Symbiosis, 26 (1999) 247–264
Balaban, Philadelphia/Rehovot

Comparison of the Lipid Profiles of Arbuscular Mycorrhizal (AM) Fungi and Soil Saprophytic Fungi

JAN JANSA[1], MILAN GRYNDLER[2*], and MIROSLAV MATUCHA[3]
[1]*Institute of Botany, Academy of Sciences, Kvetnove namesti 1, Pruhonice, 25243, Czech Republic, Tel.&Fax. +42-2-67750022, E-mail. jansa@ibot.cas.cz;* [2]*Institute of Microbiology, Academy of Sciences, Videnska 1083, Prague 4, 142 20, Czech Republic, Tel. +42-2-4752382, Fax. +42-2-4752384, E-mail. gryndler@biomed.cas.cz; and* [3]*Institute of Experimental Botany, Academy of Sciences, Videnska 1083, Prague 4, 142 20, Czech Republic, Tel. +42-2-4752484*

Received April 22, 1998; Accepted July 28, 1998

Abstract

Lipids from twelve strains of soil saprophytic fungi, *Endogone pisiformis* and eight strains of AM fungi were obtained by extraction with organic solvents. Extraction efficiency of several solvents was tested to get maximum yields. The amount of lipids in spores of AM fungi was estimated. Profiles of extracted lipids were obtained using thin layer chromatography (lipids) and gas chromatography (fatty acid methylesters (FAME) released by hydrolysis of acylesters). Majority of AM fungi gave a characteristic fatty acid (FA) profile, sharply distinct from other organisms. Three groups were distinguished among the fungi under study: the first was represented by soil saprophytic fungi, comprising also *Glomus deserticola* and *Scutellospora heterogama*, the second consisted of *Glomus* spp. (different from that mentioned above), and the third consisted of *Endogone pisiformis*, that was separated from the above groups.

Keywords: Arbuscular mycorrhiza, soil saprophytes, fungi, *Glomus*, *Endogone*, *Scutellospora*, lipids, fatty acids, chromatography

*The author to whom correspondence should be sent.

0334-5114/99/$05.50

Abbreviations

AM = arbuscular mycorrhiza(l); AMF = arbuscular mycorrhizal fungi; FA = fatty acid; FAME = fatty acid methylester; TLC = thin layer chromatography; GC = gas chromatography; MS = mass spectrometry.

1. Introduction

AM fungi, like other heterotrophic organisms, depend on sweeping for reduced carbon compounds. These compounds are supposed to be saccharides, synthesized as a result of photosynthesis in a host plant. No mycorrhiza-specific sugar compounds were found in AM fungi, e.g. such as described in ectomycorrhizas (trehalose, mannitol), that would have a function of "biochemical valve" (Hayman, 1974; Bevege et al., 1975). In AM fungi, this function is supposed to be ensured by lipids. An increased translocation of lipids into mycorrhizal roots was demonstrated by radiochemistry (Bevege et al., 1975; Cox et al., 1975). The above-mentioned results suggest that plant photosynthates are converted to lipids, lipoproteins, aminoacids and ketoacids.

Lipids that accumulate during the lifetime of mycorrhiza are stored in resting structures of the fungi (spores and vesicles) and are consumed during spore germination and subsequent growth (Gaspar et al., 1997). The transfer of lipids into plant roots from germ-tubes of AM fungi is negligible. During the symbiotic phase of a mycorrhizal root, the net synthesis of lipids is significantly enhanced, compared to nonmycorrhizal roots, due to the activity of the fungi (Gaspar et al., 1997a). This increased synthesis of lipids in mycorrhizal roots is caused by photosynthate drain to the roots and, therefore, there is usually observed a growth depression of the host plant during the early stage of the plant development (Buwalda and Goh, 1982; Bowen 1981).

Higher levels of lipids in mycorrhizal roots are mainly due to higher amounts of triacylglycerols and phosphatidylethanolamine, the levels being significantly increased after colonization of the roots by the mycorrhizal fungus. A lipid analysis of AM roots during development of the infection showed a proportional decrease of phospolipids and an increase of trialcylglycerols and free fatty acids (FA) (Gaspar et al., 1997a). Triacylglycerols constitute 38% of the spore fresh weight of *Glomus versiforme* (Gaspar et al., 1994), the main fatty acid in them being palmitoleic acid (more than 50% of the lipid material) and the other fatty acids being represented by palmitic and oleic acids and some polyunsaturated FA of the ω3 and ω6 families.

The aim of this work was to estimate the amount of lipids in spores of AM fungi and to analyse the lipids for their chemical composition. Several strains of arbuscular mycorrhizal fungi and also *Endogone pisiformis* (an axenically culturable species formerly accepted as a close relative to the group of *Glomales*, e.g. Gerdemann and Trappe, 1974) were compared to soil saprophytic *Zygomycetes* and the genus *Fusarium*, a representative of the anamorphs of soil *Ascomycetes*. The purpose was to find some distinction between the lipid profiles of symbiotic and saprophytic fungi. The existence of such a distinction would be very useful for both qualitative and quantitative estimation of mycorrhizae if the variability of chemical composition of lipids were not too high.

2. Materials and Methods

Cultivation of fungi

Axenically culturable fungi were cultivated on liquid media (malt extract medium - 120 g of brewery malt extract concentrate per liter of distilled water, pH 5.5) in stationary cultures for 5–20 days (duration depended on a strain's growth rate). Endogone pisiformis was cultivated on the liquid MRM medium (Mitchell and Read, 1981) for 40 days.

The following isolates of axenically culturable fungi were used:

- *Linderina penispora* Raper & Fennell (CCF 1574)
- *Mortierella elongata* Linnem. (CCF 1143)
- *Mortierella alpina* Peyronel (CCF 1525)
- *Syncephalastrum racemosum* Schröt. (CCF 1667)
- *Cunninghamella elegans* Lendner (CCF 1672)
- *Micromucor ramannianus* Möler var. *ramannianus* (CCF 2644)
- *Zygorrhynchus moelleri* Vuill. (CCF 2585)
- *Absidia coerulea* Bain. (CCF 1020)
- *Mucor plumbeus* Bonord. (CCF 1618)
- *Mucor hiemalis* Wehmer (CCF 1668)
- *Fusarium moniliforme* Sheld. var. *moniliforme*
- *Rhizopus stolonifer* (Ehrenb.: Fries) Vuill.
- *Endogone pisiformis* Lk.:Fr. (DAOM 198006).

AM fungi were cultivated on the roots of *Allium ampeloprasum* L. ssp. *porrum* for 5 months in 8×8×10 cm black plastic pots in a soil-sand mixture (1:2 v/v), autoclaved and recolonized by soil bacteria for three weeks. The pots were protected from air-borne contamination by spores of AM fungi by glass cylinders (diameter 12 cm, length 30 cm) covered with Petri dish lids. This

plant was used for both previously observed facts that this perennial host plant stimulates sporulation of AM fungi and for the easiness of protecting these plants by the glass cylinders.
The following strains of fungi were used:
- *Glomus fistulosum* Skou & Jakobsen (BEG 23)
- *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerdemann
- *Glomus mosseae* Gerdemann & Trappe (BEG 25)
- *Glomus geosporum* (Nicolson & Gerdemann) Walker (BEG 11)
- *Glomus geosporum* (isolate 24A - CZ)
- *Glomus deserticola* Trappe, Bloss & Menge
- *Scutellospora heterogama* (Nicol. & Gerd.) Walker & Sanders (95/16 Rothamsted)
- *Glomus etunicatum* Becker & Gerdemann (95/2 CZ).

Cultivation of maize (Zea mays L.) for checking mycorrhiza-specific lipids in planta

Both uninoculated and inoculated (100 spores of Glomus fistulosum BEG 23 per pot) maize plants were grown in 8×8 cm plastic pots filled with a soil-sand mixture (1:5 v/v). The cultivation took 8 weeks in a growth chamber (16 h day - 8 h night, 23–18°C, 70–75% rel. humidity). Maize was chosen to obtain maximum yield of recognizably yellow mycorrhizal roots.

Isolation of lipids from saprophytic fungi

The mycelium of soil saprophytes obtained from liquid culture was filtered through an asbestos filter, washed with 2% $(NH_4)_2SO_4$, homogenized in a mortar at 4°C, and extracted for 30 min with an extraction mixture of chloroform-methanol (2:1) using the method described by Folch et al. (1957). The technique was also used by Radwan et al. (1996) on fungal material. The extraction mixture was then twice diluted with 1% NaCl and the obtained emulsion centrifuged (10 min; 3000 g). The lower (nonpolar) phase was collected, evaporated using a vacuum evaporator at 30°C, and redissolved in hexane. Other extraction mixtures used to carry out preliminary observations are described in the corresponding experiments (Table 1).

Isolation of lipids from spores of AM fungi

Spores of AM fungi were wet sieved from soil and isolated according to Gerdemann and Nicolson (1963), separated from the soil particles by a step-

gradient centrifugation in 2.5 M sucrose-distilled water gradient at 3000 g for 45 min.

For estimation of the lipid content of the AMF spores, about 10,000 purified spores were used (done for *Glomus fistulosum* BEG 23).

For the lipid analysis by gas chromatography (GC), about 1000 spores from each isolate (species) were used. The spores were homogenized in a Potter-Elvehjem glass homogenizer using the above mentioned extraction mixture (2 ml). Another 2 ml of the extraction mixture were used for washing the homogenizer to ensure the reproducibility of the extraction procedure. A volume of 2 ml of 1% sodium chloride was then added to the extract. The emulsion obtained was centrifuged in glass vials at 3000 g for 10 min. The lower (nonpolar) phase was collected, vacuum evaporated and redissolved in hexane as above.

Isolation of lipids from plant roots (Zea mays L.)

The extraction of lipids from roots was similar to that from the mycelium of saprophytic fungi, the homogenization in a mortar being followed with extraction by a chloroform-methanol mixture (2:1), and subsequent shaking with 1% NaCl. The emulsion obtained was centrifuged as in the previous case. The nonpolar phase was collected, vacuum evaporated and redissolved in hexane.

Quantification of lipids in the extracts of spores

The amount of organic matter in lipid extracts of spores was processed according to the method of Brangdon (1951). The organic matter was oxidized in a potassium dichromate-sulfuric acid mixture (2% w/v) at 110°C for 45 min. The reaction mixture was then diluted (1:1) with water. The amount of organic matter was estimated colorimetrically at a wavelength of 580 nm. Triolein and anhydrous glucose were used as the reference standards.

Chromatographic analysis

TLC of lipid extracts was run on Silicagel plates (Silufol). As developing solvent, a 70:30:0.5 (v/v/v) mixture of petrolether-diethylether-ammonium hydroxide was used to be favourable for the detection of neutral lipids. The spots were detected using UV fluorescence and iodine vapours.

The gas chromatographic (GC) analysis was carried out using a Perkin-Elmer S3600 gas chromatograph equipped with a capillary column SPB-5 (30 m length, 0.25 mm width, 0.25 μm coating) and a flame-ionization detector.

Nitrogen was used as a carrier gas, at a flow rate of 2 ml per min. The samples were hydrolyzed and fatty acids methylated by derivatization with 1M methanolic trimethylchlorosilane. The samples were immediately vacuum evaporated and redissolved in hexane. A volume of 1 µl of a sample was injected (split injector 1:25 at 250°C) and the analysis run under the following temperature program: 3 min 150°C, then a temperature gradient of 5° per min to 220°C. The latter temperature was kept for 45 min. The peaks were identified by comparing with the results of gas chromatography-mass spectrometry (GC-MS) analysis of fatty acid methyl esters of *Glomus fistulosum* BEG23.

The statistical evaluation was performed using the SOLO statistical package (BMDP Software, Los Angeles, California, 1991). Division of the objects into groups was carried out using cluster analysis.

Euclidean distance (D_e) between groups was counted according to Hebak and Hustopecky (1987), following the equation:

$$D_e(i,i') = \sqrt{(x_{i1}-x_{i'1})^2 + (x_{i2}-x_{i'2})^2 + \ldots + (x_{ij}-x_{i'j})^2}$$

where x_{ij} and $x_{i'j}$ are the percentages of a specific (j) fatty acid in the total fatty acids present in the sample in i and i' organisms.

3. Results

Use of petrolether and benzene as the extraction agents resulted in a precipitation during oxidization of the evaporated samples.

As TLC analysis has shown, great majority of lipids in the spores of *Glomus fistulosum* were neutral fats having R_f values similar to that of triolein that was used as standard. TLC chromatography with complexing agents revealed the presence of desaturated lipids in the extracts of AM fungi. Free fatty acids and steroid compounds were also detected in the extracts. Free fatty acids of AMF were more polar than that of saprophytic fungi.

The total carbon content of an "average spore" of *Glomus fistulosum* was about 1.9 µg of triolein or 5.5 µg of glucose.

Gas chromatography

The results of the analyses are presented in Table 4. The groups of fatty acids, where the determination of specific compounds was difficult, were summed; difference between the contents of major fatty acids in mycorrhizal fungi and soil saprophytes is shown in Figs. 2 and 3. Majority of lipid material

Table 1. Extraction of lipids from spores of *Glomus fistulosum* by various solvents (per cent of total oxidizable matter in the spores)

Solvent	Percent of extracted organic matter
Folch mixture (chloroform-methanol 2:1)	81–92
Ethylacetate	75–80
Hexane	40–45

Table 2. Cluster analysis of fatty acid profiles of mycorrhizal and saprophytic fungi

Species	Number of the cluster determined using FAME profiles
Scutellospora heterogama	1
Glomus geosporum (24A)	2
Glomus geosporum (BEG11)	2
Glomus mosseae (BEG25)	2
Glomus etunicatum	2
Glomus caledonium	2
Glomus deserticola	1
Glomus fistulosum (BEG23)	2
Endogone pisiformis	3
Mortierella elongata	1
Mortierella alpina	1
Rhizopus stolonifer	1
Fusarium moniliforme	1
Syncephalastrum racemosum	1
Mucor hiemalis	1
Mucor plumbeus	1
Zygorrhynchus moelleri	1
Absidia coerulea	1
Linderina penispora	1
Micromucor ramannianus	1
Cunninghamella elegans	1

in mycorrhizal fungi is represented by compounds containing 16 carbon atoms in the molecule (16C FA), mainly palmitoleic acid, and also by considerable amounts of compounds containing 20 carbon atoms (20C FA). In contrast the saprophytic fungi synthesized mostly fatty acids having 18 atoms in the molecule (18C FA). In *Glomus deserticola* and *Endogone pisiformis* the majority of fatty acids had, surprisingly, a chain including 18 carbon atoms.

Table 3. Percent of variation unexplained by creating clusters

Number of clusters	Percent of variation
1	100
2	82.2
3	41.2
(4)	(32.5)

Mean fatty acid profiles of the fungi representing the clusters:

Group 1: Saprophytic fungi, *Glomus deserticola, Scutellospora heterogama*	
Palmitoleic acid	3.7
Palmitic acid	17.9
Oleic acid	40.1
other 18C acids	19.2
20C acids	2
long chain fatty acids	1.1
Group 2: *Glomus* spp. - AM fungi	
Palmitoleic acid	59.6
Palmitic acid	12.4
Oleic acid	10.4
other 18C acids	4.1
20C acids	9.6
long chain fatty acids	Traces
Group 3: *Endogone pisiformis*	
Palmitoleic acid	0
Palmitic acid	16.6
Oleic acid	59.6
other 18C acids	7.1
20C acids	Traces
long chain fatty acids	13.2

The results show close similarity between *Glomus* spp. (except *Glomus deserticola*), that constitute a rather homogeneous group. Lipid profiles of *Glomus deserticola* and *Scutellospora heterogama* show a closer similarity to saprophytic fungi than to AMF. The reason for such a difference from other AM fungi is not clear. However, similar results were obtained by Jabaji-Hare (1988) for *Gigaspora margarita*. No significant quantities of the specific compounds characteristic of the group of AM fungi (represented mainly by *Glomus* spp.) were found in this species.

Table 4. Percentages of fatty acids in fungal lipid extracts

Fatty acid	Palmitoleic acid	Palmitic acid	Oleic acid	Sum of other 18CFA	Sum of 20C FA	Sum of long chain FA (longer than 20C)
Scutellospora heterogama	27.2	25.3	15.3	16.6	3.7	1.0
Glomus geosporum (24A)	70.8	9.0	11.7	2.0	5.5	0
Glomus geosporum (BEG11)	59.4	16.3	9.7	6.3	3.8	0
Glomus mosseae (BEG 25)	70.2	6.6	12.9	4.8	2.4	0
Glomus etunicatum	55.3	12.9	10.3	4.9	14.6	0.6
Glomus caledonium	63.8	8.6	12.3	5.0	6.8	0
Glomus deserticola	8.5	24.8	54.3	4.9	1.9	0.7
Glomus fistulosum (BEG 23)	38.3	20.7	5.2	1.7	24.7	0
Endogone pisiformis	0	16.6	59.6	7.1	0	13.2
Mortierella elongata	0	13.9	45.5	15.8	17.7	1.7
Mortierella alpina	0	11.6	20.9	20.4	4.7	3.7
Rhizopus stolonifer	0.3	23.7	47.8	23.8	0	3.4
Fusarium moniliforme	0	24.3	40.4	34.0	0	0.5
Syncephalastrum racemosum	0	22.1	49.4	7.8	0	0
Mucor hiemalis	1.5	14.4	41.0	18.6	0	1.0
Mucor plumbeus	1.9	18.7	43.4	13.5	0	0.5
Zygorrhynchus moelleri	0	12.5	41.8	15.4	0	0.6
Absidia coerulea	0.5	16.3	23.9	34.3	0	1.3
Linderina penispora	11.1	11.5	40.3	24.8	0	0.1
Micromucor ramannianus	0.2	15.5	50.4	17.8	0	1.4
Cunninghamella elegans	0.4	16.0	46.5	21.5	0	0

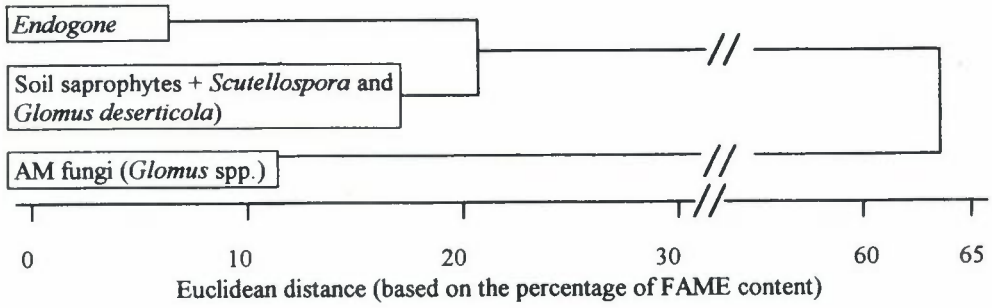

Figure 1. Dendrogram of fungal groups based on the Euclidean distance between FAME profiles in the groups.

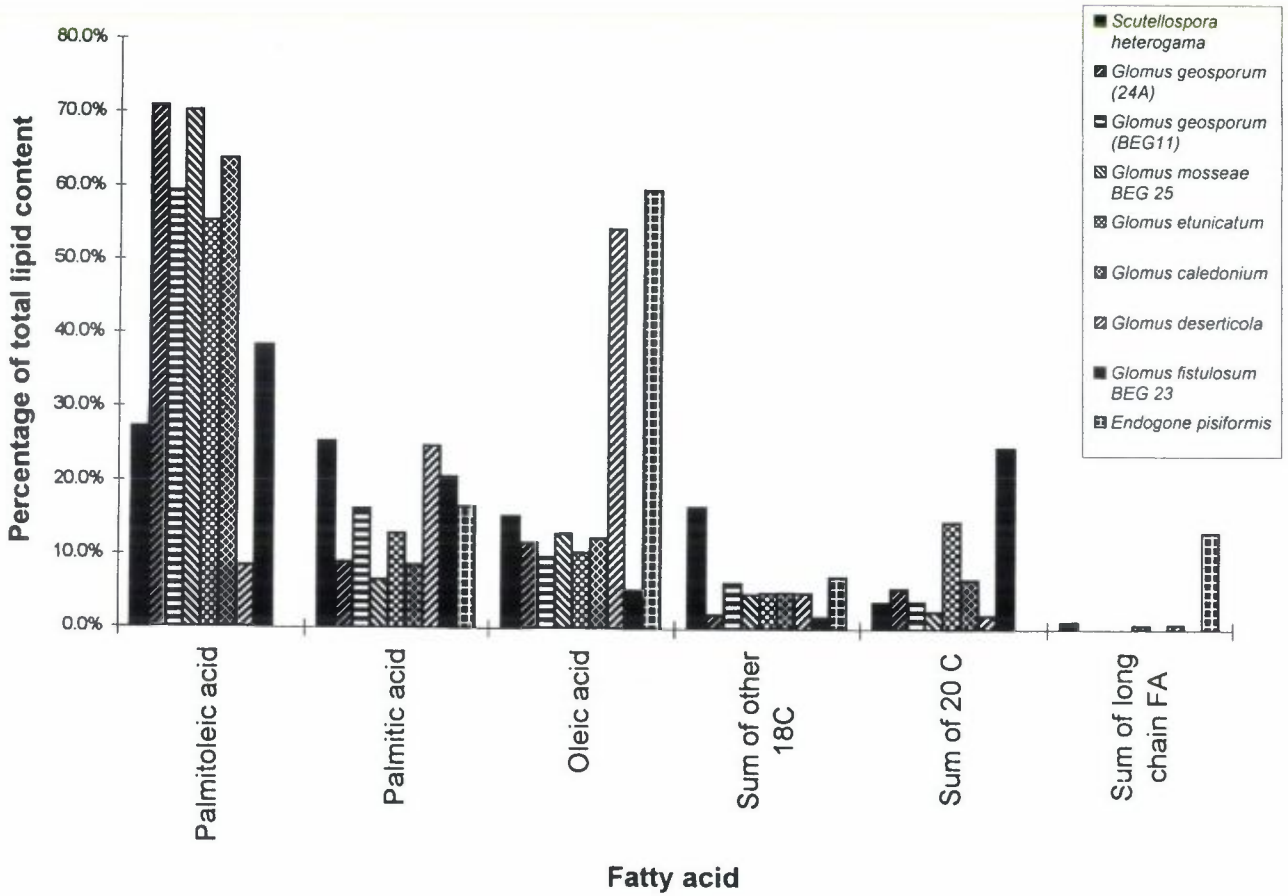

Figure 2. Lipid profiles of mycorrhizal fungi (incl. *Endogone* sp.).

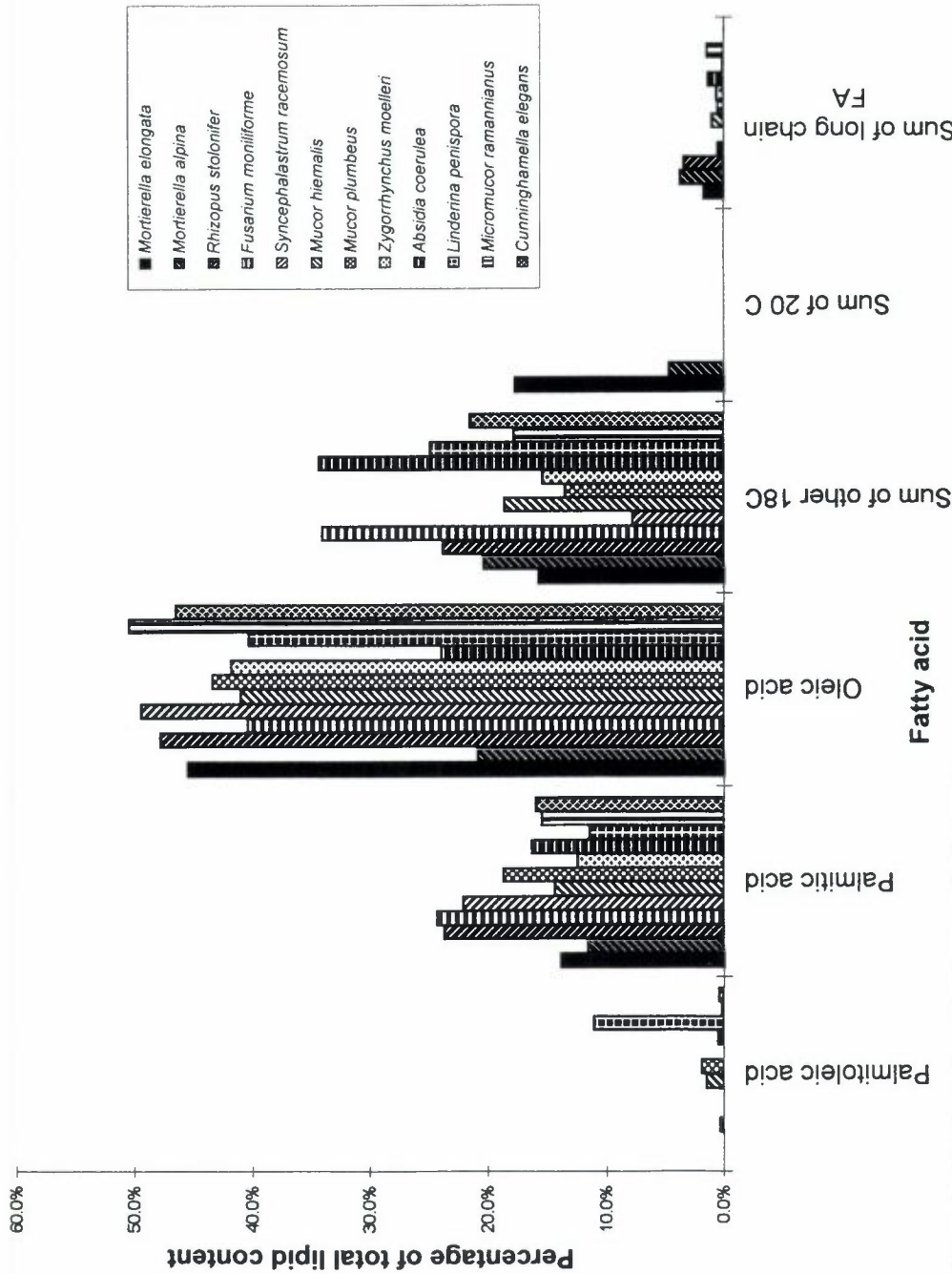

Figure 3. Lipid profiles of soil saprophytic fungi.

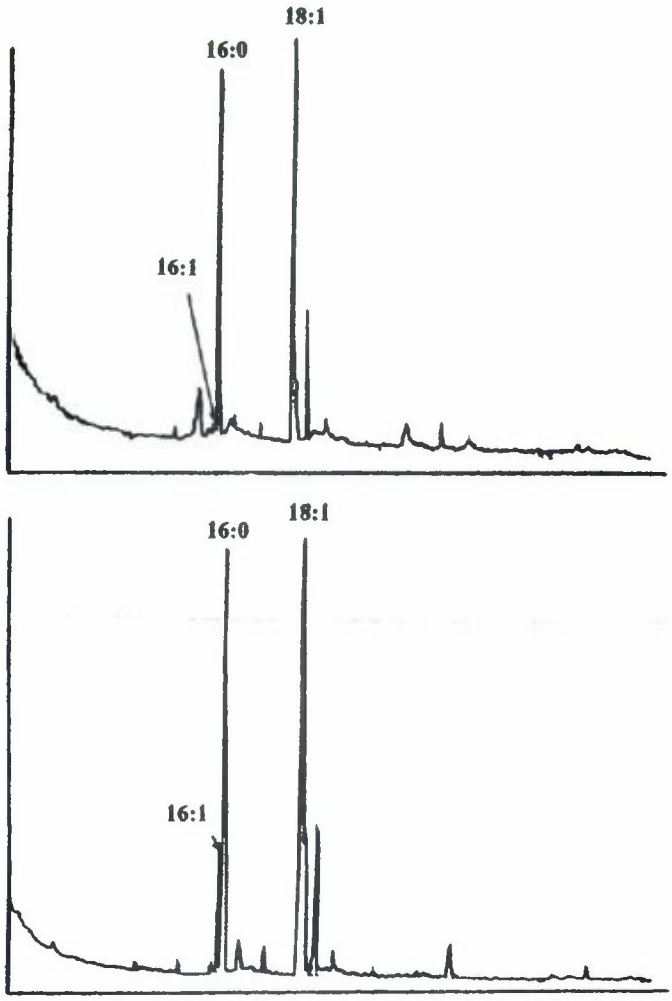

Figure 4. Fatty acid profile of root extracts of mycorrhizal and nonmycorrhizal plants. Gas chromatography-flame ionization detection, position of palmitoleic acid marked with an arrow. A. Nonmycorrhizal root extract (*Zea mays* L.), fatty acid methyl esters. B. Mycorrhizal root extract (*Zea mays* L. and *Glomus fistulosum* BEG23), fatty acid methyl esters.

A comparison of root FAME profiles of mycorrhizal and non-mycorrhizal plants is shown in the Fig. 4. The main difference is in the content of palmitoleic acid.

4. Discussion

The results presented suggest that lipids form the majority of carbon compounds in spores of AM fungi. Depending on the extraction procedure used, 40 to 90% of the total carbon can be extracted by organic solvents from the spores (Table 1). This is a rather high value, compared to the measurements of lipid contents in other fungi. For *Aspergillus nidulans*, the amount of lipids in mycelium under the lipid synthesis-supporting conditions was estimated to be about 12–20% of the carbon compounds (Wynn and Ratledge, 1977). In commercial baker's yeasts, the amount of lipids (the total of neutral lipids, free fatty acids, sterols and phospholipids) in cells is 23–32.2% of the biomass dry weight (Murakami et al., 1996). In *Mucor* species, lipid profiles were analyzed by Dexter and Cooke (1984). The total lipid content was estimated to vary from 10.3% (*M. strictus*) to 19.6% (*Mucor rufescens*) of the total organic matter. Gaspar et al. (1994) estimated the lipid content of spores of *Glomus versiforme* to be 38% of the spore fresh weight. High values of lipid contents in AM fungi shown in this work probably reflect the carbon- and energy-storage functions of spores of AM fungi. Lipid content of vegetative mycelium of AM fungi may better correspond to measurements of lipids in mycelium of other fungi (Dexter and Cooke, 1984).

The extraction mixture of chloroform-methanol (2:1) (Folch et al., 1957) was the best among the solvents used for the extraction of lipids from fungal spores (Table 1), for several reasons. The method was developed for extractions of lipids from tissues containing a high percentage of water and, we believe, this feature makes this method very useful for our purpose. Compared to the extraction with ethyl acetate (giving also a high recovery rate), a chloroform-containing mixture is more suitable because, after combining it with the water solution (1% NaCl), the nonpolar phase remains under the water phase and is thus easily obtainable from the vial.

Triacylglycerols represent the main energy store in both the AM fungi and the majority of soil saprophytic fungi, as proved here by TLC. Some studies on lipid composition and metabolism of AM fungi have been available (Beilby, 1980; Beilby and Kidby, 1980; Bentivenga and Morton, 1994; Cooper and Lösel, 1978; Jabaji-Hare et al., 1984; Jabaji-Hare 1988; Gaspar et al., 1994, 1997), but these characteristics are still not routinely used in taxonomy as is the case of various other organisms (e.g. Brondz and Olsen, 1990; Augustyn et al., 1992).

Two major patterns of fatty acids profiles were distinguished during the analyses. These two groups correspond well with the classification of fungi according to their symbiotic and/or saprophytic status (Table 2). The unified profile consisting mainly of 18C fatty acids, typical for the saprophytes under study (Fig. 3), strongly contrasted with the stable pattern observed in AM fungi, characterized by a high percentage of 16C fatty acids (Fig. 2). The fatty acid

detected in AM fungi, 9-hexadecenoic (palmitoleic) acid, was rarely observed at higher percentages in saprophytic fungi. In five zygomycetous species (*Syncephalastrum racemosum, Absidia anomala, Cunninghamella elegans, Mortierella hyalina* and *Thamnidium elegans*), Radwan et al. (1996) found that palmitoleic acid represented only up to 2.6% of the total fatty acids present. A significant part of organic compounds in the AM fungi also consists of 20C and higher fatty acids. Our results (Table 4) agree with those of Pacovsky and Fuller (1988), who observed palmitoleic acid as a major fatty acid in storage lipids in vesicles of an AM fungus. High amounts of triglycerides, diglycerides and free fatty acids in mycorrhizal roots and AM fungi were also reported by other authors (Cooper and Lösel, 1978; Jabaji-Hare, 1988).

The difference in FA profiles between AM fungi and saprophytes has still not been directly studied, but there are some reports on the lipid composition of the fungi in the literature which can be compared to the results presented in this work concerning AM fungi (Bentivenga and Morton, 1994; Jabaji-Hare, 1988).

Members of the genus *Aspergillus*, cultivated at various conditions, contain about 15–20% palmitic acid (16:0), 5–9% stearic acid (18:0), 68–73% of oleic acid (18:1) and 4–6% linoleic acid in the growing mycelium (Fernando and Bean, 1986). Dexter and Cooke (1984) studied fatty acid profiles in *Mucor* strains. They found that palmitoleic acid did not exceed 4.2% of the total FA in several *Mucor* species. The majority of lipid material was present in the form of 18C fatty acids group (mainly 18:1, 18:2 and 18:3).

A complex analysis of the lipid composition of several *Mucor* species cultivated at different temperatures (15–25°C) showed a variation in the 16C and 18C fatty acid contents that did not exceed a value of 10% with respect to the changing environmental conditions. A greater variation was found in the case of 14C and 20C fatty acids (Hammonds and Smith, 1986). In *Mortierella* spp., Kamisaka et al. (1988) observed that 50 to 80% of dry cell weight was comprised of lipids whose composition was influenced by metal ions in the culture media and the cultivation temperature. Nevertheless, biochemically different fungal strains exist, classified as the members of one species or a lower taxon. For example, a variation of production of specific FA by a mutant strain of a zygomycetous fungus *Mortierella ramanniana* is the production rate of 18% of total lipids of γ-linolenic acid (Hiruta et al., 1996).

A higher content of 18C fatty acids to the detriment of 16C ones in the extract of *Endogone pisiformis* (Table 4, Fig. 2) is explainable by the taxonomic position of the fungus (separation of *Endogonales* from *Glomales* during their evolution) (Morton and Benny, 1990). The aberration in lipid composition observed in our experiments in *Scutellospora heterogama* may reflect the evolution gap between the genera of *Gigasporinae* and *Glominae* (Morton and Benny, 1990; Jabaji-Hare, 1988). In the case of *Glomus deserticola*, the aberration of lipid

profile making it different from that of the group may be a result of geographic separation of this strain since it originated in the USA (Fig. 1).

As to the *Endogone* lipid composition, similar results were also obtained by Jabaji-Hare et al. (1988), who reported that majority of fatty acids was present as 18:1 and 16:0 (60% and 20%, respectively, in the neutral lipid fraction), 16:1 fatty acid being present only in trace amounts.

In other fungal groups, a considerable amount of 16:1 (palmitoleic) acid was found only in *Pythium* (6%) and *Phytophthora* (3%) (Müller et al., 1994). Other taxons of the analysed fungi (other Phycomycetes, Asco- and Basidiomycetes) contained up to 1% of the total content of fatty acids. Only in the commercial baker's yeasts (Murakami et al., 1996) was the percentage of palmitoleic acid in lipids higher. Main fatty acids included in lipids were: 16:1 > 18:1 > 16:0 > 18:0. In the basidiomycete *Heterobasidion annosus* (Müller et al., 1995), the majority of lipid material is composed of 18:2 and 18:0 fatty acids (30% and 20%, respectively). The other fatty acids are 16:0 (11%) and 18:1 (3%). Sterols represent 13% of total lipids. Fatty acid composition of lipids of another basidiomycetous fungus, *Ganoderma australe* was shown by Martínez et al. (1991) to have the respective proportions of 18:1, 18:2, 16:0, 18:0, 24:0, 16:1Δ9 and 16:1Δ3 acids: 44, 32, 11, 3, 2.5, 1.6 and 0.2%.

Comparing the content of fatty acids in mycorrhizal and nonmycorrhizal roots, we found the content of palmitoleic acid to be a specific sign of mycorrhization (see Figs. 4a and b). This observation confirms the results of Ölsson et al. (1995) who considered the content of 16:1(ω5), 20:4 and 20:5 fatty acids to be highly significant markers of root colonization by *Glomus* sp. and *Glomus caledonium*. When used for soil samples, the estimation may be inaccurate due to some bacteria present (Walker, 1969). The bacteria, in contrast, usually lack polyunsaturated compounds such as 20:5, which makes these FA potential candidates for mycorrhiza marker compounds. On the other hand this compound was detected in zygomycetous fungi (Amano et al., 1992).

Gaspar et al. (1997) found the amount of palmitoleic acid to be about 6 times higher in roots colonized by *Glomus versiforme* than in the control. A correlation between the 16:1ω5 fatty acid content and the colonization of the root by *Glomus* spp. was observed by Graham et al. (1995). As Beilby (1980) found a similar lipid pattern, compared to that referred above, also with *Acaulospora* sp., a combination of 16:1 fatty acids and polyunsaturated 20 carbon compounds seems to be a good biochemical marker for AM fungi belonging to the suborder Glomineae. The suborder Gigasporineae is characterized by a different pattern of FA, which is obvious from our present results on *Scutellospora heterogama* and from the results of Jabaji-Hare (1988) who found the dominant FA by *Gigaspora margarita* to be 16:0 (palmitic) FA.

Stability of the production of marker fatty acids by glomalean endomycorrhizal fungi grown on different host plants and the possibility of storage

of spores up to 22 months (at 4°C), that did not significantly change their lipid composition, were proved by Bentivenga and Morton (1994). They were able to distinguish *Gigaspora* from other fungi (due to 18:1 content), but they did not find a specific marker for distinguishing *Glomus* spp. from *Scutellospora*.

In conclusion, it is obvious that 9-hexadecenoic acid is a compound characteristic for a majority of *Glomus* species studied. Regarding the scarcity of this FA in soil zygomycetous saprophytes and the presence of this compound in some yeasts, lipid profiles of glomalean fungi seem to be more similar to those of ascomycetous yeasts than to those of filamentous *Zygomycetes*. Even though AMF are considered to be an evolutionarily conserved group (Pirozynski and Dalpé, 1989), this work shows rather a high variation of lipid profiles within *Glomus* spp.

The content of palmitoleic acid is a prospective marker for determination of AM infection in roots. It could be used as a reproducible and easily applicable method for routine estimations that had so far been performed almost exclusively by microscopical observation of stained root samples.

Acknowledgement

We express our gratitude to Dr. Yolande Dalpé for kindly providing the isolate of *Endogone pisiformis* and to Dr. Marie Vanova (Culture Collection of Fungi, Charles University, Prague) for kindly providing pure strains of soil saprophytic fungi for analytical studies. We thank Mrs. Jana Rouckova for her outstanding technical assistance during cultivation of the mycorrhizal fungi. This work was supported by the European Communities and the Ministry of Education, Youth and Sports of the Czech Republic, project COST-OC 821.10.

REFERENCES

Amano, N., Shinmen, Y., Akimoto, K., Kawashima, H., and Amachi, T. 1992. Chemotaxonomic significance of fatty acid composition in the genus *Mortierella* (Zygomycetes, Mortierellaceae). *Mycotaxon* **44**: 257–265.

Augustyn, O.P.H., Kock, J.L.F., and Ferreira, D. 1992. Differentiation between yeast species, and strains within a species, by cellular fatty acid analysis 5: A feasible technique? *Systematic and Applied Microbiology* **15**: 105–115.

Beilby, J.P. and Kidby, D.K. 1980. Biochemistry of ungerminated and germinated spores of the vesicular arbuscular fungus, *Glomus caledonius*: changes in neutral and polar lipids. *Journal of Lipid Research* **21**: 739–750.

Beilby, J.P. 1980. Fatty acids and sterol composition of ungerminated spores of the vesicular arbuscular fungus, *Acaulospora laevis*. *Lipids* **15**: 949–952.

Bentivenga, S.P. and Morton, J.B. 1994. Stability and heritability of fatty acid methyl ester profiles of glomalean endomycorrhizal fungi. *Mycological Research* **98**: 1419–1426.

Bevege, D.I., Bowen G.D., and Skinner, M.F. 1975. Comparative carbohydrate physiology of ecto- and endo-mycorrhizas. In: *Endomycorrhizas*. F.E. Sanders, B. Mosse and P.B. Tinker, eds. Academic Press, London, pp. 149–174.

Bowen, G.D. 1985. The fate of assimilates and the cost of the vesicular-arbuscular mycorrhizal symbiosis. TECDOC-338, International Atomic Energy Agency (IAEA), Vienna, 1985.

Brangdon, J.H. 1951. Colorimetric determination of blood lipids. *Journal of Biological Chemistry* **190**: 513–516.

Brondz, I. and Olsen, I. 1990. Multivariate analyses of cellular carbohydrates and fatty acids of *Candida albicans, Torulopsis glabrata* and *Saccharomyces cerevisiae*. *Journal of Clinical Microbiology* **28**: 1854–1857.

Buwalda, J.G. and Goh, K.M. 1982. Host fungus competition for carbon as a cause of growth depressions in vesicular-arbuscular mycorrhizal ryegrass. *Soil Biology and Biochemistry* **14**: 103–106.

Cooper, K.M. and Lösel, D.M. 1978. Lipid physiology of VA mycorrhiza. I. Composition of lipids in roots of onion, clover and ryegrass infected with *Glomus mosseae*. *New Phytologist* **80**: 143–151.

Cox G., Sanders, F.E., Tinker P.B., and Wild J.A. 1975. Ultrastuctural evidence relating to host endophyte transfer in a vesicular-arbuscular mycorrhiza. In: *Endomycorrhizas*. F.E. Sanders, B. Mosse and P.B. Tinker, eds. Academic Press, London, pp. 297–312.

Fernando, T. and Bean G. 1986. Effects of tricyclazole on growth, release of aflatoxin, and sterol and fatty acid content, by *Aspergillus* isolates. *Transactions of the British Mycological Society* **87**: 445–449.

Folch, J., Lees, M., and Sloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**: 497–509.

Gaspar, L., Pollero, R., and Cabello M. 1997. Partial purification and characterization of lipolytic enzyme from spores of the arbuscular mycorrhizal fungus *Glomus versiforme*. *Mycologia* **89**: 610–614.

Gaspar, L., Pollero, R., and Cabello M. 1997a. Variations in the lipid composition of alfalfa roots during colonization with the arbuscular mycorrhizal fungus *Glomus versiforme*. *Mycologia* **89**: 37–42.

Gaspar, M.L., Pollero, R.J., and Cabello, M.N. 1994. Triacylglycerol consumption during spore germination of VAM fungi. *Journal of American Oil Chemists Society* **71**: 449–452.

Gerdemann, J.W. and Trappe, J.M. 1974. The Endogonaceae in the Pacific Northwest. *Mycologia Memoir* No. 5, The New York Botanical Garden, 76 pp.

Graham, J.H., Hodge, N.C., and Morton, J.B. 1995. Fatty acid methyl ester profiles for characterization of Glomalean fungi and their Endomycorrhizae. *Applied and Environmental Microbiology* **61**: 58–64.

Hammonds, P. and Smith, S.N. 1986. Lipid composition of a psychrophilic, a mesophylic and thermophylic *Mucor* species. *Transactions of the British Mycological Society* **86**: 551–560.

Hayman, D.S. 1974. Plant growth responses to vesicular-arbuscular mycorrhiza. IV. Effect of light and temperature. *New Phytologist* **73**: 71–80.

Hebak, P. and Hustopecky, J. 1987. Vicerozmerne statisticke metody s aplikacemi. SNTL, Praha, 454 pp.

Hiruta, O., Futamura, T., Takebe, H., Satoh, A., Kamisaka, Y., Yokodi, T., Nakabara, T., and Suzuki, O. 1996. Optimization of scale-up of γ-linolenic acid by *Mortierella ramanniana* MM15-1, a high γ-linolenic acid producing mutant. *Journal of Fermentation and Bioengineering* **82**: 366–370.

Jabaji-Hare, S. 1988. Lipid and fatty acid profiles of some vesicular-arbuscular mycorrhizal fungi: contribution to taxonomy. *Mycologia* **80**: 622–629.

Jabaji-Hare, S.H., Deschene, A., and Kendrick, B. 1984. Lipid content and composition of vesicles of a vesicular-arbuscular mycorrhizal fungus. *Mycologia* **76**: 1024–1030.

Jabaji-Hare, S., Tremblay, M.F., and Parent, S. 1988. Changes in concentrations of total fatty acids, soluble proteins, nitrogen and phosphatase activities in mycelia of *Endogone pisiformis* (Endogonaceae) during growth. *Mycologia* **80**: 54–62.

Martínez, A.T., Barrasa J.M., Prieto A., and Blanco M.N. 1991. Fatty acid composition and taxonomic status of *Ganoderma australe* from Southern Chile. *Mycological Research* **95**: 782–784.

Mitchell, D.T. and Read, D.J. 1981. Utilization of inorganic and organic phosphates by the mycorrhizal endophytes of *Vaccinium macrocarpon* and *Rhododendron ponticum*. *Transactions of the British Mycological Society* **76**: 255–260.

Morton, J.B. and Benny, G.L. 1990 Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order *Glomales*, two new suborders: Glominae and Gigasporinae and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. *Mycotaxon* **37**: 471–491.

Müller, M.M., Kantola, R., Korhonen, K., and Uotila, J. 1995. Combined fatty acid and sterol profiles of *Heterobasidion annosum* intersterility groups S, P and F. *Mycological Research* **99**: 1025–1033.

Müller, M.M., Kantola, R., and Kitunen, V. 1994. Combining sterol and fatty acid profiles for the characterization of fungi. *Mycological Research* **98**: 593–603.

Murakami, Y., Yokoigawa, K., Kawai, F., and Kawai, H. 1996. Lipid composition of commercial baker's yeast having different freeze-tolerance in frozen dough. *Bioscience, Biotechnology and Chemistry* **60**: 1874–1876.

Ölsson, P.A., Baath, E., Jakobsen, I., and Söderström, B. 1995. The use of phospholipids and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. *Mycological Research* **99**: 623–629.

Pacovsky, R.S. and Fuller, G. 1988. Mineral and lipid composition of *Glycine-Glomus-Bradyrhizobium* symbiosis. *Physiologia Plantarum* **72**: 733–746.

Radwan, S.S., Zreik, M.M., and Mulder, J.L. 1996. Distribution of arachidonic acid among lipid classes during culture ageing of five Zygomycete species. *Mycological Research* **100**: 113–116.

Walker, R.W. 1969. Cis-11-hexadecenoic acid from *Cytophage hutchinsonii* lipids. *Lipids* **4**: 15–18.

Wynn, J.P. and Ratledge, C. 1997. Malic enzyme is a major source of NADPH for lipid accumulation by *Aspergillus nidulans*. *Microbiology* (Reading) **143**: 253–257.