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COMPLETE FATTY ACID COMPOSITION OF SELECTED ASPERGILLUS SPECIES

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The goal of this work was to perform a detailed analysis of fatty acids from Aspergillus species in a search for biomarkers to differentiate the species. In the course of this work, we isolated various classes of lipids (neutral lipids, glycolipids, phospholipids and lipopolysaccharides) from six Aspergillus species by chloroform/methanol extraction and multiple column fractionations. Further separation of structurally different fatty acids from phospholipids was also performed. The identities of isolated fatty acids from all lipid fractions were tentatively assigned using gas chromatography/mass spectrometry (GC/MS). The experimental methodology was found to be suitable for complete characterization of the fatty acids from various cellular sources. While the methods used in this work are standard techniques, the detail obtained in the fatty acid composition of each of the Aspergillus species is much greater than previously described.

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Introduction

Aspergillus species belong to ascomycetes within the subdivision deuteromycota because most do not produce sexual spores. Asexual spores are formed by the aerial mycelium and in *Aspergillus* species they are called conidiospores [1]. These spores can be easily disseminated through the air and, therefore, inhalation is a common route of transmission into the body and a cause of pulmonary infection. Some *Aspergillus* species act as opportunistic pathogens causing numerous diseases, ranging from allergic reactions to fatal infections that spread from the lungs to other body tissues and organs [1].

The rate of fungal infections in the clinical setting has been increasing over the last several years due to the larger number of immunocompromised patients [2]. Aspergilosis is one of the most common infections in this group of patients and very serious infections occur, especially in lung transplant recipients [3].

The most frequent fungal infections, approximately 90 % [4], are caused by *Aspergillus fumigatus*. Other *Aspergillus* species causing disease are less common but include, for example, *Aspergillus terreus* infections associated with high mortality rate [5]. Also *Aspergillus niger,* while not a frequent pathogen, has been isolated from the gastrointestinal tract contaminated, potentially, from a hospital water distribution system [6].

Aspergillus species are most commonly identified by morphological characteristics and, therefore, laboratory growth is necessary to identify the species. Other conventional methods currently used in clinical laboratories for diagnosis of fungal infections include antigen detection, detection of fungus-specific metabolites, detection of cell wall components, detection of fungus-specific nucleic acids, and DNA-based methods such as polymerase chain reaction (PCR) and modifications [7].

Alternative for classical microbiological techniques for microorganism detection in a clinical setting are analytical chemistry methodologies. Gas chromatography/mass spectrometry (GC/MS) was employed as a general, sensitive and fast bioanalytical technique by many investigators for taxonomical classification of microorganisms. The greatest advantage of GC/MS microbiological applications was its universality, which allows analysis of all fungal pathogens of interest. Gas chromatography methodology is commercially available for chemotaxonomic classification of yeast and bacteria based on the fatty acid methyl esters (FAMEs) and is distributed under the name of MIDI (Microbial Diagnostics, Inc. from Newark, Delaware) [8]. GC/MS determination of fatty acid composition from microorganisms has been shown to be a fast, reliable and convenient method for the determination [9,10].

Analysis of the lipid composition of *Aspergillus niger* has been performed previously, but the results show only the weight percentage of fatty acids from C16 to C18 in chain-length, with different degrees of unsaturation, and the ratios

between the saturated and unsaturated fatty acids [11]. Another study has described fatty acids from lipids of cell-wall components of *Aspergillus fumigatus* and the results also showed only fatty acids from C16 to C18 with different degrees of saturation [12]. In the case of *Aspergillus nidulans,* determination of the fatty acid composition was described [13], and provided a more detailed analysis of the fatty acid composition than the earlier studies described, with fatty acids from C13 to C24 and different degrees of saturation being identified during growth of the fungi. Other studies only reported analysis of total lipids in different *Aspergillus* species [14,15].

In this work we investigated the fatty acid composition of six different *Aspergillus* species: *A. versicolor*; *A. niger, A. terreus, A. clavatus, A. fumigatus,* and *A. nidulans.* The fatty acids were isolated from different cellular sources (neutral lipids, glycolipids, phospholipids, and lipopolysaccharides) and the phospholipids were separated into functional classes and characterized by GC/MS. The composition of lipids was observed in greater detail than that reported in existing literature. The fatty acid composition characteristics (quantitative and qualitative) were also used for taxonomical classification of individual *Aspergillus* species.

Materials and Methods

Sample Description

The following *Aspergillus* fungal cultures were purchased from ATCC (Manassas, VA, USA): *A. versicolor (*9577*)*: ATCC medium 336; *A. niger (*9642-U*)*: ATCC medium 336; *A. terreus (*1012*)*: ATCC medium 325; *A. clavatus (*9192*)*: ATCC medium 325; *A. fumigatus (*96918*)*: ATCC medium 325; *A. nidulans (*10074*)*: ATCC medium 336. Growth media: ATCC medium 336 (potato dextrose agar), ATCC medium 325 (malt extract agar-Blakeslee's formula).

Extraction procedure

Aspergillus specimens (50 mg) were dissolved in phosphate buffer (pH = 7.4) (1 ml) and extracted with methanol:chloroform (1:1; v/v) (2 ml). The neutral lipids, glycolipids, phospholipids and lipopolysaccharides were separated by solid phase extraction (SPE) column described by Zelles [16]. After saponification by mild alkaline methanolysis and acidic hydrolysis, the free fatty acids were liberated and transesterified to FAMEs. The structurally different fatty acids were also separated on SPE columns [16]. Three different types of columns were used during the separation: SPE-Si (silica) columns for separation of neutral lipids, glycolipids and

phospholipids; SPE-NH_2 (aminopropyl) columns for separation of unsubstituted, hydroxy substituted and unsaponifiable fatty acids from phospholipids; and SPE-SCX (benzensulfonyl) columns for separation of saturated, monounsaturated and polyunsaturated fatty acids from phospholipids. Different fatty acids were washed out of the SPE columns using various organic solvents to match their relative polarity. At this point, the separation was a sample preparation step prior GC/MS analysis, so no quantitative measurements were done during the column separation. Fractions containing saturated FAMEs (SATFAs), monounsaturated FAMEs (MUFAs), polyunsaturated FAMEs (PUFAs) and unsaponifiable FAMEs with a non-ester bond in the phospholipid (UNSFAs) were dissolved in ethyl acetate (30 μl) and analyzed directly by GC/MS. Fatty acids liberated from neutral lipids, glycolipids and the lipopolysaccharides were derivatized using BSTFA (*N,O,*-bis(trimethylsilyl)trifluoroacetamide) with 1 % TMCS (trimethylchlorsilane) (25 μl) and pyridine (5μl) (Pierce Biotechnology, Inc. Rockford, IL,USA). The samples were heated for 1 hour at 90 °C, cooled, and injected into the GC/MS. The same derivatization procedure was used for fractions containing hydroxy-substituted FAMEs with an ester bond in phospholipids (PLOH) and hydroxy-substituted FAMEs with an unsaponifiable ester bond in phospholipid (UNSOH) in order to derivatize hydroxy groups present in FAMEs; these derivatives were also analyzed by GC/MS.

The peaks representing FAMEs were identified using the NIST-MS Search 2.0 spectral library (Agilent Technologies, Palo Alto, CA, USA) and were also compared with a commercial standard of fatty acids (Supelco 37 Component FAME Mix; Supelco, Bellefonte, PA, USA).

Experimental GC/MS

FAMEs were analyzed by GC/MS (HP5890/HP5970) using a fused silica capillary column SPB-5 (30 m × 0.25 mm × 0.25 μm, Supelco, Bellefonte, PA, USA). Each sample (1 ml) was injected by an autosampler (HP 6890 series). The initial temperature on the column was 70 °C and was held constant for 2 minutes and a temperature gradient of 10 °C min^{-1} was finished at 280 °C (held for 7 min). The detector temperature was 290 °C and the injector temperature was 250 °C. Ultra-pure helium was used as a carrier gas (1ml min^{-1} flow rate). The ionization energy was 70 eV. All measurements were conducted in triplicate (the same sample injected three times) to ensure reproducibility and the mean values of the fatty acid levels were used for relative quantitation. Relative quantitation results were expressed as percentages of individual fatty acids compared to the base peak (100 %). The absolute concentrations of individual fatty acids were not determined since the relative ratios between fatty acids were used for the classification as opposed to absolute fatty acid concentration. The absolute amounts can vary

between individual species due to culturing conditions and, thus, relative concentrations are usually used instead. All the fatty acids were measured at the same response factor.

Results

Using the previously described extraction procedure, we analyzed the fatty acid components of the following fungi: *A. versicolor*; *A. niger, A. terreus, A. clavatus, A. fumigatus,* and *A. nidulans.* The main objective was to identify differences in the fatty acid components in each fraction that are characteristic of the individual species of *Aspergillus*. Because the fatty acid composition varies with the conditions used to culture the organisms [17], the values reported in this paper are valid only for the described growth conditions.

Neutral Lipids (summarized in Table I)

The neutral lipid fraction was found to contain fatty acids from C9 to C24 in chain-length with different degrees of unsaturation and to posses cyclopropyl-, dicarboxylic, hydroxy- and oxo- fatty acids. Even though this fraction contained a total of 44 different fatty acids, the differences between individual fungi were, in general, limited to variations in relative percent (RP) of the individual fatty acids. There were, however, a few unique components: nonanoic acid (C9:0) is present only in *A. fumigatus* and *A. clavatus*; methyl-dodecanoic acid (C12:0 met) in *A. versicolor* and *A. nidulans*; 9,10-epoxyoctadecanoic acid (C18:0 oxo) in *A. clavatus* and 9,10-dihydroxy-, and octadecanedioic acid (C18:0 (2OH) diCOOH) in *A. niger*.

By comparing the results presented in Table I, the fatty acid present in each of the *Aspergillus* species could be observed. The fatty acid profiles of *A. versicolor* and *A. nidulans* show the same components, but differ in the percent composition and can, thus, be differentiated.

The major fatty acid in all the species was hexadecanoic acid (C16:0) and, therefore, this component was the base peak (100 relative percent (RP)) in the chromatogram. The *A. niger* profile has the lowest number of fatty acids, which can be used to differentiate it from the other *Aspergillus* species. Also the amount of some fatty acids in *A. niger* were different from the other species. For example 9,10,12,13,18-pentahydroxyoctadecanoic acid (C18:0 (5OH)) was present in 18.45 RP, while in the other species it was from 0.62 to 3.19 RP; docosanoic acid (C22:0) occured in 24.15 RP, which was much higher then observed in the other species (3.57 – 6.50 RP); and, finally, 2,3-dihydroxypropyl octadecanoate (1-monostearin) was present in 14.77 RP in *A. niger* and in the other species was

Table I Relative percent (RP) of fatty acids in the *Aspergillus* species in the neutral lipid fraction. Values in italics indicate the major differences between the species. The same emphasis is used in all tables

Fatty acids	*Aspergillus versicolor*	*Aspergillus terreus*	*Aspergillus nidulans*	*Aspergillus fumigatus*	*Aspergillus clavatus*	*Aspergillus niger*
C9:0	-	-	-	*0.52*	*0.65*	-
C9:0 oxo	0.27	0.59	0.61	0.65	1.09	-
C9:0 diCOOH [a]	0.58	0.43	0.77	-	1.24	-
C10:0	0.27	0.44	0.56	0.75	0.91	-
C12:0	10.23	4.53	17.46	10.93	12.69	13.87
C12:0 met [b]	*0.38*	-	*0.35*	-	-	-
C13:0	0.64	0.56	0.82	0.96	1.12	-
C13:0 met [b]	1.28	1.28	1.16	1.05	1.70	-
C14:1	6.71	5.69	7.26	6.91	7.55	-
C14:0	50.30	43.82	56.42	53.46	55.93	19.61
C14:0 met A [bc]	2.31	2.34	2.65	2.39	2.72	-
C14:0 met B [bc]	6.12	6.49	5.79	6.27	7.77	-
C15:1	1.10	1.19	0.91	0.82	1.53	-
C15:0	9.06	10.06	9.62	9.61	11.27	6.33
C15:0 met [b]	2.08	2.73	2.52	2.46	2.82	-
C16:1	11.04	12.10	14.16	12.02	12.13	-
C16:0	100.00	100.00	100.00	100.00	100.00	100.00
C16:0 met A [bc]	3.46	4.60	3.74	3.65	4.91	-
C16:0 met B [bc]	3.54	4.69	4.05	3.69	4.12	-
C17:0 cyc [e]	1.94	2.84	3.07	2.97	2.35	-
C17:0	4.88	6.62	6.11	5.91	7.02	5.86
C17:0 met [b]	1.12	1.34	1.20	1.13	1.49	-
C18:3	0.22	0.69	0.42	-	0.35	-
C18:2 A [c]	11.84	27.41	18.63	16.91	11.36	3.90
C18:2 B [c]	2.07	3.18	2.33	2.30	2.82	-
C18:2 C [c]	2.00	2.68	2.21	1.90	3.29	-
C18:1 A [c]	80.92	89.95	84.94	78.51	81.17	24.00
C18:0	47.59	54.75	47.97	43.60	53.05	75.42
C18:0 (9,10)diOH [d]	2.49	3.68	2.88	1.18	3.60	12.32
C18:0 (5OH) [d]	0.62	3.19	1.73	1.13	2.13	*18.45*
C18:0 oxo	-	-	-	-	*1.10*	-
C18:0 (2OH) diCOOH [ad]	-	-	-	-	-	*13.75*
C19:0 cyc [e]	0.98	2.02	1.42	1.14	1.51	-
C19:0	0.89	1.47	1.04	0.72	1.39	-
C20:4	0.72	1.49	0.74	0.68	0.85	-
C20:3	0.59	1.38	0.65	0.60	0.88	-
C20:1	1.62	2.89	1.43	1.24	1.77	-
C20:0	1.61	2.97	2.27	1.34	2.28	4.25
C21:0	0.67	1.46	0.63	-	0.65	-
C22:1	1.32	2.81	1.51	0.65	1.80	-
C22:0	4.68	6.50	5.28	3.57	5.91	*24.15*
C23:0	0.65	0.97	0.99	0.59	0.55	-
C24:0	0.68	0.82	1.07	0.72	0.75	-
1-monostearin	1.19	1.55	1.63	1.41	1.53	*14*

[a] diCOOH – dicarboxylic acid; [b] met – methyl group (unknown position); [c] A, B, C – different positions of methyl group or double bond; [d] (OH) – hydroxyl group (known or unknown position); [e] cyc – cyclopropyl group in chain; C9:0 oxo – 9-oxononanoic acid; C18:0 oxo – 9,10-epoxyoctadecanoic acid; 1-monostearin – 2,3-dihydroxypropyl octadecanoate

from 1.19 to 1.63 RP. Other similarities and differences can be gleaned by examination of the data in Table I. Thus, while the neutral lipid fraction was the most complex of all the fractions, only variation in the RP of the individual fatty acids was observed, at least in most cases, and the neutral lipids could not be used to differentiate the species, with some minor exceptions.

Glycolipids (summarized in Table 2)

The glycolipid fraction contained saturated fatty acids from C9 to C24 in chain-length, with some components being hydroxy- and/or oxo-substituted. There were significant differences in the composition of fatty acids between all of the species. Some fatty acids were unique or present only in two *Aspergillus* species; 9-oxo-nonanoic acid (C9:0 oxo) in *A. versicolor* and *A. niger*; methyl-tetradecanoic acid (C14:0 met B) and methyl-pentadecanoic acid (C15:0 met) in *A. versicolor* and *A. nidulans*; heneicosanoic acid (C21:0) in *A. nidulans*; 2-hydroxydocosanoic acid (C22:0 (OH)), 2-hydroxytricosanoic acid (C23:0 (OH)), and 2-hydroxy-tetracosanoic acid (C24:0 (OH)) in *A. clavatus*.

Table II Relative percent (RP) of fatty acids in the *Aspergillus* species in the glycolipid fraction.

Fatty acids	*Aspergillus versicolor*	*Aspergillus terreus*	*Aspergillus nidulans*	*Aspergillus fumigatus*	*Aspergillus clavatus*	*Aspergillus niger*
C9:0 oxo	*1.79*	-	-	-	-	*6.97*
C12:0	10.15	3.10	10.14	3.21	18.08	38.38
C14:0	25.73	21.26	17.97	16.11	11.43	28.05
C14:0 met B [bc]	*3.75*	-	*0.87*	-	-	-
C15:0	5.52	5.66	4.33	4.8	6.01	7.19
C15:0 met [b]	*1.71*	-	*2.24*	-	-	-
C16:0	100.00	100.00	100.00	100.00	100.00	100.00
C16:0 met B [bc]	4.38	-	3.33	4.01	3.47	-
C17:0	4.14	3.55	3.83	3.23	4.13	3.82
C18:0	43.74	45.33	54.72	54.74	74.09	59.78
C18:0 (OH) [d]	-	32.36	13.77	4.25	10.69	-
C18:0 (9,10)diOH [d]	1.31	-	2.18	2.03	4.57	-
C20:0	2.70	-	-	3.70	4.10	-
C21:0	-	-	*2.05*	-	-	-
C22:0	28.93	37.43	53.11	35.70	89.61	29.50
C22:0 (OH) [d]	-	-	-	-	*1.66*	-
C23:0	6.96	11.39	22.48	9.09	24.39	-
C23:0 (OH) [d]	-	-	-	-	*4.66*	-
C24:0	3.84	5.97	15.23	5.71	16.15	-
C24:0 (OH) [d]	-	-	-	-	*2.34*	-
1-monostearin	6.58	6.21	17.08	9.99	51.05	39.52

[b] met – methyl group (unknown position); [c] A, B, C – different positions of methyl group or double bond; [d] (OH) – hydroxyl group (known or unknown position); C9:0 oxo – 9-oxononanoic acid; 1-monostearin – 2,3-dihydroxypropyl octadecanoate

The major fatty acid was hexadecanoic acid (C16:0), which was assigned as the base peak (100 RP) in the chromatogram. There were also significant differences in the RP of the fatty acids present in each species of *Aspergillus*. The most pronounced changes were observed in association with the following fatty acids: dodecanoic acid (C12:0) from 3.10 to 38.38 RP; hydroxyoctadecanoic acid (C18:0 (OH)) from 4.25 to 32.36 RP; docosanoic acid (C22:0) from 28.93 to 89.61 RP; 2,3-dihydroxypropyl octadecanoate (1-monostearin) from 6.21 to 51.05 RP.

Unsubstituted Fatty Acids from Phospholipids (summarized in Table III)

Unsubstituted fatty acids included three fractions: saturated fatty acids (SATFAs) ranging from C12 to C20 in chain-length; monounsaturated fatty acids (MUFAs) ranging from C16 to C20 in chain-length, and polyunsaturated fatty acids (PUFAs) ranging from C8 to C20 chain-length.

Differences were observed in the composition of fatty acids in all species, including SATFAs. For example dodecanoic acid (C12:0) was only present in *A. terreus* and *A. nidulans* while eicosanoic acid (C20:0) was only present in *A. versicolor* and *A. clavatus*. In turn, *A. versicolor* could be distinguished from *A. clavatus* by the presence of tetradecanoic acid (C14:0). Examination of SATFAs indicated that each of the *Aspergillus* species can be unambiguously differentiated from the others.

Information from the other two groups of fatty acids (MUFAs and PUFAs) was complementary to the SATFAs and provided more accurate determination of the *Aspergillus* species. The composition of fatty acids in these two groups did not allow differentiation of the *Aspergillus* species from each other even though some compounds were present only in some species. For example nonadecenoic acid (C19:1) (MUFAs) was only present in *A. nidulans* and *A. clavatus* but the composition of fatty acids in these two species was the same and therefore cannot be used for their differentiation. In PUFAs, 8-oxooctanoic acid (C8:0 oxo) was observed only in *A. versicolor* and *A. clavatus*, but these species could not be distinguished from each other, and the pattern of PUFAs did not permit differentiation of the species.

The major fatty acid found in SATFAs fraction was octadecanoic acid (C18:0), which was considered as the base peak (100 RP) in the chromatogram. The amounts of the fatty acids were different in all the species, with the largest difference being observed in the RP of hexadecanoic acid (C16:0) where the percentage ranged from 40.57 to 97.56 RP.

The MUFAs fraction had octadecenoic acid (C18:1 A) as a major fatty acid and is the base peak (100 RP) in the chromatograms of all the species. There were differences observed in the relative percentage of fatty acids of different *Aspergillus* species, but the changes were not sufficient to allow discrimination of

Table III Relative percent (RP) of fatty acids in the *Aspergillus* species in the phospholipid fraction containing unsubstituted FAMEs

	Fatty acids	*Aspergillus versicolor*	*Aspergillus terreus*	*Aspergillus nidulans*	*Aspergillus fumigatus*	*Aspergillus clavatus*	*Aspergillus niger*
SATFAs	C12:0	-	8.54	12.32	-	-	-
	C14:0	3.08	-	8.88	4.95	-	-
	C16:0	55.32	40.57	86.27	74.50	46.79	97.56
	C17:0	4.81	3.22	4.75	3.07	3.93	-
	C18:0	100.00	100.00	100.00	100.00	100.00	100.00
	C20:0	4.51	-	-	-	5.52	-
MUFAs	C16:1	3.22	5.08	4.97	1.28	2.07	-
	C17:0 cyc [c]	1.59	-	1.56	-	1.35	-
	C18:1 A [c]	100.00	100.00	100.00	100.00	100.00	100.00
	C19:1	-	-	2.70	-	2.56	-
	C20:1	5.15	-	5.61	-	6.12	-
PUFAs	C8:0 oxo	9.33	-	-	-	4.84	-
	C9:0 oxo	28.00	7.75	3.23	2.44	16.13	-
	C18:2 A [c]	100.00	100.00	100.00	100.00	100.00	-
	C20:4	4.10	4.81	4.84	5.69	4.03	66.67
	C20:3	6.67	6.81	7.26	8.94	6.45	100.00

[c] A, B, C – different positions of methyl group or double bond; [c]cyc – cyclopropyl group in chain; C8:0 oxo – 8-oxooctanoic acid; C9:0 oxo – 9-oxononanoic acid

each species from the others.

In the PUFAs fraction, octadecadienoic acid (C18:2 A) was the base peak (100 RP) in the chromatogram, with the exception of *A. niger*, where this acid was not present and the base peak fatty acid was eicosatrienoic acid (C20:3); this absence of octadecadienoic acid (C18:2A) in *A. niger* was a significant marker for taxonomical identification of this species.

Hydroxy Substituted Fatty Acids (PLOH)

This fraction did not contain any hydroxy-substituted fatty acids, therefore, it was not possible to use this group of fatty acids for differentiation of these fungi.

Unsaponifiable Fatty Acids from Phospholipids (summarized in Table IV)

Unsaponifiable fatty acids included two fractions: unsubstituted fatty acids with a non-ester bond in phospholipids (UNSFAs) and hydroxy-substituted fatty acids with a non-ester bond in phospholipids (UNSOH). The UNSFA fraction contained

fatty acids from C9 to C24 in chain-length with different degrees of unsaturation. There were only slight differences in the composition of fatty acids between the *Aspergillus* species in the range from C14 to C20 in chain-length. Unique fatty acids or fatty acids found only in two species were: nonanoic acid (C9:0) and heneicosanoic acid (C21:0) in *A. nidulans*; 13-docosenoic acid (C22:1) in *A. versicolor* and *A. nidulans*. Thus *A. versicolor* and *A. nidulans* were uniquely identified and differentiated from the other species and from each other. Other species could not, however, be distinguished on the basis of the UNSFAs shown in Table IV.

UNSOH fraction could be used as a complement to the UNSFAs. There were no differences in composition between *A. terreus*, *A. nidulans* and *A. fumigatus*, and the intensity pattern of these species was very similar. In the case of *A. clavatus* no fatty acids were observed while *A. versicolor* and *A. niger* had the same composition. The absence of any fatty acid in this fraction for *A. clavatus* was most striking, but the other species did not have unique compositions allowing differentiation based on the UNSOH shown in Table IV.

Table IV Relative percent (RP) of fatty acids in the *Aspergillus* species in the phospholipid fraction containing unsaponifiable FAMEs

	Fatty acids	*Aspergillus versicolor*	*Aspergillus terreus*	*Aspergillus nidulans*	*Aspergillus fumigatus*	*Aspergillus clavatus*	*Aspergillus niger*
UNSFAs	C9:0	-	-	*7.46*	-	-	-
	C14:0	6.74	9.51	5.36	13.60	9.77	6.24
	C15:0	5.01	6.28	4.25	5.93	7.05	4.61
	C15:0 met [b]	1.52	0.87	0.82	0.65	0.68	1.15
	C16:1	3.09	4.71	1.99	2.05	5.53	4.06
	C16:0	100.00	100.00	100.00	100.00	100.00	100.00
	C16:0 met B [bc]	3.90	2.83	5.79	6.13	11.50	9.11
	C17:0	6.52	5.93	5.58	5.05	5.89	5.29
	C18:2 A [c]	5.69	1.90	3.76	2.74	2.49	5.67
	C18:1 A [c]	27.92	11.13	38.39	20.07	14.94	33.79
	C18:0	88.47	62.77	70.16	61.15	65.89	69.95
	C20:0	4.81	2.94	3.66	2.24	2.95	2.21
	C21:0	-	-	*1.90*	-	-	-
	C22:1	*2.76*	-	*2.12*	-	-	-
	C22:0	9.61	3.51	8.26	3.99	3.78	4.09
	C24:0	1.21	1.13	1.30	-	1.07	-
UNSOH	C16:0 (OH) [d]	15.80	9.53	5.82	8.85	-	13.60
	C18:0 (?,?)diOH [d]	-	12.07	12.07	12.55	-	-
	C18:0 (9,10)diOH [d]	10.72	30.09	42.26	34.86	-	14.51
	C22:0 (OH) [d]	100.00	100.00	100.00	100.00	-	100.00

[b] met – methyl group (unknown position); [c] A, B, C – different positions of methyl group or double bond; [d] (OH) – hydroxyl group (known or unknown position)

Hexadecanoic acid (C16:0) was the base peak (100 RP) in the chromatogram of the UNSFAs fraction. There were also differences in the RP of the fatty acids among all of the species, although the differences were not dramatic. The greatest differences were, for example, in methylhexadecanoic acid (C16:0 met B) ranged from 2.83 to 11.50 RP or octadecenoic acid (C18:1A) ranged from 14.94 to 38.39 RP, both present in all the species.

2-Hydroxydocosanoic acid (C22:0 (OH)) was the base peak (100 RP) in the chromatogram of UNSOH fraction. Changes in the relative percentage were most significant in the case of 9,10-dihydroxyoctadecanoic acid (C18:0 (9,10)diOH) which ranges from 10.72 to 42.26 RP.

Lipopolysaccharides (summarized in Table V)

Fatty acids in the lipopolysaccharide fraction ranged from C5 to C24 in chain-length with varying degrees of unsaturation and cyclopropyl-, dicarboxylic- and oxo- fatty acids were observed, along with the straight chain compounds. There were significant differences in the composition of fatty acids between all of the species examined, and also some unique fatty acids were present. The fatty acids which were unique or present in only two species include: 4-oxopentanoic acid (C5:0 oxo) in *A. terreus* and *A. niger*; hepta-2,4-dienoic acid (C7:2) in *A. terreus*; tridecanoic acid (C13:0) and methyltridecanoic acid (C13:0 met) in *A. nidulans* and *A. clavatus*; pentadecenoic acid (C15:1), 2-hexylcyclopropaneoctanoic acid (C17:0 cyc) and nonadecanoic acid (C19:0) in *A. versicolor*; methylhexadecanoic acid (C16:0 metB) in *A. versicolor* and *A. terreus*; tricosanoic acid (C23:0) in *A. clavatus*.

The most abundant fatty acid was hexadecanoic acid (C16:0), which was assigned as the base peak (100 RP) in the chromatogram. The changes in the amounts of the fatty acids are listed in Table V. Significant differences were also observed in the abundance of these fatty acids; especially tetradecanoic acid (C14:0) which ranged from 12.31 to 37.66 RP and octadecenoic acid (C18:1 A) being 10.58 to 50.14 RP.

Discussion

By separating lipids into four different groups and performing fatty acid analysis by GC/MS, we determined the composition of lipids in considerably greater detail compared to the direct analysis in one step as reported in previous study. Further separation of phospholipids into unsubstituted, hydroxy-substituted and unsaponifiable provided additional information for the *Aspergillus* species examined in this study. Because of the different composition of fatty acids in the

Table V Relative percent (RP) of fatty acids in the *Aspergillus* species in the lipopolysaccharide fraction

Fatty acids	*Aspergillus versicolor*	Aspergillus terreus	Aspergillus nidulans	Aspergillus fumigatus	Aspergillus clavatus	Aspergillus niger
C5:0 oxo	-	*12.18*	-	-	-	*5.05*
C7:2	-	*1.45*	-	-	-	-
C9:0 diCOOH [a]	1.62	6.54	0.87	-	1.66	-
C12:0	3.56	11.90	0.83	-	3.76	-
C13:0	-	-	*0.91*	-	*1.69*	-
C13:0 met [b]	-	-	*0.85*	-	*1.06*	-
C13:0 diCOOH [a]	1.39	3.61	2.57	-	2.74	-
C14:0	13.76	37.66	17.30	23.63	23.02	12.31
C14:0 met A [bc]	-	1.66	1.85	-	2.57	-
C14:0 met B [bc]	6.69	5.48	3.13	3.14	3.95	2.41
C15:1	*2.36*	-	-	-	-	-
C15:0	14.19	16.05	12.69	8.49	14.99	7.45
C15:0 met [b]	2.29	1.37	1.54	1.63	2.29	-
C16:1	12.08	3.63	-	18.68	-	14.70
C16:0	100.00	100.00	100.00	100.00	100.00	100.00
C16:0 met A [bc]	6.38	3.48	-	5.78	-	-
C16:0 met B [bc]	*3.37*	*0.69*	-	-	-	-
C17:0	6.43	5.55	7.13	6.71	7.71	8.13
C17:0 cyc [c]	*2.95*	-	-	-	-	-
C18:1 A [c]	16.00	10.58	-	50.14	-	42.25
C18:1 B [c]	7.52	3.16	-	7.91	-	-
C18:0	33.71	32.52	43.22	44.46	38.64	61.49
C19:0	*0.87*	-	-	-	-	-
C20:0	2.49	2.11	1.79	-	2.70	4.70
C22:1	-	-	-	-	-	*4.87*
C22:0	-	-	3.08	-	1.75	3.11
C23:0	-	-	-	-	1.23	-
C24:0	0.97	1.23	2.16	-	2.15	3.96

[a] diCOOH – dicarboxylic acid; [b] met – methyl group (unknown position); [c] A, B, C – different positions of methyl group or double bond; [c] cyc – cyclopropyl group in chain; C5:0 oxo – 4-oxopentanoic acid

separated lipid fractions and the lipopolysaccharides, any of the fractions discussed in the results could be used for identification of the examined *Aspergillus* species.

In the case of neutral lipids, the RP differences were used for differentiation between *Aspergillus versicolor* and *Aspergillus nidulans* due to the similarities of the fatty acid composition in this fraction. In the glycolipid fraction, the differences in composition were the most pronounced and, therefore, this fraction could be used as an independent and sole source of fatty acids for taxonomical classification. Also the lipopolysaccharide fraction could be used as an independent criterion for characterization of the examined *Aspergillus* species. Unsubstituted fatty acids in phospholipids did not have to be separated into

SATFAs, MUFAs and PUFAs to achieve taxonomic classification of the studied fungi, just as unsaponifiable fatty acids in phospholipids did not have to be separated into UNSFAs and UNSOH because the results from all the groups were complementary and together provided sufficient information for differentiation between the *Aspergillus* species. Also, it was not necessary to analyze hydroxy substituted fatty acids (PLOH) fraction, since none of these compounds were observed.

This complex analysis of *Aspergillus versicolor*, *Aspergillus terreus*, *Aspergillus nidulans*, *Aspergillus fumigatus*, *Aspergillus clavatus* and *Aspergillus niger* provided us with detail information about their fatty acid composition and the relationship of the fatty acids variance to the taxonomical order.

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