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GC/MS PROFILING OF FATTY ACIDS IN MEDICALLY IMPORTANT FUNGI

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The application of gas chromatography/mass spectrometry (GC/MS) method was described for the generation of fatty acid profiles of medically important fungi. The composition of fatty acids was characteristic for different fungi and was used for identification and taxonomical purposes. The GC/MS methodology was shown to be suitable for diagnostic purposes in clinical settings thanks to its sensitivity, speed and accuracy.

Introduction

Fungal Infections: Severe fungal infections are now encountered more frequently in clinical settings compared to a decade ago, largely due to the greater number of

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immunocompromised patients. According to a recent survey of hospitals, nosocomial urinary tract infections constituted 42 % of the infections, surgical wound infections 24 %, nosocomial pneumonia 10 %, nosocomial bacteremia 5 %, and nosocomial infections at all other sites 10 % [1]. Immunocompromised patients include the following individuals: cancer patients, organ and bone marrow transplant patients, diabetics, patients with in-dwelling catheters, and people with HIV/AIDS.

The spectrum of opportunistic pathogens in immunocompromised patients has broadened over the past decade, with a wide variety of newly emerging lethal pathogens. Patients who undergo a heart transplant have a fatality rate associated with fungal infections of 36 % [2]. In bone marrow transplant patients, mortality associated with fungal infections approaches 95 % [3]. *Candida sp.* and *Aspergillus sp.* remain the most commonly encountered fungal microorganisms. *Mucor sp.* has been reported in bone marrow transplant patients with non-classical sites of manifestation; particularly pulmonary, cutaneous, and gastric region [4].

Diagnostic issues are usually addressed after clinical manifestations of the fungal infection appear, some of which have very subtle presentation. A significant factor, which complicates the diagnosis of fungal infection in immunocompromised patients, is the presence of immunosupression. The signs and symptoms of acute infections are non-specific due to the patient's inability to mount an adequate immune response. Fever is often absent, thus, allowing the fungal infection to progress into a systemic stage, which significantly limits treatment options. Also, preventive use of antibiotics is known to be one of the major risk factors for fungal infections. In a recent study, the relationship between the use of antibiotics and the incidence of a particular fungal infection was examined [5]. The incidence of *Candida albicans* was significantly higher in patients receiving antimicrobials not affecting anaerobic flora. Patients treated with third generation cephalosporins, carbapenems, glycopeptides and broad spectrum penicillins were more likely to develop invasive *Aspergillus sp.* infections.

A major difficulty in the successful management of systemic fungal infections is the inability to establish an early and correct diagnosis [6]. Most of the microbiological methods used for diagnosis of fungal infections in immunocompromised patients suffer from one or more shortcomings. A short description of disadvantages of each diagnostic method follows:

Culturing, Microscopy and Biochemical/Metabolic Assays

A significant concentration of pathogen is required for microscopic examination, thus, limiting its use for early detection. Most immunocmpromised patients suffer from multiple-organism infections (bacterial, fungal/yeast, viral) and the cross-

contamination can also affect the cultivation process. Due to the slow growth of fungi under cultured conditions, the identification can take days, prolonging the period of empirical and possibly inadequate therapy.

Immunoassay Based Methods

Measurement of antibody levels in patient serum fails in 50% of the cases because immunosupressed patients lack antibody-forming capability. Newer immunoassays based on recognition of mannans from the cell walls of *Candida* and *Aspergillus sp.* are limited by the rapid clearance of the antigen (mannan) from the patient's serum.

Molecular Methods

Polymerase chain reaction (PCR) based methods require identification of specific gene targets. Once developed, the PCR methodology can be very sensitive and relatively fast. However, most PCR assays applied in clinical diagnostics still suffer from contamination problems by ubiquitous fungal conidia and from the impossibility to distinguish between colonization and infection.

Mass Spectrometry: In order to overcome diagnostic difficulties with the early identification of fungal infections in immunocompromised patients, we propose the use of the analytical technique of gas chromatography/mass spectrometry, in order to generate a "finger-print" of a particular fungal microorganism. Analytical profiles of pure cultures can be compiled into a library of fungal "finger-prints" and characteristic features (biomarkers) will be identified for each microorganism. Then, such biomarkers will be sought in the clinical samples in order to establish the presence or absence of the microorganism. As an analytical technique, mass spectrometry is characterized by a unique combination of speed, high specificity and superb sensitivity. The use of mass spectral peaks as biomarkers for taxonomic purposes was employed in many specific applications in the past, providing identification of genus, species and even resistance toward antifungal agents.

The compound classes to be examined as a source of the biomarkers are fatty acids. Fatty acids represent a suitable class of molecules with chemotaxonomic utilities since they are major constituents of the cell membrane and they are easily accessible for mass spectral analysis. Gas chromatography is commercially available for chemotaxonomic classification of bacteria using fatty acid methyl esters (FAMEs) [7]. Various authors [8–14] have reported the presence of fatty acid chains of unusual length characteristic of various species of fungi. The occurrence of branched-chain fatty acids in fungi has been described in different fungal species [15,16]. Also, polyunsaturated fatty acids have been

described as unique to fungi, with the distribution being strongly correlated with taxonomic order [17–19].

The microbiological application of gas chromatography/mass spectrometry allows the use of a single instrument and methodology for multiple fungal microorganisms. The fungal agent does not need to be cultured in order to make a specific taxonomical classification. The presence of the fungal infection is established by using multiple biomarkers (mass spectral peaks of fatty acids), thus, reducing false positive/false negative results. The method is simple, fast and sensitive, therefore, applicable to clinical settings.

Experimental

Fungal Specimens. The following fungal microorganisms were purchased from ATCC (USA): *Aspergillus niger* (9642-U), *Aspergillus terreus* (1012), *Aspergillus fumigatus* (96918), *Candida glabrata* (2001), *Candida utilis* (9226), *Candida albicans* (14053), *Mucor rouxii* (24905), *Mucor azygosporus* (15087) *Mucor hiemalis* (8977-B).

Analytical Methods

Extraction of Fatty Acids. Freeze dried fungal specimens were dissolved in 1 ml of phosphate buffer (7.4 pH). Samples were sonicated with an ultra sonic probe at 2 Watts for 5 min (Ultrasonic Procesor, Cole-Parmer Instruments, Illinois, USA) in order to release membrane components. The samples were extracted with 4 ml $CH_3OH : CHCl_3$ (1 : 2). Following the addition of organic solvents, the mixture was vortexed for 1 min and centrifuged for 20 min at 10,000 rpm and 0 °C. The lower phase was collected and dried under a stream of nitrogen. The crude mixture of lipids was pre-separated prior to GC/MS analysis into neutral lipids (eluted with 5 ml chloroform), glycolipids (eluted with 5 ml acetone) and finally phospholipids and polar lipids (eluted with 20 ml methanol) by a SPE-Si column (Silica, 125 mg ml^{-1}, Alltech, USA). The eluates were dried under nitrogen and redissolved in 2 ml $CH_3OH : CHCl_3 : HCl$ (10 : 1 : 1). Then the mixture was kept at 60 °C in sealed vials overnight to undergo acidic hydrolysis to free the fatty acids from complex lipids. The free fatty acids were extracted into 4 ml hexane:toluene (1 : 1) after a 2 ml wash with 2 % NaCl (aqeous). The final organic fraction containing all fatty acids was dried under nitrogen and reacted with 30 µl Methyl 8 reagent in order to increase volatility. A scheme of the sample preparation is shown in Fig. 1.

GC/MS Analysis. The analysis was conducted using a Hewlett Packard GC/MS (HP 5890/HP 5970, Agilent Technologies, USA) on a capillary SPB-5 column

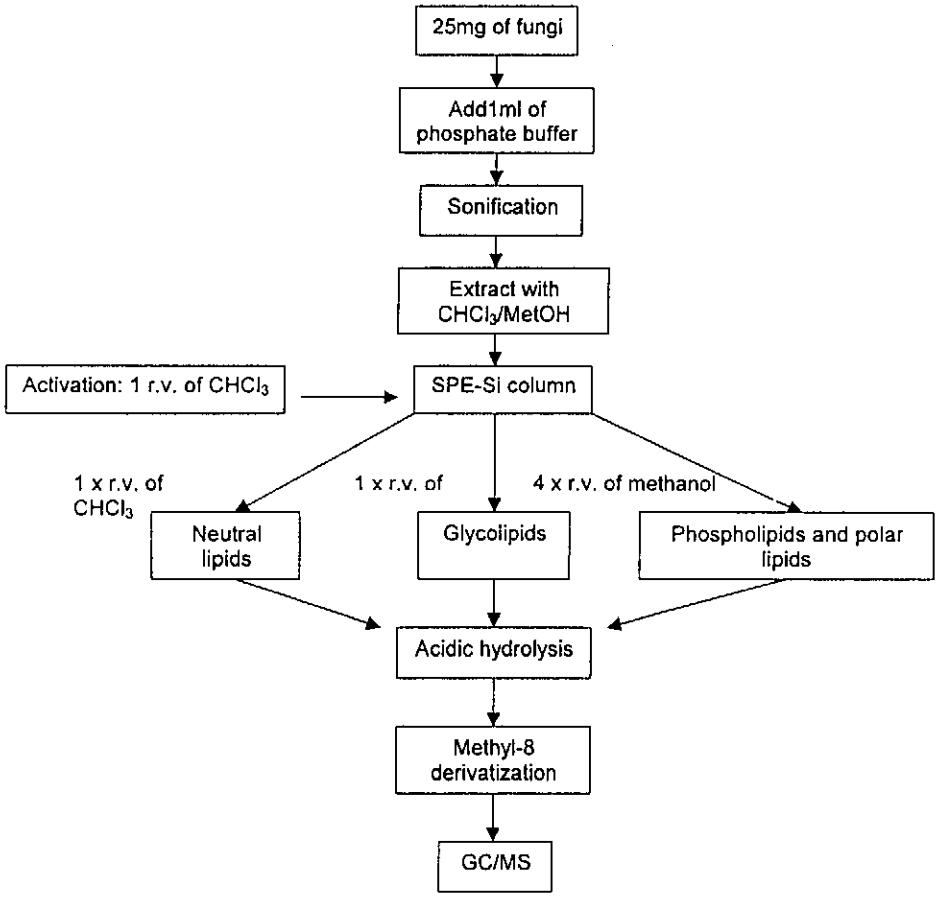

Fig. 1 Sample preparation scheme

(Supelco, USA) of the following dimensions: 30 m × 0.25 mm × 0.25 mm. Ultra pure Helium was used as carrier gas at a flow rate of 1 ml min^{-1}. The temperature gradient was started at 70 °C (initial temperature maintained for 2 min), then the temperature was increased at a rate of 10 °C min^{-1} to 280 °C (the final temperature maintained for 7 min). The injector port was kept at 250 °C while the GC/MS interface was maintained at 290 °C. Electron ionization mode was used during mass spectral analysis with positive ion detection mode.

Data Interpretation. All mass spectra were searched and fatty acids identified by using a mass spectral library (National Institute of Standard and Technology, Washington D.C., USA).

Results and Discussion

A 25 mg sample of each of the dry fungi was weighed out and processed in the manner shown in Fig. 1. After separation on the SPE-Si column, three different fractions were obtained: 1. neutral lipids, 2. glycolipids, and 3. phospholipids and polar lipids. The eluates were subjected to acid hydrolysis and the free fatty acids were methylated with Methyl-8 prior to GC/MS analysis. The GC/MS fatty acid methyl ester (FAME) profiles contained in three different fractions are summarized in Table I (FAMEs from neutral lipids), Table II (FAMEs from glycolipids), and Table III (FAMEs from phospholipids and polar lipids).

A total of 36 different FAMEs were identified in the fungal extracts, with the greatest number being found in the neutral lipid fraction (Table I), and the least in the glycolipid fraction (Table II). However, the fraction with the highest information content was the phospholipid and polar lipids fraction (Table III). All of the fatty acids in Tables I, II and III are abbreviated in the following manner: the number following the letter C indicates the number of carbons in the fatty acid chain. The presence of double bonds is indicated by a 2n symbol and the number following indicates the number of the double bonds; information regarding the position of the double bond, however, is not available. The word "(methyl)" indicates the presence of a methyl group on the fatty acid chain. The word "(Oxo)" describes the presence of an aldehyde group on the last carbon and "(Cyclo)" indicates the presence of a cyclopropyl group in the fatty acid chain and the carbon number indicates the total number of carbons in the fatty acid. DME designates a dicarboxylic acid dimethyl ester.

All of the FAMEs profiles in this fraction are unique and characteristic for individual fungi. There are differences between genera as well as well as individual species of fungi, thus allowing complete taxonomical classification based on the FAMEs profiles. Information from this fraction alone is sufficient for identification of all examined fungi.

Representative total ion chromatograms (TIC) of the three different fractions from *Aspergillus fumigatus* are shown in Fig. 2. The upper portion of the figure shows the FAMEs profile of the neutral lipid fraction, the middle frame shows the FAMEs of the glycolipid fraction, and the lower panel shows the FAMEs profile of the phospholipids and polar lipids. There are significant differences in the profiles in the number of FAMEs (27 in neutral lipids fraction, 8 in the glycolipid fraction, and 19 in the phospholipids and polar lipid fraction) and also in the distribution of FAMEs. For example the neutral lipid fraction has a wide variety of FAMEs evenly distributed. In contrast, the glycolipid fraction contains only a few FAMEs in the middle and higher retention time areas. The phospholipid and polar lipid fraction has an even distribution in the middle and higher retention times.

Typical TIC of FAMEs profiles from the phospholipid fraction of the three

Table I The GC/MS fatty acid methyl ester (FAME) profiles from neutral lipid fraction. Each identified methyl ester is assigned by a number in the increasing order of retention time. The plus sign indicates the presence of FAME in the extract, while the minus sign indicates its absence

Fatty acid ID number	Fatty acid methyl esters	Retention time	Neutral lipid fractions								
			Mucor			*Aspergillus*			*Candida*		
			rouxii	*ayzgosporus*	*hiemalis*	*tereus*	*fumigatus*	*niger*	*utilis*	*glabrata*	*albicans*
1	C10 ME	10.92	+	–	–	–	–	–	–	–	–
2	C9(Oxo) ME	12.46	–	–	–	–	–	–	+	–	–
3	C12 ME	13.68	+	+	+	+	+	–	+	+	+
4	C9 DME	13.95	–	+	–	+	+	–	–	+	+
5	C13 ME	14.95	+	–	+	+	+	–	+	+	+
6	C13(Methyl) ME	15.73	+	+	+	+	+	–	+	+	–
7	C14(2n:1) ME	16.03	+	+	+	+	+	–	–	+	–
8	C14 ME	16.18	+	+	+	+	+	+	+	+	+
9	C14(Methyl) ME	16.89	+	+	+	+	+	–	–	+	–
10	C14(Methyl) ME	16.99	+	+	–	+	+	–	–	+	–
11	C15 ME	17.31	+	+	+	+	+	–	+	+	–
12	C15(Methyl) ME	18.00	+	+	+	+	+	–	+	+	–
13	C16(2n:1) ME	18.19	+	+	+	+	+	–	+	+	+
14	C16 ME	18.47	+	+	+	+	+	+	+	+	+
15	C16(Methyl) ME	19.06	+	+	–	+	+	–	–	+	–
16	C16(Methyl) ME	19.15	+	+	+	+	+	–	–	+	–
17	C17(Cyclo) ME	19.22	+	+	+	+	+	–	–	+	–
18	C17 ME	19.43	+	+	+	+	+	+	+	+	+
19	C18(2n:3) ME	20.00	+	+	–	–	–	–	–	–	–
20	C17(Methyl) ME	20.08	+	+	+	+	+	–	+	+	–
21	C18(2n:2) ME	20.16	+	+	+	+	+	+	+	+	+
22	C18(2n:1) ME	20.25	+	+	+	+	+	+	+	+	+
23	C18(2n:1) ME	20.27	+	+	+	+	–	+	+	+	+
24	C18 ME	20.46	+	+	+	+	+	+	+	+	+

Table I – Continued

Fatty acid ID number	Fatty acid methyl esters	Retention time	Neutral lipid fractions								
			Mucor			*Aspergillus*			*Candida*		
			rouxii	*oryzae*	*hiemalis*	*tereus*	*fumigatus*	*niger*	*utilis*	*glabrata*	*albicans*
25	C18(2n:2) ME	20.63	–	–	–	–	–	–	–	–	–
26	C18(2n:2) ME	21.03	–	–	–	–	–	–	–	–	–
27	C19 ME	21.39	+	–	–	–	–	–	–	–	–
28	C20(2n:4) ME	21.73	+	–	–	–	+	–	–	+	–
29	C20(2n:3) ME	21.88	+	+	–	–	+	–	–	+	–
30	C20(2n:1) ME	22.07	+	+	+	+	+	+	–	+	+
31	C20 ME	22.28	+	+	+	+	+	+	+	+	+
32	C21 ME	23.17	–	–	–	–	–	–	–	–	–
33	C22(2n:1) ME	23.85	+	+	+	+	+	+	+	+	+
34	C22 ME	24.05	+	+	+	+	+	–	+	+	+
35	C23 ME	25.06	–	–	–	+	+	–	–	+	–
36	C24 ME	26.25	–	–	–	–	–	–	–	–	+

Table II The GC/MS fatty acid methyl ester (FAME) profiles from glycolipid fraction. The meaning of symbols is the same as in Table I

Fatty acid ID number	Fatty acid methyl esters	Retention time	Glycolipid fractions								
			Mucor			*Aspergillus*			*Candida*		
			rouxii	*oryzae*	*hiemalis*	*tereus*	*fumigatus*	*niger*	*utilis*	*glabrata*	*albicans*
1	C10 ME	10.92	–	–	–	–	–	–	–	–	–
2	C9(Oxo) ME	12.46	–	–	–	–	–	–	–	–	–
3	C12 ME	13.68	+	–	+	–	–	–	+	–	–
4	C9 DME	13.95	+	–	+	–	–	–	+	–	–
5	C13 ME	14.95	–	–	–	–	–	–	–	–	–
6	C13(Methyl) ME	15.73	+	–	–	–	–	–	+	–	–

Table II – Continued

Fatty acid ID number	Fatty acid methyl esters	Retention time	Glycolipid fractions								
			Mucor			*Aspergillus*			*Candida*		
			rouxii	*oryzae*	*hiemalis*	*terreus*	*fumigatus*	*niger*	*utilis*	*glabrata*	*albicans*
7	C14(2n:1) ME	16.03	+	–	+	–	–	–	–	–	–
8	C14 ME	16.18	+	+	+	+	+	–	+	+	+
9	C14(Methyl) ME	16.89	+	–	+	–	–	–	+	–	–
10	C14(Methyl) ME	16.99	+	–	+	–	–	–	+	–	–
11	C15 ME	17.31	+	+	+	+	+	–	+	+	+
12	C15(Methyl) ME	18.00	+	–	+	–	–	–	+	–	–
13	C16(2n:1) ME	18.19	+	–	+	–	–	–	+	+	+
14	C16 ME	18.47	+	+	+	+	+	+	+	+	+
15	C16(Methyl) ME	19.06	+	–	+	–	–	–	–	–	–
16	C16(Methyl) ME	19.15	+	–	+	–	–	–	+	–	–
17	C17(Cyclo) ME	19.22	+	–	+	–	–	–	–	–	–
18	C17 ME	19.43	+	–	+	–	+	–	+	+	+
19	C18(2n:3) ME	20.00	+	–	–	–	–	–	–	–	–
20	C17(Methyl) ME	20.08	+	–	+	–	–	–	–	–	–
21	C18(2n:2) ME	20.16	+	–	+	–	–	–	+	–	+
22	C18(2n:1) ME	20.25	+	+	+	+	+	+	+	+	+
23	C18(2n:1) ME	20.27	+	–	+	–	–	–	+	+	+
24	C18 ME	20.46	+	+	+	+	+	+	+	+	+
25	C18(2n:2) ME	20.63	–	–	–	–	–	–	–	–	–
26	C18(2n:2) ME	21.03	–	–	–	–	–	–	–	–	–
27	C19 ME	21.39	–	–	–	–	–	–	–	–	–
28	C20(2n:4) ME	21.73	–	–	–	–	–	–	–	–	–
29	C20(2n:3) ME	21.88	–	–	–	–	–	–	–	–	–
30	C20(2n:1) ME	22.07	–	–	–	–	–	–	+	–	–
31	C20 ME	22.28	+	–	+	–	–	–	+	–	–
32	C21 ME	23.17	–	–	–	–	–	–	–	–	–

Table II – Continued

Fatty acid ID number	Fatty acid methyl esters	Retention time	Glycolipid fractions								
			Mucor			*Aspergillus*			*Candida*		
			rouxii	*ayzgosporus*	*hiemalis*	*tereus*	*fumigatus*	*niger*	*utilis*	*glabrata*	*albicans*
33	C22(2n:1) ME	23.85	+	–	+	–	–	–	+	–	–
34	C22 ME	24.05	+	–	+	–	+	–	+	–	–
35	C23 ME	25.06	+	–	–	–	+	–	+	–	–
36	C24 ME	26.25	–	–	–	–	–	–	–	–	–

Table III The GC/MS fatty acid methyl ester (FAME) profiles from phospholipid and polar lipid fraction. The meaning of symbols is the same as in Table I

Fatty acid ID number	Fatty acid methyl esters	Retention time	Phospholipid fractions								
			Mucor			*Aspergillus*			*Candida*		
			rouxii	*ayzgosporus*	*hiemalis*	*tereus*	*fumigatus*	*niger*	*utilis*	*glabrata*	*albicans*
1	C10 ME	10.92	–	–	–	–	–	–	–	–	–
2	C9(Oxo) ME	12.46	–	–	–	–	–	–	–	–	–
3	C12 ME	13.68	+	–	+	+	–	–	+	–	–
4	C9 DME	13.95	+	–	+	+	–	–	+	–	+
5	C13 ME	14.95	–	–	–	–	–	–	+	–	–
6	C13(Methyl) ME	15.73	–	–	–	–	–	–	–	–	–
7	C14(2n:1) ME	16.03	+	–	+	–	–	–	–	–	–
8	C14 ME	16.18	+	+	+	+	+	+	+	+	+
9	C14(Methyl) ME	16.89	+	–	+	–	–	–	+	–	–
10	C14(Methyl) ME	16.99	+	+	+	–	+	–	+	–	–
11	C15 ME	17.31	+	+	+	+	+	–	+	–	+
12	C15(Methyl) ME	18.00	+	+	+	+	+	–	+	–	–
13	C16(2n:1) ME	18.19	+	+	+	+	+	–	+	+	+
14	C16 ME	18.47	+	+	+	+	+	+	+	+	+

Table III – Continued

Fatty acid ID number	Fatty acid methyl esters	Retention time	Phospholipid fractions								
			Mucor			*Aspergillus*			*Candida*		
			rouxii	*ayzgosporus*	*hiemalis*	*tereus*	*fumigatus*	*niger*	*utilis*	*glabrata*	*albicans*
15	C16(Methyl) ME	19.06	+	+	+	+	–	–	+	–	–
16	C16(Methyl) ME	19.15	+	+	+	+	+	–	+	+	+
17	C17(Cyclo) ME	19.22	+	+	+	+	–	–	+	–	–
18	C17 ME	19.43	+	+	+	+	+	+	+	+	+
19	C18(2n:3) ME	20.00	+	–	–	–	–	–	–	–	–
20	C17(Methyl) ME	20.08	+	–	–	–	–	–	–	–	–
21	C18(2n:2) ME	20.16	+	+	+	+	+	–	+	+	+
22	C18(2n:1) ME	20.25	+	+	+	+	+	+	+	+	+
23	C18(2n:1) ME	20.27	+	+	+	+	+	–	+	+	+
24	C18 ME	20.46	+	+	+	+	+	+	+	+	+
25	C18(2n:2) ME	20.63	+	–	+	+	–	–	–	–	–
26	C18(2n:2) ME	21.03	+	–	+	–	–	–	–	–	–
27	C19 ME	21.39	–	–	–	–	–	–	–	–	–
28	C20(2n:4) ME	21.73	+	+	+	+	+	–	+	+	–
29	C20(2n:3) ME	21.88	+	+	+	+	+	–	+	+	–
30	C20(2n:1) ME	22.07	–	–	–	–	–	–	–	–	–
31	C20 ME	22.28	+	+	+	+	+	–	+	+	–
32	C21 ME	23.17	+	+	–	+	+	–	–	–	–
33	C22(2n:1) ME	23.85	–	–	–	–	–	–	–	–	–
34	C22 ME	24.05	+	+	+	+	+	–	+	+	–
35	C23 ME	25.06	+	+	+	+	+	–	+	+	–
36	C24 ME	26.25	+	+	+	+	+	–	+	+	–

genera are shown in Figure 3 where the differences between genera can be seen by comparing the profiles of *Aspergillus niger* (top panel), *Candida albicans* (middle panel) and *Mucor rouxii* (lower panel). Six different FAME's were identified in the chromatogram of *Aspergillus niger*. A total of 11 FAMEs were

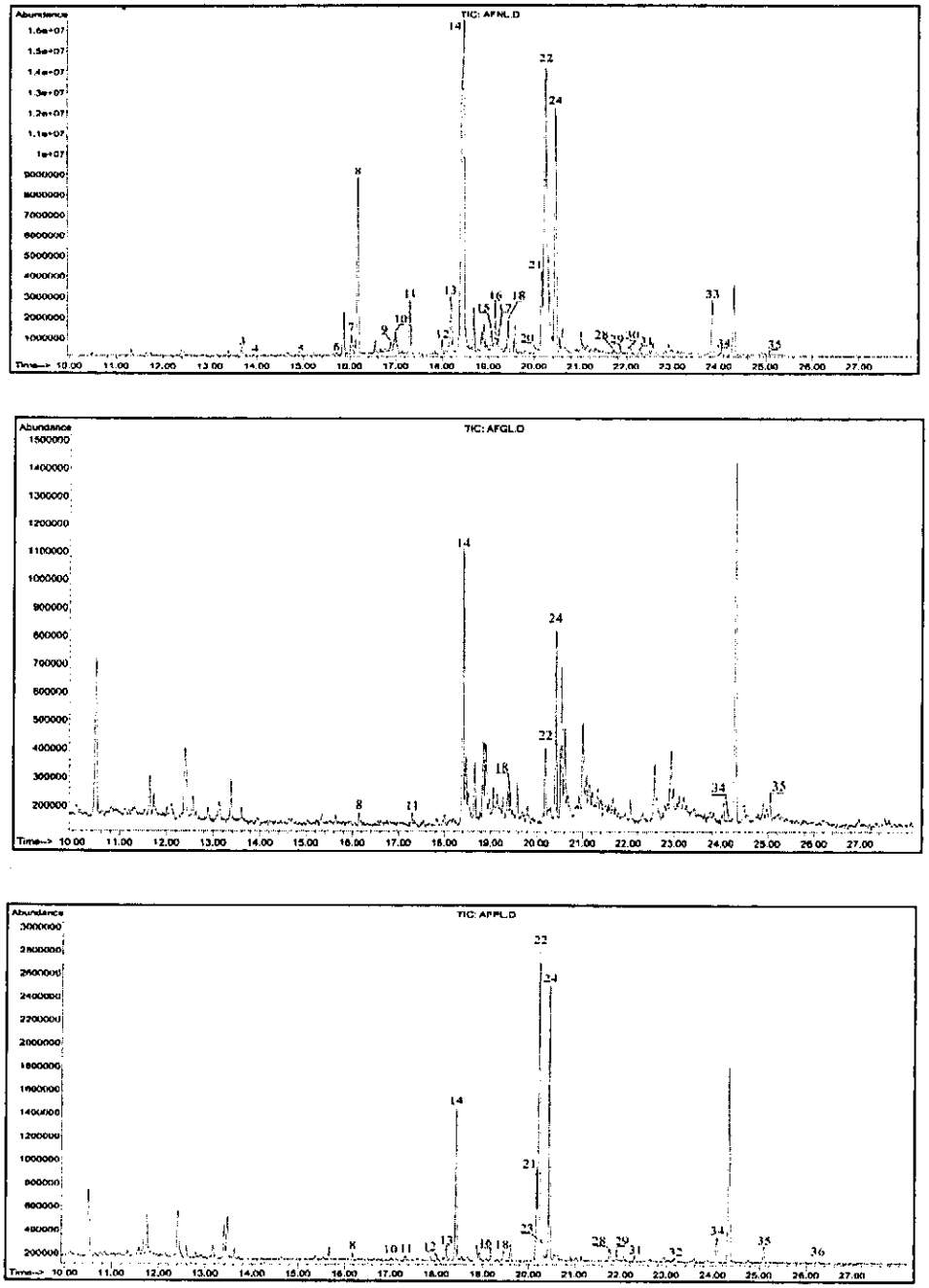

Fig. 2 Total ion chromatograms (TIC) of the three different fractions from *Aspergillus fumigatus*. Upper panel shows FAME profile of the neutral lipid fraction, the middle panel shows FAME profile of the glycolipid fraction, and the lower panel shows FAME profile of the phospholipid and polar lipid fraction. Unmarked peaks originated from the Methyl-8 reagent or contaminants

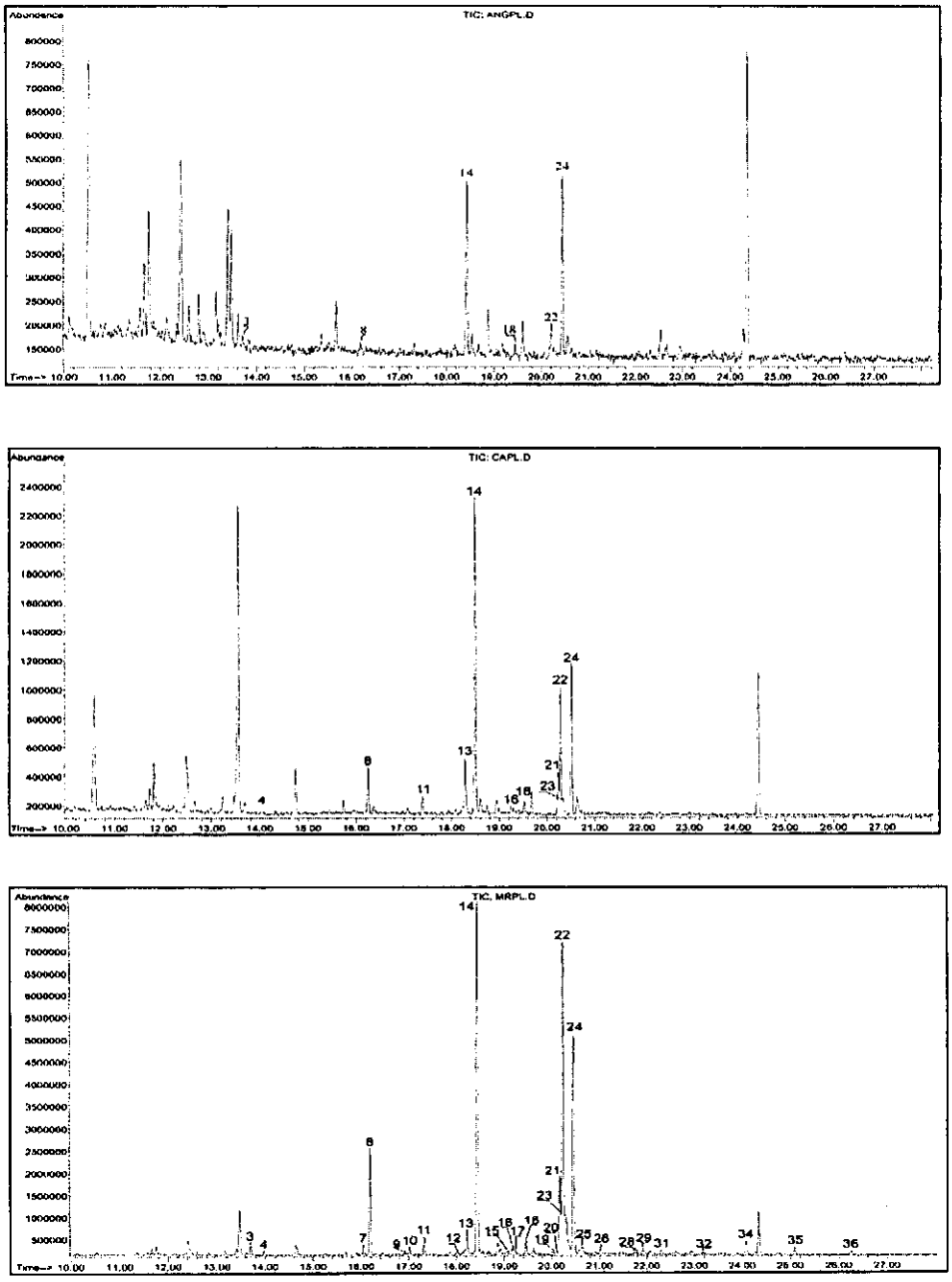

Fig. 3 Total ion chromatograms (TIC) from the phospholipid and polar lipid fraction of three different genera: upper panels shows FAME profile of *Aspergillus niger*, the middle panel shows FAME profile of *Candida albicans*, and the lower panel shows FAME profile of *Mucor rouxii*. Unmarked peaks originated from the Methyl-8 reagent or contaminants

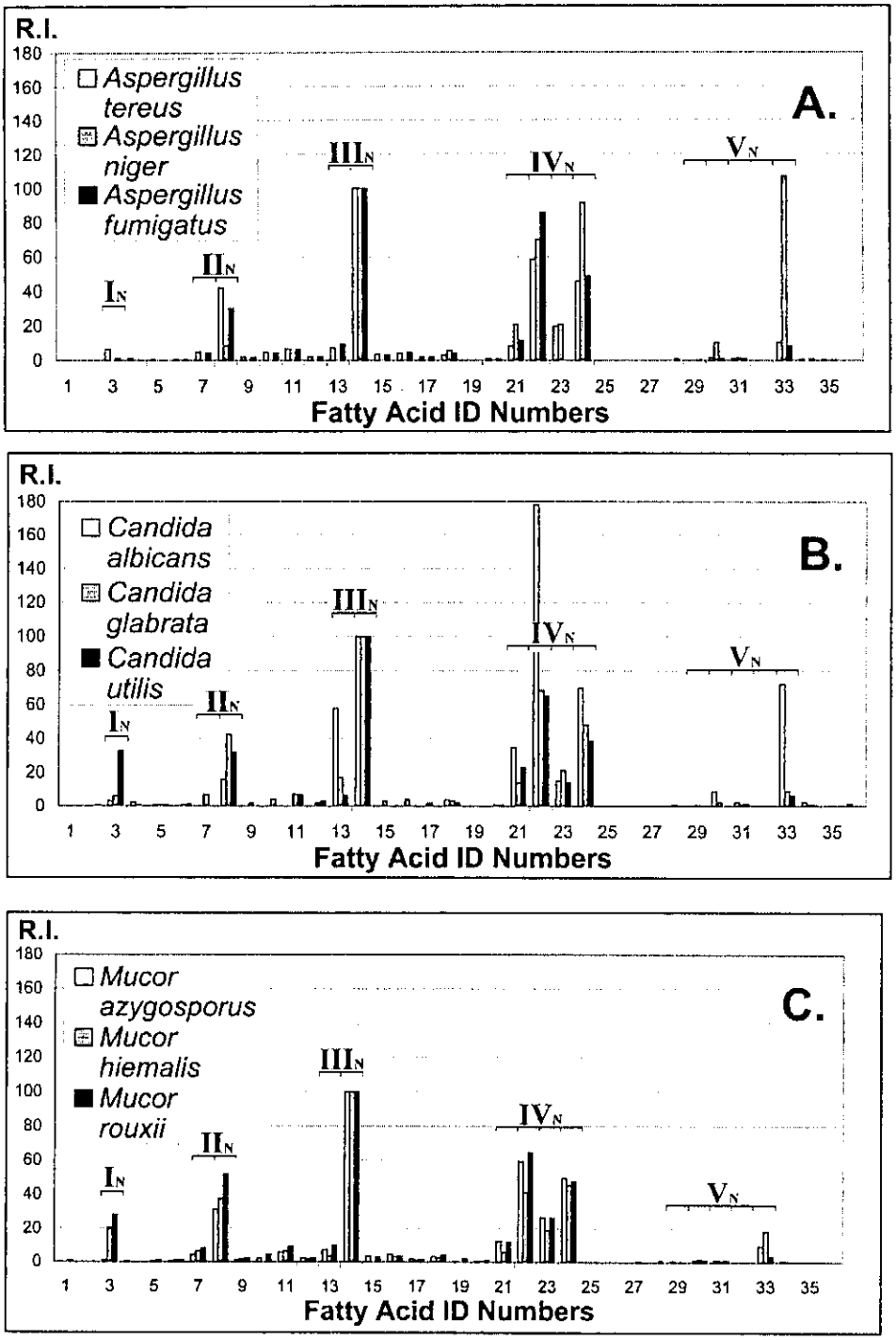

Fig. 4 FAME profiles of the neutral lipid fraction(N) from all nine fungi

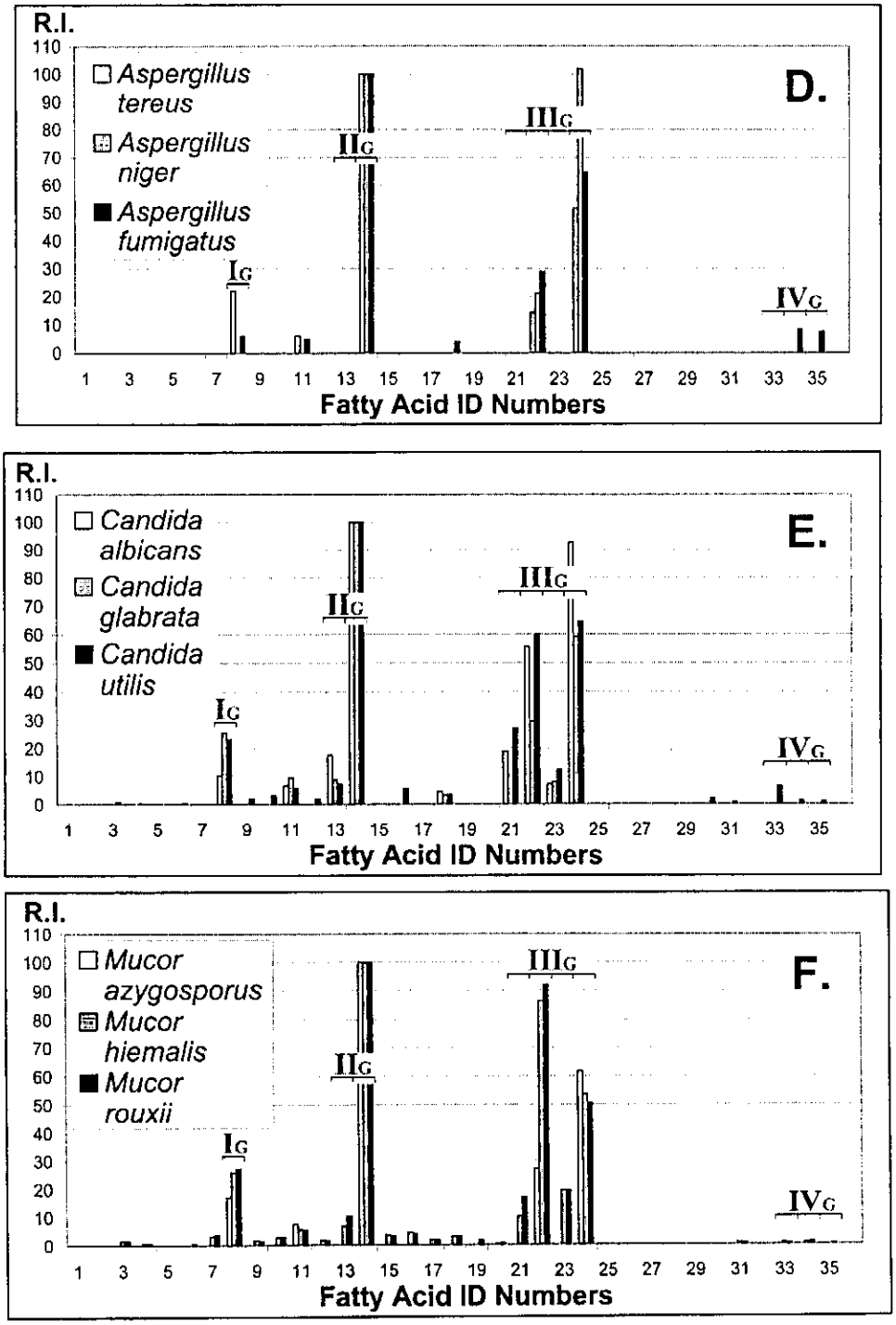

Fig. 5 FAME profiles of the glycolipid fraction (G) from all nine nungi

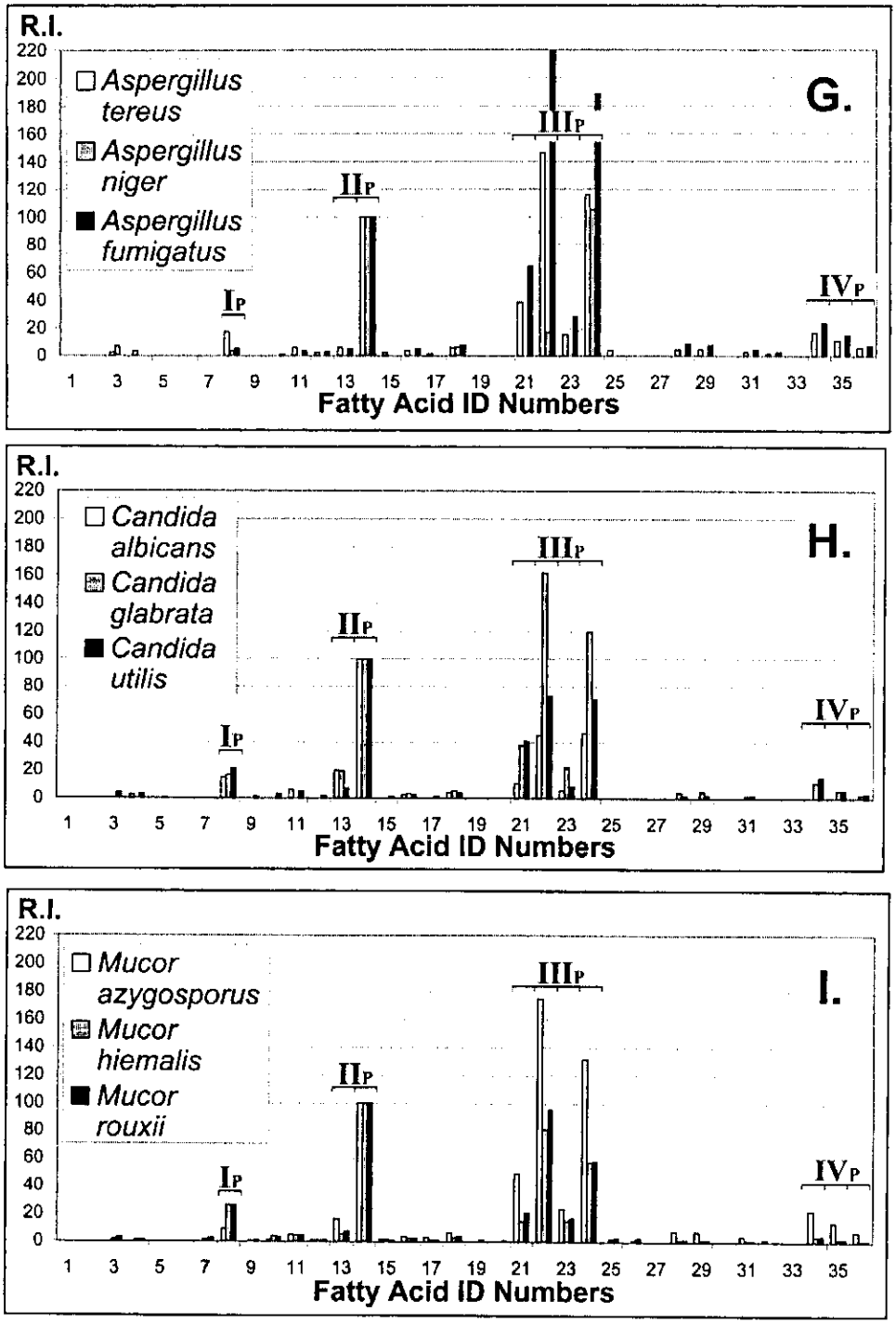

Fig. 6 FAME profiles of the phospholipid and polar lipid fraction (P) from all nine fungi

identified in the chromatogram of *Candida albicans* and a total of 29 different FAMEs were identified in the chromatogram of *Mucor rouxii*. Each individual fungus provided a unique composition profile of FAMEs in the phospholipids and polar lipid fraction. All FAMEs were identified using the mass spectral library search program and all resulting hits exceeded 90% similarity of searched mass spectrum with the library spectrum of a FAME standard.

The patterns formed by the different FAME profiles can be displayed graphically for easier comparisons. Figure 4 shows the FAME profiles of the neutral lipid fraction of all nine fungi. Ignoring the minor components, there are five regions of interest for all three panels: I N) # 3, II N) # 7–8, III N) #13–14, IV N) #21–24, and V N) # 29–33 (N stands for neutral lipid fraction). Differences in composition are observed in the *Aspergillus sp.* (Panel A), the *Candida sp.* (Panel B) and the *Mucor sp.* (Panel C); intensity differences are also observed between the species (white, grey and black column) in each of the figures, confirming the unique composition of FAMEs in individual species of fungi.

Figure 5 shows the FAMEs profiles of the glycolipid fraction for each fungus studied. Differences in composition are observed in the *Aspergillus sp.* (Panel D), the *Candida sp.* (Panel E) and the *Mucor sp.* (Panel F). There are a significantly smaller number of FAMEs, since in glycolipids the major portion of the molecules consist of carbohydrates. Major regions of interest for all three panels in this fraction are: I G) # 8, II G) # 13–14, III G) # 21–24, and IV G) # 33–35 (G stands for glycolipid fraction). Even though differences in the FAME profiles of individual fungi are not as pronounced as in the other two fractions, they are sufficient for taxonomical classification.

The FAMEs pattern from the last fraction, phospholipids and polar lipids, is shown in Fig. 6. Differences in composition are observed in the *Aspergillus sp.* (Panel G), the *Candida sp.* (Panel H) and the *Mucor sp.* (Panel I). There are four regions of interest for all three panels for the purpose of comparison: I P) # 8, II P) # 13–14, III P) # 21–24, and IV P) # 34–36 (P stands for phospholipid and polar lipid fraction). The differences in the FAME profile of the phospholipids are the most pronounced and the easiest for identification. This is most obvious in the III P region, where the differences in the composition and intensities of FAMEs are most distinguishable.

Conclusion

The data from this study showed that lipid derived molecules, fatty acid methyl esters, are suitable biomarkers for taxonomic classification of clinically significant fungi. FAME profiles from three different fractions (neutral lipids, glycolipids,

and phospholipids and polar lipids) are complementary and can be successfully used for identification of the fungal pathogens in clinical specimens. The neutral lipid fraction contained a wide variety of FAMEs evenly distributed throughout the entire retention interval. In contrast, the glycolipid fraction contained only a few FAMEs in the middle and higher retention time areas. The phospholipid and polar lipid fraction contained an even distribution in the middle and higher retention times. The phospholipid and polar lipids fraction had the highest information content. All of the FAME profiles in this fraction are unique and characteristic for individual fungi. There are differences between genera as well as well as individual species of fungi, thus allowing complete and most accurate taxonomical classification based on the FAME profiles.

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