differentiated from one another on this basis and how minor variations in culture temperature and age affect this characteristic. Many fungi were found to possess the same fatty acids but produced different relative concentrations of each. Some fungi differed in both the fatty acids produced and in the relative concentrations of others. Multivariate discriminant analysis demonstrated that all of the species included in this study had significantly different ($P < 0.001$) fatty acid profiles. Each of the three phyla from which representative species were analyzed and the sterile forms had distinctive fatty acid profiles. Significant differences in fatty acid composition were also found at the intraspecific level. Both culture temperature and age affected fatty acid composition in the fungi examined, but when these factors were held constant, variance in fatty acid composition was not a problem and fungal fatty acid profiles could be differentiated statistically.

As part of an effort to develop methods to facilitate the analysis of microbial communities in soil, we have been evaluating the use of cellular fatty acid profiles as a method to rapidly characterize and differentiate fungal isolates. When compared with standard morphological methods, this approach requires significantly less time and taxonomic expertise to characterize unknown isolates. Physiological data provided by fatty acid profiles, when used in conjunction with morphological characteristics, have the potential to increase the accuracy and resolution of sorting large numbers of fungal isolates.

Analysis of cellular fatty acid composition is now used routinely to characterize, differentiate, and identify genera, species, and strains of bacteria (14–17, 22, 23). Taxa are distinguishable by the fatty acids produced and their relative concentrations. Fungi produce fewer different fatty acids than bacteria do (11), and in the past, fatty acids have been considered to have little taxonomic value for this group of organisms. However, recent work has shown that cellular fatty acid profiles can be used to differentiate and identify genera, species, and strains of yeasts and yeast-like organisms (2, 4, 8, 21, 26).

The objectives of this work have been to (i) analyze the cellular fatty acid compositions of a diverse group of filamentous fungi to determine if they each have a unique fatty acid profile and can be differentiated on this basis, (ii) determine how minor variation in culture methods affects fatty acid composition in these fungi, and (iii) determine what ecological or taxonomic information can be obtained from fatty acid profiles. This technique is not intended to be used for identification of fungi but rather to characterize and differentiate large numbers of isolates.

MATERIALS AND METHODS

Fungi examined. A total of over 100 different fungi were included in this study (Table 1). Twenty-eight of these fungi are known species from culture collections and include oomycetes, zygomycetes, ascomycetes, basidiomycetes, and Fungi Imperfecti. The remaining fungi were isolated from soils on the Kellogg Biological Station (KBS) Long Term Ecological Research (LTER) site. Multiple isolates of many of the KBS soil fungi were obtained, and for many of these species, a number of isolates were examined so that possible intraspecific variation in fatty acid composition could be detected. Fungi isolated from KBS LTER soils were identified by the use of keys in the *Compendium of Soil Fungi* (6). All unidentified fungi were morphologically distinct from one another and the identified species.

Culture of fungi. All fungi were grown in 100 ml of malt broth (10 g of malt extract, 10 g of dextrose, 0.5 g of Bacto Peptone, each per liter) in 250-ml flasks in a slow-shake culture (125 rpm). Our standard method involved growth of cultures at room temperature (24 to 26°C) for 3 to 5 days or until enough biomass was produced for fatty acid extraction. Slower-growing fungi (some basidiomycetes) were cultured for up to 10 days. The growth period for each species was kept uniform to minimize variation in fatty acid composition.

To examine the effect of length of growth period of culture on fatty acid composition of individual species, a number of fungi were grown for 3, 4, and 5 days before harvest and fatty acid extraction. To determine the influence of temperature on fatty acid composition, an experiment was conducted in which a number of species were grown at 17 to 19, 24 to 26, and 31 to 33°C.

Extraction of fatty acids. Fatty acids were extracted from fungal tissue by a five-step procedure: (i) harvest—removal of fungal tissue from malt broth; (ii) saponification—lysis of cells and liberation of fatty acids from cellular lipids; (iii) methylation—formation of methyl esters of fatty acids; (iv) extraction—transfer of fatty acid methyl esters from an aqueous phase to an organic phase; (v) base wash—removal of free fatty acids and residual reagents from the organic extract. This method is thought to extract fatty acids from all cell components, including the plasma membrane, mitochondria, and lipid inclusions.

Fungal mycelium was harvested from broth by vacuum filtration through a Nytex filter (40- or 150-μm-pore-size mesh) in a Büchner funnel. The harvested biomass was rinsed with nanopure H_2O while still in the funnel and then placed on a lipid-free paper towel for several minutes to remove excess moisture. One-gram (wet weight) samples of fungal tissue were then placed into 4.0 ml of a saponification reagent (150 g of NaOH in 1 liter of 50% methanol) and homogenized with a Tissuemizer tissue grinder. The homogenate was then divided into four equal samples and placed in clean screw-top test tubes (13 by 100 mm; with Teflon cap liners). Subsamples were saponified in a 100°C water bath for 30 min and cooled in a room-temperature water bath. To methylate the liberated fatty acids, 2.0 ml of 54% 6 N HCl in methanol was added to each tube. Subsamples were then placed in an 80°C water bath for 10 min and immediately cooled to room temperature. To extract fatty acid methyl esters from the aqueous phase, 1.25 ml of 50% hexane–50% methyl *tert*-butyl ether was added to each tube, and the tubes were rotated end-over-end for 10 min. Next, the aqueous phase (bottom of tube) containing fungal debris was removed with a Pasteur pipette, and 3.0 ml of 1.2% NaOH in H_2O was added to each tube; the tube was then rotated end-over-end for 5 min. Finally, the organic phase (top of tube) containing the fatty acid methyl esters was removed from the tubes and placed in a crimp-top gas chromatography vial. When necessary, the samples were stored for up to 2 weeks at −20 or −70°C.

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TABLE 1. Fungi examined[a]

Fungal species or isolate
From culture collections
Oomycetes
Pythium sp.
Zygomycetes
Cunninghamella elegans
Mortierella isabellina
Rhizopus stolonifer
Syncephalastrum racemosum
Thamnidium ctenidium
Zygorhynchus moellerii
Ascomycetes
Aspergillus alliaceus
Chaetomium perlucidum
Emericella spectabilis
Gymnoascus sp.
Sordaria fimicola
Basdiomycetes
Bjerkandra adusta
Cyathus stercoreus
Gleophyllum trabeum
Lentinus edodus
Phanerochaete chrysosporium
Phlebia brevispora
Pleurotus ostreatus
Pleurotus sajor-caju
Poria placenta
Trametes versicolor
Deuteromycetes
Acremonium persicinum
Microdochium bolleyi
Paecilomyces lilacinus
Penicillium paraherquei
Penicillium restrictum
Penicillium simplicissimum
KBS LTER soil fungi
101 (*Penicillium verruculosum*)
102 (*Ramichloridium* sp.)
103 (*Phialophora malorum*)
104 (*Botrytis* sp.)
105
106 (*Volutella collectotricoides*)
107 (*Gonytrichum macrocladium*)
108 (*Phoma* sp. A)
109 (*Rhizoctonia* sp.)
110, 111, 112, 113, 114
115 (*Penicillium variabile*)
116, 117, 118, 119
120 (*Penicillium herquei*)
121, 124, 125
128 (*Phoma* sp.)
131, 134, 138, 141, 142, 145
211, 212, 213, 214, 215, 216, 217
218 (*Chrysosporium* sp.)
219
2110 (*Penicillium variabile*)
2111
2112 (*Chrysosporium* sp.)
2113
2114 (*Rhizoctonia* sp.)
2115, 2116, 2117, 2118, 2119, 2120, 2121, 2122
2123 (*Phoma* sp.)
2124, 2125
8231, 8232, 8234
8235 (*Trichosporiella cerebriformis*)
8236
8237 (*Penicillium frequentans*)
8238, 82310, 82311
82312 (Mycelia sterilia)
8239, 82310, 82311
82312 (Mycelia sterilia)
82313, 82314, 82315, 82316
82317 (Mycelia sterilia)
82318 (Mycelia sterilia)
82319, 82320, 82321, 82322
82323 (*Pythium* sp.)
82324 (*Acremonium percicinum*)
82325

[a] For all KBS LTER soil fungi, an isolate number is given and, where possible, a species identification. Isolate numbers not followed by a species name have not yet been identified; all unidentified isolates are morphologically different.

Analysis of fatty acids. Fatty acid extracts were analyzed by gas-liquid chromatography with the Microbial Identification System (MIS) developed by Microbial I. D., Inc. (Newark, Del.). This system was designed to analyze the fatty acid composition of unknown bacteria and to identify them by matching the fatty acid composition of an unknown organism to one of the fatty acid profiles in its computer database of known organisms. The MIS consists of a chromatographic unit (chromatograph, integrator, and autosampler) coupled to a computer system. The gas-liquid chromatograph is equipped with a nonpolar fused silica capillary column (20 m by 0.2 mm [inside diameter]; Hewlett-Packard, Wilmington, Del.) and a flame ionization detector. Data from the chromatographic analysis are sent to the computer system, where fatty acids are identified on the basis of their retention times relative to known standards and quantified relative to other fatty acids in the sample on the basis of peak width and area data. The system is calibrated with known fatty acid standards when it is started and after every 10th sample. Results of each sample analysis are printed out in a fatty acid composition report and also stored on a hard disk within the computer.

Samples were run through the gas chromatography column for 38 min, long enough for fatty acids up to 28 carbons long to pass through. The initial oven temperature was 170°C, and the final oven temperature of 300°C was reached 26 min after injection. The injector temperature was 250°C, and the detector temperature was 300°C. The flow rates of the carrier gases nitrogen, hydrogen, and air were 30, 30, and 400 ml min^{-1}, respectively.

Fatty acid nomenclature. Fatty acids are designated as the number of carbon atoms in the chain followed by a colon, the number of double bonds, and in some cases the configuration and location of the double bonds from the carboxyl end of the molecule. For example, 18:2(cis 9, 12) indicates linoleic acid which has an 18-carbon chain with two double bonds involving the number 9 and 12 carbon atoms. The presence and location of substituent groups on the carbon chain is designated accordingly, i.e., 18:0(3OH).

Generation of fatty acid profiles. Fatty acid profiles of individual fungi reported in this paper are based on analysis of the cellular fatty acid content of at least four independently grown cultures, and some profiles are compiled from as many as 25. The fungal biomass from each culture was divided into four subsamples; thus, analyses of at least 16 samples were used to generate each profile (a minimum of 10 subsamples was required to statistically differentiate isolates). The value for each fatty acid in a given profile is the mean from all analyzed cultures of that fungus. Concentrations of each fatty acid are expressed as a percentage of the total fatty acid content.

Comparison of fungal fatty acid profiles. A number of methods were employed to compare fatty acid profiles of individual fungi and to determine whether fungi can be differentiated on the basis of fatty acid composition. Cluster analyses (MIS software) were used to examine relationships among all of the fungi analyzed in this study and to indicate which fungi had the most-similar fatty acid compositions. The fatty acid profiles of the most-similar species were then compared by an index of similarity and a multivariate statistical test. To quantify the similarity in fatty acid composition of two fungi, an index-of-relationship value (9) was calculated by the following formula:

$$R_{xy} = (C_x/C_y)_1 (C_x + C_y/200)_1 + \ldots + (C_x/C_y)_n (C_x + C_y/200)_n$$

where x and y represent the fungi being compared, C is the mean fatty acid concentration expressed as a percentage of the total content for 1 through n fatty acids, and (C_x/C_y) represents the minor ratio of the fatty acids being used in this calculation. To determine if the fatty acid compositions of two fungi were statistically different, discriminant analysis was used to test the hypothesis that

TABLE 2. Fatty acid compositions of fungi from culture collections

Fungus	% Of total fatty acid content (mean ± SD)							
	12:0	14:0	15:0	16:0	16:1	17:0	17:1	18:0
Oomycetes								
Pythium sp.	0.38 ± 0.31	7.37 ± 3.28		13.07 ± 3.92	14.93 ± 8.70		0.38 ± 0.21	1.42 ± 0.93
Zygomycetes								
Cunninghamella elegans		1.02 ± 0.18		19.73 ± 0.73	1.56 ± 0.23			3.40 ± 0.66
Mortierella isabellina	T	1.44 ± 0.24		24.78 ± 0.40	4.77 ± 0.88			1.57 ± 0.30
Rhizopus stolonifer		1.14 ± 0.42	0.48 ± 0.24	21.01 ± 1.42	0.60 ± 0.42			9.19 ± 1.07
Syncephalastrum racemosum		0.64 ± 0.22	T	21.79 ± 3.37	0.95 ± 0.34			5.16 ± 0.89
Thamnidium ctenidium	0.61 ± 0.64	1.90 ± 0.98	0.29 ± 0.05	19.25 ± 1.22	2.67 ± 1.12	0.40 ± 0.11	0.39 ± 0.08	5.91 ± 2.05
Zygorhynchus moelleri	0.22 ± 0.08	1.81 ± 1.70		24.47 ± 3.38	3.68 ± 3.86			3.24 ± 0.64
Ascomycetes								
Chaetomium perlucidum			0.42 ± 0.33	18.95 ± 0.92	T			6.76 ± 0.45
Emericella spectabilis		T	16.0 ± 1.04	0.45 ± 0.28	T			4.53 ± 0.47
Gymnoascys sp.		0.27 ± 0.28	0.28 ± 0.28	18.44 ± 1.66	0.93 ± 0.09			4.25 ± 0.33
Sordaria fimicola		0.88 ± 0.10	T	26.59 ± 1.98	5.27 ± 0.77			2.30 ± 0.33
Basidiomycetes								
Cyathus stercoreus		0.33 ± 0.36	0.61 ± 0.68	15.66 ± 0.67	2.08 ± 1.68			2.15 ± 0.41
Gleophyllum trabeum			0.31 ± 0.42	14.57 ± 0.63				2.65 ± 0.92
Lentinus edodes		T	T	18.06 ± 0.36	0.71 ± 0.08	0.48 ± 0.11	0.43 ± 0.06	4.61 ± 0.65
Poria placenta				14.24 ± 1.12				0.80 ± 0.49
Phanerochaete chrysosporium		T	T	18.28 ± 0.69	0.63 ± 0.20	0.30 ± 0.23	0.41 ± 0.23	4.27 ± 0.71
Trametes versicolor		T	T	16.63 ± 2.34	T			1.60 ± 0.35
Deuteromycetes								
Acremonium persicinum				12.20 ± 7.91	16.80 ± 9.64			1.86 ± 1.20
Aspergillus alliaceus				15.74 ± 1.73				5.32 ± 1.44
Microdochium bolleyi		0.17 ± 0.04		16.93 ± 0.60	0.73 ± 0.13			3.36 ± 0.87
Paecilomyces lilacinus		0.51 ± 0.20		31.60 ± 0.20	1.92 ± 0.16			2.65 ± 0.15
Penicillium paraherquei		0.29 ± 0.16	0.80 ± 0.13	13.73 ± 0.26	0.46 ± 0.11	2.42 ± 0.23	2.67 ± 0.41	3.30 ± 0.50
Penicillium restrictum		0.34 ± 0.10	0.22 ± 0.14	19.52 ± 0.60	0.34 ± 0.03	0.43 ± 0.10	0.21 ±0.04	7.23 ± 0.51
Penicillium simplicissimum		0.49 ± 0.27	0.61 ± 0.29	16.93 ± 0.82	0.39 ± 0.05	0.77 ± 0.08	0.48 ± 0.04	5.90 ± 0.67

[a] In this column, the types of fatty acids are shown in parentheses.
[b] T, trace (less than 0.15%).

TABLE 2—*Continued*

% Of total fatty acid content (mean ± SD)									
18:1	18:2	18:3	20:0	20:1	20:2	20:4	22:0	24:0	Others[a]
									1.50 ± 1.18 (14:1), 4.59 ± 1.95 (20:5)
32.50 ± 3.84	9.27 ± 2.43	2.23 ± 3.14	T[b]	2.56	T				
48.15 ± 2.59	13.74 ± 0.07	9.92 ± 1.10		0.42 ± 0.03				0.15 ± 0.11	
45.09 ± 2.03	16.58 ± 1.44	4.38 ± 0.73							
37.55 ± 1.04	8.95 ± 0.35	18.56 ± 1.30	0.41 ± 0.12		T			0.41 ± 0.03	
51.09 ± 3.01	8.91 ± 1.28	9.10 ± 2.35	T						
33.02 ± 3.90	17.12 ± 3.56	14.61 ± 2.01	T	T					0.88 ± 1.03 (10:0)
34.97 ± 1.08	14.28 ± 1.68	14.48 ± 3.37		T	T				
7.38 ± 5.79	63.48 ± 3.71								
17.70 ± 9.41	52.18 ± 3.91								
40.24 ± 0.99	34.81 ± 2.90		T						
22.69 ± 3.04	39.82 ± 0.75								
1.88 ± 1.86	74.81 ± 1.79								
17.80 ± 3.16	62.96 ± 2.86								
22.77 ± 2.12	50.49 ± 2.24								
7.42 ± 1.19	76.54 ± 2.32								
20.76 ± 3.75	53.22 ± 4.80								
20.87 ± 11.73	55.59 ± 6.31				1.14 ± 0.44				
16.80 ± 1.32	48.58 ± 15.95								
20.28 ± 7.97	58.39 ± 5.96								
33.0 ± 1.26	45.15 ± 2.81		T						
38.24 ± 1.63	23.79 ± 1.80								
25.41 ± 0.23	40.46 ± 0.61								0.32 ± 0.04 (19:1)
34.49 ± 1.24	36.42 ± 1.62		0.24 ± 0.02						
25.98 ± 0.60	46.81 ± 1.36								

TABLE 3. Fatty acid compositions of different isolates of the same species

Fungal species and isolate no.	% Of total fatty acid content (mean ± SD)														
	12:0	14:0	15:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	20:4	22:0	24:0	Others
Penicillium verruculosum																		
KBS LTER 101-1		0.25 ± 0.24		28.07 ± 1.70	0.47 ± 0.31			5.86 ± 0.98	20.56 ± 2.47	44.19 ± 4.25								
KBS LTER 101-2		0.36 ± 0.19		28.08 ± 0.70	0.52 ± 0.28			5.73 ± 1.35	21.41 ± 2.26	43.61 ± 3.73								
KBS LTER 101-3		0.34 ± 0.19		28.67 ± 1.34	0.59 ± 0.19			5.70 ± 1.15	21.94 ± 2.84	42.56 ± 4.83								
KBS LTER 101-4		0.25 ± 0.23		28.30 ± 1.28	0.56 ± 0.24			5.97 ± 1.13	22.35 ± 2.52	41.05 ± 4.87								
KBS LTER 101-5		0.35 ± 0.19		28.93 ± 1.08	0.54 ± 0.29			5.68 ± 1.06	22.16 ± 2.12	41.96 ± 3.67								
KBS LTER 101-6		0.32 ± 0.20		27.42 ± 0.53	0.61 ± 0.08			5.25 ± 0.82	20.51 ± 1.88	45.54 ± 2.75								
KBS LTER 101-7		T[a]		26.91 ± 2.97	0.24 ± 0.34			5.12 ± 0.36	18.68 ± 2.80	49.00 ± 6.08								
Phialophora malorum																		
KBS LTER 103-1		0.48 ± 0.03		32.77 ± 0.49	1.49 ± 0.04			4.18 ± 0.26	41.50 ± 0.84	18.38 ± 0.74								
KBS LTER 103-2		0.57 ± 0.04		35.41 ± 0.52	2.09 ± 0.17			3.19 ± 0.25	38.85 ± 1.44	19.35 ± 1.03								
KBS LTER 103-3		0.47 ± 0.05		32.16 ± 1.48	1.66 ± 0.44			3.76 ± 0.73	40.10 ± 0.45	20.14 ± 1.60								
Rhizoctonia sp.																		
KBS LTER 109-1		0.32 ± 0.05		26.45 ± 3.04	1.12 ± 0.30	0.17 ± 0.15	T	6.14 ± 1.81	33.06 ± 3.53	31.82 ± 5.35		T						
KBS LTER 109-2		0.34 ± 0.09		26.51 ± 2.71	1.26 ± 0.31	0.19 ± 0.04	T	5.95 ± 1.03	36.41 ± 2.63	27.77 ± 3.91		0.19 ± 0.13						
Volutella colletotricoides																		
KBS LTER 106-1		1.14 ± 0.17		31.38 ± 2.30	2.18 ±0.28			2.69 ± 0.77	36.61 ± 1.33	25.23 ± 2.78								
KBS LTER 106-2		1.03 ± 0.12		31.06 ± 1.39	2.15 ± 0.20			2.60 ± 0.27	36.75 ± 2.13	25.78 ± 3.42								
KBS LTER 106-3		0.80 ± 0.18		27.92 ± 2.92	2.11 ± 0.33			3.07 ± 0.66	39.70 ± 1.58	25.80 ± 2.46								

[a] T, trace (less than 0.15%).

TABLE 4. Comparison of pairs of fungi with the most-similar fatty acid compositions

Fungi compared	Index-of-relationship value	Discriminant analysis (F statistic)
Pleurotus ostreatus vs. *Pleurotus sajor-caju*	0.96	12.375[a]
Gleophyllum trabeum vs. *Pleurotus ostreatus*	0.88	24.742[a]
Phoma sp. A vs. *Myrothecium* sp. B	0.92	33.441[a]
Rhizoctonia sp. A vs. *Penicillium funiculosum*	0.94	37.563[a]
Unidentified hyphomycete A vs. *Trichoderma koningii*	0.90	40.809[a]
Phoma sp. A vs. *Phoma* sp. C	0.91	48.719[a]
Acremonium sp. A vs. *Penicillium funiculosum*	0.87	64.620[a]
Rhizoctonia sp. A vs. *Acremonium* sp. A	0.88	85.999[a]
Gleophyllum trabeum vs. *Pleurotus sajor-caju*	0.91	90.478[a]
Myrothecium sp. A vs. *Penicillium funiculosum*	0.90	104.080[a]
Phoma eupyrena vs. unidentified coelomycete A	0.91	107.768[a]
Penicillium paraherquei vs. *Penicillium simplicissimum*	0.80	124.610[a]
Cunninghamella elegans vs. *Syncephalastrum racemosum*	0.86	142.227[a]
Bjerkandra adusta vs. *Cyathus stercoreus*	0.93	210.090[a]
Penicillium restrictum vs. *Gymnoascus* sp.	0.88	268.408[a]

[a] $P < 0.001$.

species x (fa_1, fa_2, fa_3. . .fa_n) = species y (fa_1, fa_2, fa_3. . .fa_n), where fa is fatty acid. Also, seven species within the genus *Penicillium* were compared by these methods to determine how well congeners could be differentiated. Finally, in four other species, the fatty acid profiles of a number of isolates were compared to detect possible intraspecific differences.

The ability of the MIS to differentiate fungi on the basis of fatty acid composition was evaluated by testing its accuracy at matching the fatty acid composition of individual fungal samples to the corresponding library fatty acid profile for that fungus. The MIS uses multivariate pattern recognition techniques to compare individual samples with established fatty acid profiles in the library database. Samples are matched to library profiles by using a covariance matrix, principal-component analysis, and calculations of cross terms (e.g., ratios between fatty acid concentrations).

RESULTS

Fatty acids present in fungi examined. The fungi analyzed contained fatty acids of carbon chain lengths ranging from 12 to 24. The most common and abundant fatty acids extracted were 16:0, 18:0, 18:1(cis 9), and 18:2(cis 9, 12), which often made up greater than 95% of the total fatty acid content of the dikaryotic fungi (Tables 2 and 3). These four fatty acids were present in all of the fungi examined in this study. In zygomycetes, oomycetes, and sterile forms, 18:3(cis 6, 9, 12), 18:0 (3OH), and 20:4 were also present in relatively high concentrations. Other fatty acids (12:0, 14:0, 15:0, 16:1, 17:0, 17:1, 20:0, 20:2, 20:5, and 24:0) were commonly detected but usually represented less than 5% of the total fatty acid content.

Species of fungi and sometimes strains within a species differed in the number and kinds of fatty acids present and in the relative concentrations of each type (Tables 2 and 3). The number of fatty acids extracted from a fungus ranged from 4 to 19. The relative concentration of an individual fatty acid ranged from less than 1% of the total fatty acid content to over 75%.

Differentiation of fungi based on fatty acid composition. Comparisons of pairs of fungi with the most similar fatty acid compositions (as indicated by cluster analysis) demonstrated that these species have statistically different fatty acid compositions (Table 4). Discriminant analysis showed that the fatty acid profiles of these species are different at high levels of significance ($P < 0.001$). Index-of-relationship values for these pairs of fungi ranged from 0.80 to 0.96. In other comparisons

TABLE 5. Comparison of seven *Penicillium* species based on fatty acid profiles

Species	Index-of-relationship value[a]						
	Penicillium verruculosum	*Penicillium variabile*	*Penicillium herquei*	*Penicillium frequentans*	*Penicillium paraherquei*	*Penicillium restrictum*	*Penicillium simplicissimum*
Penicillium verruculosum	1.00	0.85 (462.17)*	0.90 (434.85)*	0.82 (3,957.3)*	0.72 (1,104.2)*	0.73 (1,695.5)*	0.81 (475.66)*
Penicillium variabile		1.00	0.78 (257.62)*	0.89 (101.38)*	0.79 (177.92)*	0.81 (29.04)*	0.89 (81.42)*
Penicillium herquei			1.00	0.83 (1,110.5)*	0.67 (1,160.3)*	0.66 (981.77)*	0.79 (325.35)*
Penicillium frequentans				1.00	0.75 (3,236.6)*	0.78 (702.11)*	0.92 (737.99)*
Penicillium paraherquei					1.00	0.72 (630.55)*	0.78 (124.61)*
Penicillium restrictum						1.00	0.78 (407.90)*
Penicillium simplicissimum							1.00

[a] Results of the Hotelling-Lawley multivariate test are shown in parentheses (df = 10, 9). *, values are significant at the 0.001 probability level.

of representatives of different fungal phyla (data not shown), the index-of-relationship values were all less than 0.60. Within-phyla comparisons (other than those given in Table 4) all yielded values between 0.55 and 0.91.

Comparison of the seven *Penicillium* species demonstrated that all of these species also have distinct fatty acid profiles (Tables 2 and 5). The index-of-relationship values for these seven congeners ranged from a low of 0.66 to a high of 0.92 (Table 5). Results of multivariate statistical tests comparing these fatty acid profiles showed that even the most similar species (based on cluster analyses and index of relationship values) were statistically different, with high levels of significance ($P < 0.001$).

Examination of intraspecific differences in fatty acid composition indicated that different isolates of the same species can also have disparate fatty acid compositions and may be statistically differentiated on this basis (Tables 3, 6, and 7). Discriminant analysis revealed that our three isolates of *Phialophora malorum* and two isolates of *Rhizoctonia* sp. have different fatty acid compositions (Table 6). Comparison of three isolates of *Volutella colletotricoides* indicated that isolates 106-1 and 106-2 are not statistically different and 106-3 is distinct. Analysis of seven isolates of *Penicillium verruculosum* showed that one, 101-7, has a fatty acid composition that is significantly different from all others (Table 7). Isolates 101-1, 101-2, 101-3, 101-4, and 101-5 all have very similar fatty acid profiles as indicated by high index-of-relationship values and nonsignificant discriminant analysis results.

TABLE 6. Comparison of fatty acid compositions of different isolates of the same species

Isolates compared	Index-of-relationship value	Discriminant analysis (F statistic)[a]
Phialophora malorum		
KBS LTER 103-1 vs. KBS LTER 103-2	0.91	33.293*
KBS LTER 103-1 vs. KBS LTER 103-3	0.94	18.710*
KBS LTER 103-2 vs. KBS LTER 103-3	0.92	16.767*
Rhizoctonia sp.		
KBS LTER 109-1 vs. KBS LTER 109-2	0.92	13.296*
Volutella colletotricoides		
KBS LTER 106-1 vs. KBS LTER 106-2	0.98	0.904
KBS LTER 106-1 vs. KBS LTER 106-3	0.91	11.408*
KBS LTER 106-2 vs. KBS LTER 106-3	0.92	32.074*

[a] *, $P < 0.001$.

Tests of the MIS computer and software showed that it has the capability to differentiate fungal fatty acid profiles with a high degree of accuracy. The MIS correctly matched individual sample analyses of cultures of the seven *Penicillium* species to the corresponding library fatty acid profiles with an overall efficiency of 98% (Table 8). All samples of five of the seven species were correctly matched, and only one sample in each of the other two species was mismatched. In an additional test, of 200 randomly chosen sample analyses from the complete database of approximately 2,000 samples, 190 were correctly matched to the corresponding library fatty acid profile and 10 were mismatched, giving an overall accuracy of 95%. This level of precision also demonstrates the low variation in fatty acid composition among samples of the same fungus.

Effect of temperature on fatty acid composition. Our experiment demonstrates that the temperature at which a fungus is cultured affects its fatty acid composition (Tables 9 and 10). The degree to which fatty acid composition was altered by temperature, however, was dependent on the fungus. For example, index-of-relationship values comparing the fatty acid

TABLE 7. Comparison of seven isolates of *Penicillium verruculosum* based on fatty acid profiles

Isolate no.	Index-of-relationship value[a]						
	101-1	101-2	101-3	101-4	101-5	101-6	101-7
101-1	1.00	0.98 (1.646)	0.96 (1.671)	0.94 (0.426)	0.95 (0.708)	0.97 (3.415)*	0.91 (23.487)
101-2		1.00	0.97 (0.219)	0.95 (0.684)	0.97 (0.652)	0.96 (2.759)	0.90 (16.776)***
101-3			1.00	0.97 (1.722)	0.99 (0.675)	0.94 (2.709)	0.88 (13.029)***
101-4				1.00	0.97 (0.808)	0.92 (6.762)**	0.86 (22.027)***
101-5					1.00	0.93 (0.638)	0.87 (16.718)***
101-6						1.00	0.94 (31.063)***
101-7							1.00

[a] Results of the Hotelling-Lawley multivariate test are shown in parentheses (df = 6, 13). *, **, and ***, values are significant at the <0.05, <0.01, and <0.001 probability levels.

TABLE 8. Ability of the MIS to differentiate seven *Penicillium* species on the basis of fatty acid composition

Species	No. of individual samples subjected to test	No. of individual samples correctly matched to library entry	No. of individual samples incorrectly matched to library entry	% Accuracy[a]
Penicillium frequentans	8	8	0	100
Penicillium herquei	18	18	0	100
Penicillium paraherquei	12	12	0	100
Penicillium restrictum	8	7	1	87
Penicillium simplicissimum	14	13	1	93
Penicillium variabile	21	21	0	100
Penicillium verruculosum	32	32	0	100

[a] Overall accuracy, 98% (112 of 114 samples correctly matched).

compositions of *Cyathus stercoreus* in the three temperature treatments were between 0.93 and 0.98, indicating relatively small changes. In contrast, index-of-relationship values for the sterile fungus KBS LTER 8238 ranged from 0.46 to 0.67, attesting to major shifts in fatty acid composition. In the other six fungi included in this experiment, index-of-relationship values for between-treatment comparisons ranged from 0.72 to 0.93, with most values (12 of 18) between 0.80 and 0.89.

In general, the same fatty acids were present in a fungus when grown at different temperatures but their relative amounts were different. In all eight species, the relative amount of 16:0 fatty acid decreased with decreasing temperature, and in six of

TABLE 9. Fatty acid compositions of fungi grown at three different temperatures

Fungus	Treatment[a]	% of total fatty acid content (mean ± SD)							
		12:0	14:0	15:0	16:0	16:1	17:0	17:1	18:0
Aspergillus alliaceus	HT			0.67 ± 0.18	16.56 ± 0.34				5.14 ± 1.18
	RT				15.16 ± 0.31				5.45 ± 1.56
	LT				13.05 ± 2.25		0.45 ± 0.25		8.17 ± 1.06
Cyathus stercoreus	HT		0.43 ± 0.41	1.01 ± 0.23	17.02 ± 0.80	1.26 ± 0.31			2.31 ± 0.56
	RT		0.30 ± 0.38	1.03 ± 0.03	15.72 ± 0.18	1.41 ± 0.06			1.62 ± 0.10
	LT		0.63 ± 0.55	0.68 ± 0.20	15.86 ± 1.41	0.78 ± 0.40			1.72 ± 0.93
Phanerochaete chrysosporium	HT		T	0.49 ± 0.16	19.37 ± 0.98	0.27 ± 0.33	T	T	3.37 ± 0.66
	RT		T	0.30 ± 0.03	19.52 ± 0.61	0.63 ± 0.04	0.37 ± 0.03	T	4.65 ± 0.38
	LT				17.45 ± 0.32	0.71 ± 0.04	0.31 ± 0.34	0.37 ± 0.41	4.60 ± 0.19
Phialophora malorum (KBS LTER 103)	HT		0.75 ± 0.03		36.93 ± 1.04	1.63 ± 0.14			4.52 ± 0.12
	RT		0.50 ± 0.11		33.80 ± 2.44	1.53 ± 0.25			4.62 ± 0.15
	LT		0.34 ± 0.07		28.87 ± 1.80	1.40 ± 0.18			5.94 ± 1.40
Poria placenta	HT		0.63 ± 0.01	T	19.40 ± 0.25				0.86 ± 0.02
	RT		T	0.54 ± 0.01	15.15 ± 0.14		0.81 ± 0.02		0.90 ± 0.02
	LT			0.85 ± 0.02	14.10 ± 0.12				
Syncephalastrum racemosum	HT		1.04 ± 0.06	0.43 ± 0.02	28.54 ± 0.23	0.96 ± 0.05			3.67 ± 0.29
	RT		0.78 ± 0.13	T	23.59 ± 3.40	0.88 ± 0.10			4.82 ± 0.36
	LT		0.66 ± 0.08	T	21.89 ± 2.50	1.45 ± 0.09			4.32 ± 0.73
Zygorhynchus moelleri	HT	T	2.00 ± 0.13	T	31.81 ± 0.22	2.64 ± 0.11			2.99 ± 0.19
	RT	0.27 ± 0.05	1.97 ± 0.15	T	26.64 ± 0.74	3.13 ± 0.22			2.51 ± 0.19
	LT	0.33 ± 0.05	1.40 ± 0.34	T	22.70 ± 2.60	3.15 ± 0.23			2.74 ± 0.21
Mycelia sterilia (KBS LTER 8238)	HT		1.29 ± 0.02	T	24.66 ± 0.14	0.64 ± 0.07			5.01 ± 0.22
	RT		0.66 ± 0.11	T	19.07 ± 0.98	0.32 ± 0.06			5.11 ± 0.44
	LT		1.22 ± 0.28		14.97 ± 0.56	0.23 ± 0.03			6.73 ± 0.49
Mycelia sterilia (KBS LTER 82312)	RT		1.16 ± 0.25	T	16.34 ± 0.07	0.30 ± 0.01	T		5.17 ± 0.57
	LT		2.65 ± 0.07		18.63 ± 0.27	0.40 ± 0.01			4.83 ± 0.32

[a] HT, high-temperature treatment (31 to 33°C); RT, room-temperature treatment (24 to 26°C); LT, low-temperature treatment (17 to 19°C).
[b] In this column, the types of fatty acids are shown in parentheses.
[c] T, trace (less than 0.15%).

the eight species, the amount of 18:2 fatty acid increased with decreasing temperature. Changes in the other common and abundant fatty acids, 18:0 and 18:1, were inconsistent. In the sterile fungus KBS LTER 8238, the amount of 18:1 fatty acid decreased about 24% and that of 20:4 increased nearly 30% from the high-temperature treatment to the low-temperature treatment.

Effect of age of culture on fatty acid composition. The length of time a fungus was cultured also had an effect on its fatty acid composition (Tables 11 and 12). However, varying culture time by up to 48 h did not appear to cause major changes in any of the three species examined. Index-of-relationship values comparing fatty acid compositions in the different treatments were no lower than 0.85, and most were above 0.90 (Table 12).

The same fatty acids were present in a fungus over the time periods tested (with the exception of the appearance of small amounts of 14:0 fatty acid in *Penicillium paraherquei* after 4 days), but their relative amounts changed. Trends were difficult to detect from this limited sample size, but it appears that the amount of 18:1 fatty acid increased slightly with age and that of 18:2 fatty acid decreased slightly.

DISCUSSION

The results demonstrate that all of the species of fungi included in this study have disparate fatty acid compositions and can be differentiated from one another on this basis. Many of the fungi analyzed contained the same fatty acids but differed in the relative amounts of each; some fungi differed in both the kinds of fatty acids produced and in the amounts of others (Table 2). Multivariate statistical tests show that even the species with the most-similar fatty acid profiles are statistically different (Table 4). Our data also indicate that significant intraspecific differences in fatty acid composition may exist in filamentous fungi (Tables 3, 6, and 7). Analysis and comparison of a wide variety of yeast species and strains within species have previously demonstrated that these fungi can be distinguished from one another by fatty acid composition (1, 3, 13, 21). Results of the present study and the cited studies of yeasts suggest that most fungi have disparate fatty acid profiles and that this may be a reliable technique to characterize species and strains. Cellular fatty acid composition has already

TABLE 9—*Continued*

% of total fatty acid content (mean ± SD)									
18:1	18:2	18:3	20:0	20:1	20:2	20:4	22:0	24:0	Others[b]
19.49 ± 3.39	57.22 ± 5.87								
24.68 ± 3.65	53.67 ± 6.05								
18.60 ± 2.84	43.29 ± 3.81								14.95 ± 8.3 [18:1(w8)]
4.15 ± 1.05	72.89 ± 2.41								
3.54 ± 0.10	75.67 ± 1.12								
3.51 ± 1.21	76.10 ± 2.99								
18.16 ± 5.03	57.19 ± 8.29								
21.43 ± 2.14	51.39 ± 2.71								
14.29 ± 0.56	61.06 ± 0.30								
40.53 ± 0.88	13.74 ± 0.35								
40.41 ± 0.80	18.21 ± 3.06								
42.58 ± 0.39	20.56 ± 0.59								
8.32 ± 0.02	68.44 ± 0.27								
5.75 ± 0.04	75.00 ± 0.34								
5.57 ± 0.12	79.48 ± 0.02								
43.22 ± 2.70	11.21 ± 1.41	9.14 ± 1.49							
48.26 ± 3.53	9.71 ± 0.77	9.45 ± 0.56							
48.11 ± 0.98	10.00 ± 0.43	11.87 ± 1.44							
35.16 ± 0.93	10.42 ± 0.15	11.81 ± 0.47		T					
30.97 ± 1.76	15.69 ± 0.24	16.27 ± 2.13		T	T				
35.99 ± 3.83	13.85 ± 1.07	17.62 ± 2.28		T	T				
33.15 ± 0.51	5.63 ± 0.05	5.99 ± 0.09		1.88 ± 0.03	T	16.30 ± 0.22	0.25 ± 0.02	0.28 ± 0.02	
17.88 ± 2.77	7.08 ± 0.58	6.51 ± 0.67		2.27 ± 0.37	0.21 ± 0.02	31.84 ± 5.63	0.25 ± 0.01	0.27 ± 0.02	6.74 ± 0.59 [18:0(3OH)], 0.32 ± 0.17 (20:5)
9.12 ± 0.78	6.92 ± 0.41	8.50 ± 0.37		3.25 ± 0.44	0.52 ± 0.03	45.16 ± 3.07	0.23 ± 0.03	0.24 ± 0.01	
26.32 ± 1.50	7.93 ± 0.52	7.80 ± 1.00		2.50 ± 0.28	0.29 ± 0.06	23.59 ± 1.81	T	0.32 ± 0.02	4.63 ± 1.71 [18:0(3OH)], (20:5)
11.44 ± 0.23	7.06 ± 0.12	9.28 ± 0.18	1.06 ± 0.02	2.47 ± 1.16	0.32 ± 0.04	26.75 ± 0.25		T	7.06 ± 0.12 [18:0(3OH)], 5.41 ± 0.08 (20:5)

TABLE 10. Comparison of fatty acid compositions of fungi grown at different temperatures

Species	Index-of-relationship value[a]		
	HT-RT	RT-LT	HT-LT
Aspergillus alliaceus	0.89	0.72	0.72
Cyathus stercoreus	0.94	0.98	0.93
Phanerochaete chrysosporium	0.88	0.81	0.88
Phialophora malorum (KBS LTER 103)	0.91	0.89	0.82
Poria placenta	0.85	0.92	0.81
Syncephalastrum racemosum	0.85	0.93	0.83
Zygorhynchus moelleri	0.79	0.85	0.80
Mycelia sterilia (KBS LTER 8238)	0.61	0.67	0.46

[a] For details, see the text. HT-RT, high-temperature treatment (31 to 33°C)–room-temperature treatment (24 to 26°C) comparison; RT-LT, room-temperature treatment–low-temperature treatment (17 to 19°C) comparison; HT-LT, high-temperature treatment–low-temperature treatment comparison.

proven to be a valuable method in the differentiation and identification of bacteria (25).

Multivariate discriminant analysis can provide a powerful tool for statistical comparison of fungi based on fatty acid composition (2, 3, 13). All of the species compared by this method in our study were statistically different at the P of $<$ 0.001 level of significance. Discriminant analysis also revealed significant disparities in fatty acid composition among several morphologically indistinguishable isolates (Tables 6 and 7). As the number of replicate analyses and variables (fatty acids) used to generate a fatty acid profile are increased, so is the statistical power of this test. Index-of-relationship values (9) provide a simple and rapid method for preliminary comparison of the similarity of the fatty acid compositions of two fungi. These relationship values can be calculated with mean concentrations for each fatty acid in a profile. This index has a weakness, however, in that it does not give much weight to a fatty acid which is produced in small amounts in one fungus but not at all in the other. These minor fatty acids may be very important in differentiating fungi by discriminant analysis. The discrepancy in these two methods is illustrated by the lack of close correlation between the values given in Tables 4, 5, and 6. Data from our study indicate that two fungi with fatty acid profiles as similar as 0.97 based on the index of relationship may be statistically different at the P of <0.05 level of significance (Table 7). Species and strains with relationship values as high as 0.94 were statistically different at the P of <0.001 level. However, in our tests, all pairs of fungi with relationship values of 0.92 or less were statistically different at the P of <0.01 or <0.001 level.

Tests of the ability of the MIS multivariate pattern recognition software to match fatty acid compositions of individual fungal samples to corresponding profiles in the library database demonstrated that this system can differentiate fungi on the basis of fatty acid composition with a high degree of accuracy (98%) (Table 8). The efficiency of the MIS to distinguish between fatty acid profiles can probably be increased by expanding the library database and editing out faulty and outlying samples.

In general, the more closely related two fungi were taxonomically or phylogenetically, the more similar they were in terms of fatty acid composition. For example, in comparisons of isolates of the same fungal species, index-of-relationship values ranged from 0.86 to 0.99 and comparisons of different species within the same genus yielded values from 0.66 to 0.96. Index-of-relationship values for two species within the same subphylum (ascomycetes or basidiomycetes) fell between 0.55

TABLE 11. Fatty acid compositions of fungi cultured for different lengths of time

Fungus	Growth period (days)	% Of total fatty acid content (mean ± SD)								
		12:0	14:0	15:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2
Penicillium paraherquei	3		0.47 ± 0.19	13.66 ± 0.29	0.33 ± 0.14	1.47 ± 0.24	1.46 ± 0.29	3.21 ± 0.57	24.05 ± 2.06	53.84 ± 2.79	
	4	T[a]	0.61 ± 0.06	13.61 ± 0.27	0.29 ± 0.12	1.88 ± 0.38	1.87 ± 0.35	3.81 ± 0.67	25.79 ± 0.64	49.62 ± 1.88	
	5	0.22 ± 0.01	0.68 ± 0.03	13.64 ± 0.10	0.33 ± 0.01	1.98 ± 0.06	2.26 ± 0.16	3.24 ± 0.19	24.64 ± 0.59	50.55 ± 0.80	
Acremonium persicinum	4			18.13 ± 0.08	0.38 ± 0.33			2.65 ± 0.19	24.04 ± 0.48	54.80 ± 0.48	
	5			18.90 ± 0.15	0.31 ± 0.31			2.88 ± 0.32	24.38 ± 0.14	53.52 ± 0.17	
	6			19.04 ± 0.21	0.87 ± 0.05			2.40 ± 0.20	31.09 ± 0.26	46.61 ± 0.31	
Microdochium bolleyi	3			18.18 ± 3.94	0.76 ± 0.16			2.84 ± 0.60	26.98 ± 0.51	50.27 ± 10.44	
	4			17.85 ± 0.68	0.66 ± 0.11			3.25 ± 0.58	32.62 ± 0.23	45.14 ± 1.60	
	5			16.62 ± 0.91	0.87 ± 0.09			2.32 ± 0.17	31.88 ± 0.30	47.50 ± 1.12	

[a] T, trace (less than 0.15%).

TABLE 12. Comparison of fatty acid compositions of fungi grown for different lengths of time

Species	Index-of-relationship values for comparison[a]:				
	1	2	3	4	5
Acremonium persicinum			0.97	0.87	0.85
Microdochium bolleyi	0.88	0.94	0.90		
Penicillium paraherquei	0.91	0.94	0.93		

[a] Comparisons: 1, 3-day-old vs. 4-day-old cultures; 2, 3-day-old vs. 5-day-old cultures; 3, 4-day-old vs. 5-day-old cultures; 4, 4-day-old vs. 6-day-old cultures; 5, 5-day-old vs. 6-day-old cultures. For details, see the text.

and 0.96; for different species within the same phylum, they were between 0.47 and 0.96. Species of fungi from different phyla had the least-similar fatty acid compositions, and these comparisons gave index-of-relationship values between 0.21 and 0.59.

Each of the three phyla of fungi from which representative species were analyzed (Oomycota, Zygomycota, and Dikaryomycota) had unique characteristics by which they could be distinguished from one another. The two strains of *Pythium* sp. (Oomycota) produced 12 and 15 different fatty acids, including 20:5 fatty acid that was not detected in any of the zygomycetes or dikaryotic fungi. Our data were in agreement with previous literature in that only the lower fungi were found to produce arachidonic acid (20:4). All of the zygomycetes examined produced a greater number of fatty acids than the dikaryotic fungi (ascomycetes and basidiomycetes) did, and 18:3 fatty acid was not produced by any of the dikaryotic fungi. Zygomycetes, in general, produce fewer fatty acids than the *Pythium* spp. and never contain 20:5 fatty acid. Dikaryotic fungi were composed of the fewest different fatty acids of any group (between 5 and 10 [usually, 6 or 7]) and never produced 20:5 or 18:3 fatty acid. No characteristic in fatty acid composition was found by which ascomycetes could be consistently distinguished from basidiomycetes. The five sterile forms (mycelia sterilia) analyzed produced the greatest diversity of fatty acids (between 15 and 20), 22:0 fatty acid, which was not found in any other taxon, and relatively high amounts of 18:0(3OH) fatty acid and 20:4 fatty acid, which were produced only in small amounts or not at all by other fungi. Studies of bacteria have, in general, shown that phenotypic relationships among organisms as indicated by fatty acid composition are in agreement with phylogenetic associations based on DNA and rRNA homology (7, 16, 18).

When employing fatty acid profiles to differentiate or compare fungi, it is important to minimize sources of variation in fatty acid composition from culture conditions. As with most other organisms, temperature is known to affect the fatty acid composition of fungi (12, 24), and in this study, significant disparities in fatty acid composition were observed with 7°C differences in culture conditions. In general, as the temperature of culture conditions is decreased, the degree of unsaturation in fatty acids is increased (5, 12, 20). In the present study, variation in fatty acid composition was minimal when fungi were grown at room temperature (24 to 26°C) and was not a problem in the statistical differentiation of fatty acid profiles. However, when fatty acid profiles are used to characterize and/or differentiate fungi, it is important to grow cultures at the same temperature and minimize deviation from that temperature. Results of this study indicate that good results can be obtained when the culture temperature is maintained within a range of 3°C.

Culture age is also known to influence fatty acid composition in fungi (12, 24), and this phenomenon was observed over the 48-h period we examined in our experiment. Smit et al. (19) reported that in the yeasts they studied, a high degree of variation in relative fatty acid concentrations was observed during the exponential and early stationary growth phases and relatively stable fatty acid concentrations were obtained from cultures harvested in the late stationary phase. Our results are consistent with this observation in that comparisons of fatty acid compositions of fungi harvested after 4 days of growth with those harvested after 5 days were more similar than those of 3- and 4-day-old cultures. These results indicate that to minimize variation among cultures of a particular fungus, they should be harvested during the late stationary growth phase.

The fatty acid composition of a fungus can provide valuable taxonomic and physiologic information as to its relationship with other fungi that may not be available from morphologic characteristics. This information is especially useful at the subspecies level, where morphological disparities are limited, and for fungi such as mycelia sterilia and vesicular-arbuscular mycorrhizal endophytes, in which morphological differentiation is minimal. For example, fatty acid analyses of several vesicular-arbuscular mycorrhizal fungi (10), which have no known sexual stage and are tentatively placed in the phylum Zygomycota, have revealed that these fungi have fatty acid profiles more similar to those of Protoctistan fungi than other zygomycetes. Examination of the fatty acid composition of four morphologically indistinguishable mycelia sterilia isolates from soil in this study indicated that all four are physiologically different fungi. Comparisons of the fatty acid profiles of these sterile forms by the index of relationship yielded similarity values between 0.24 and 0.57. Fatty acid analysis revealed that one of the seven morphologically identical isolates of *Penicillium verruculosum* examined in this work was physiologically distinct from the others. This isolate also had a lower growth rate in malt broth and on solid media than the other six and is probably a divergent strain of this fungus.

Individual species and strains of bacteria have been shown to have characteristic fatty acid profiles and are now being identified on this basis (14, 17, 23). This method can also be used for the identification of yeasts (8, 13, 21, 26). Results of the present study suggest that fatty acid composition has potential for use in the identification of filamentous fungi as well. In initial trials, unknown fungal isolates from KBS LTER soils could be reliably matched to (and identified as) or differentiated from previously characterized isolates in our library database of fatty acid profiles of KBS LTER soil fungi. Subsequent analyses of morphological and cultural traits, in most cases, verified our identifications.

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