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EXPERIMENTAL ARTICLES

Fatty Acids in the Species of Several Zygomycete Taxa

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Abstract—The composition of fatty acids synthesized de novo by thirty strains of zygomycetes from various taxa was studied. The qualitative fatty acid compositions of the fungal lipids were found to be virtually identical, but there were significant differences in the contents of individual acids. Highly active producers of essential C_{18} fatty acids, with their content exceeding 30–40% of total fatty acids, were discovered among the fungi of the families Mucoraceae, Pilobolaceae, and Radiomycetaceae. Linoleic acid was found to predominate in the fungi of the genera *Radiomyces*, *Mycotypha*, and *Circinella*, and linolenic acid (identified as its γ-isomer by gas-liquid chromatography), in the fungi of the genera *Absidia*, *Circinella*, *Pilaira*, and *Hesseltinella*. The total yield (mg/l) of bioactive acids ($C_{18:3}$, $C_{18:2}$, $C_{18:1}$) varied from 761.4 in *Pilaira anomala* to 3477.9 in *Syncephalastrum racemosum*; the total yield of essential acids, from 520.7 in *Pilaira anomala* to 1154.5 in *Hesseltinella vesiculosa*; of linoleic acid, from 279.7 in *Pilaira anomala* to 836.3 in *Mycotypha indica*; and of linolenic acid, from 120.8 in *Mycotypha indica* to 708.0 in *Hesseltinella vesiculosa*. The data on the efficient synthesis of these acids make the actively producing strains promising for biotechnological synthesis of commercially valuable lipids. *Linderina pennispora* VKM F-1219, a zygomycete of the family Kickxellaceae, which was earlier singled out into the order Kickxellales, was shown to differ from zygomycetes of the order Mucorales in having a high content of *cis*-9-hexadecenoic (palmitoleic) acid, reaching 37.0% of the fatty acid total.

Key words: fatty acids, zygomycetes, order Mucorales, order Kickxellales.

Studies on lipogenesis in filamentous fungi of the class Zygomycetes reveal their high lipogenic activity and a high diversity of fatty acids, which include C_{12}–C_{24} compounds dominated in most zygomycetes by long-chain unsaturated fatty acids [1–3]. Species- and strain-specific features of fatty acid spectra are also detected; for example, different species of zygomycetes of the genus *Entomophthora* predominantly contain either short-chain saturated or long-chain unsaturated fatty acids [3, 4]. Within the genus *Mortierella*, several strains of one species, *M. alpina*, have the highest levels of arachidonic acid synthesis [5].

In the past five years, studies on fatty acids of micromycetes were actively pursued to develop methods of fast analysis of polyunsaturated acids and to apply fungal fatty acid spectra for characterization of fungal isolates and soil samples [6]. Efforts also continue to employ composition of lipids, including sterols and fatty acids, in chemotaxonomy of closely related fungal taxa [7]. Fatty acid spectra are used for characterization of individual fungal strains of the order Mucorales.

The increasing use of polyunsaturated fatty acids as efficient drugs and dietary supplements [8] necessitates screening of microorganisms for strains actively synthesizing essential eicosapolyenoic fatty acids. Lower filamentous eukaryotic microorganisms are especially promising as producers of valuable lipids, having a number of advantages over the traditional sources of animal or plant origin [9, 10].

In this paper, we have investigated the composition of fatty acids in lower filamentous fungi belonging to poorly studied and unstudied zygomycete taxa.

MATERIALS AND METHODS

This study covered 30 zygomycete strains stored in the All-Russia Collection of Microorganisms (VKM) of the Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, representing 21 species from 14 genera belonging to seven families of the order Mucorales, including *Absidia corymbifera* F-649, F-1524, F-515, F-965, *Benjaminiella poitrasii* F-1353, *Circinella muscae* F-659, *Circinella rigida* F-860, *Circinella naumovii* F-1250, *Chaetocladium brefeldii* F-1047, F-1112, *Chaetocladium jonesii* F-1046, *Fennellomyces linderi* F-1220, *Helicostylum pulchrum* F-1418, F-1051, *Helicostylum elegans* F-1045, *Hesseltinella vesiculosa* F-1523, *Mycotypha africana* F-1214, *Mycotypha indica* F-3498, *Mucor psychrophilus* F-1441, *Pilaira anomala* F-1322, *Pilaira caucasica* F-1246, *Pirella circinans* F-1722, *Radiomyces embreei* F-1352, *Radiomyces spectabilis* F-1354, *Syncephalastrum racemosum* F-438, F-623, F-1768, *Thamnidium elegans* F-696, F-2427, and the fungus *Linderina pennispora* F-1219 of the family Kickxellaceae of the order Kickxellales. The studied zygomycetes are widespread in Russia and other territories of Europe, America, and Africa. These fungi are mostly saprotrophic, often colonizing rotting fruit, berries, other plant remains, animal feces, and soil. Some zygomycetes are psychrotolerant fungi, preferring temperatures 10–18°C [11]. In this work psychrotolerant fungi were represented by strains of the genera *Chaetocladium*, *Helicostylum*, and the strain *Mucor psychrophilus* VKM F-1441.

The cultures were grown on wort agar; a water suspension of a 7-day culture grown on this medium was used as the inoculum. Submerged cultivation was performed in 250-ml flasks, each flask containing 50 ml of the following medium prepared in tap water: 40 g/l glucose, 20 g/l Bacto-peptone, 1.4 g/l KH_2PO_4, 0.25 g/l $Mg_2SO_4 \cdot 7H_2O$, 1 g/l yeast extract. The cultures were grown in an orbital shaker at 220 rpm at 26–28°C (11–12°C for the psychrotolerant cultures).

In the screening experiments, fatty acids were analyzed at the end of the exponential phase, generally corresponding to 72 h of growth but in some cultures reached after 48 or 96 h. If necessary, growth and lipogenesis were assayed in 7-day time-course experiments. The fungal growth was assayed by weight of the biomass after drying it at 96°C until stable weight. The lipid content of the biomass and amounts of individual fatty acid components were determined by established methods [12]. Lipids were extracted by two consecutive treatments with chloroform–methanol (2 : 1 and 1 : 2, v/v). Fatty acids were converted to their methyl esters by acid methanolysis. The methyl esters of fatty acids were separated, identified and quantified by gas-liquid chromatography using a Model 3700 chromatograph (Russia) equipped with a flame ionization detector. The column (glass tube, 1 m × 3 mm i.d.) was packed with WAW-DMCS-HP chromosorb (80 to 100 mesh) coated with 17% diethyl glycol succinate. Argon was used as the carrier gas (50 ml/min); the chromatography was performed isothermically at 180°C, the injection port was kept at 250°C. Individual fatty acids in the mixture were identified by their retention time compared with the standard. Content of the individual fatty acids in the mixture was determined by the triangulation method.

RESULTS AND DISCUSSION

Our results indicate that the composition of fatty acids in the lipids of the studied fungal species of the order Mucorales is similar to that of zygomycetes of the genera *Blakeslea*, *Cunnighamella*, *Mucor*, and *Rhizopus*, studied by us earlier [3, 13].

Table 1 shows the content of short-chain fatty acids (C_{14}–C_{16}) and their main constituent, palmitic acid ($C_{16:0}$); the content of long-chain fatty acids (C_{18}), including saturated stearic acid; the total of unsaturated fatty acids; the data on growth of the fungi and on their lipogenic activity. Intervals of the contents of selected fatty acids in some fungal species (such as the *Absidia* strains) derived from both screening and time course experiments are shown in Table 2. Figures 1a, 1b illustrate the intervals of the contents of essential fatty acids and their individual components.

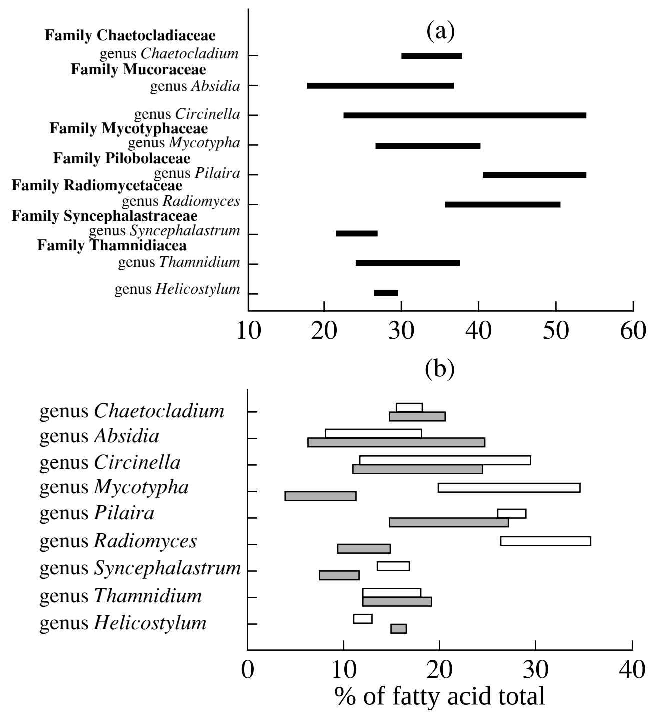

Fig. 1. Intervals of variation in the content of essential fatty acids in species from different zygomycete families: (a) total essential fatty acids and (b) individual fatty acids (open bars, $C_{18:2}$; hatched bars, $C_{18:3}$).

As can be seen from Table 1, fungal biomass reached 2.7 g/dl, with the lipid content 21–22% of the dry weight. The biomass of the psychrotolerant fungi, in general, did not exceed 0.66 g/dl after 9-day growth at 11–12°C. The analysis of fatty acids showed that in the species of the order Mucorales short-chain acids (14–16 carbon atoms in the chain) amount to 17.0–41.6% of the total cellular fatty acids and are mainly represented by saturated components, primarily palmitic acid. Myristic and palmitoleic acids are minor components, $C_{14:0}$ acid is most often found in trace amounts or constitutes no more than 6.0% of the total fatty acids, with only *Pilaira caucasica* and *Thamnidium elegans* containing 8.5% and 9.4%, respectively (Table 2). $C_{12:0}$ acid is found in trace amounts, as is the fraction comigrating on gas chromatography with the $C_{15:0}$ acid standard (no more than 2.7%) (Table 8). Some samples contained trace amounts of nonidentified acids.

Long-chain fatty acids constitute more than 60%, sometimes reaching 80% of total fatty acids, and were usually represented by eighteen-carbon, mainly unsaturated acids with one to three double bonds (Tables 1–3,

Table 1. Fatty acids in zygomycetes of different taxa*

No.	Strains	Fatty acids, % of fatty acid total					Biomass, g/dl	Lipids % of dry biomass
		C_{14}–C_{16} total	$C_{16:0}$	C_{18} total	$C_{18:0}$	unsaturated C_{18} total		
	Family Chaetocladiaceae							
1	*Chaetocladium brefeldii* F-1047	22.9	17.4	76.4	10.4	66.0	0.41	9.8
2	*Chaetocladium brefeldii* F-1112	22.9	18.9	73.3	7.3	66.0	2.70	16.1
3	*Chaetocladium jonesii* F-1046	25.2	18.3	74.8	12.2	62.6	0.38	10.0
	Family Mucoraceae							
4	*Absidia corymbifera* F-649	28.9	25.8	71.1	1.8	69.3	2.11	15.1
5	*Absidia corymbifera* F-1524	32.1	29.5	67.9	9.2	58.7	2.60	14.5
6	*Absidia corymbifera* F-515	35.3	33.4	64.6	5.4	59.2	1.88	21.9
7	*Absidia corymbifera* F-965	23.7	19.8	76.8	7.1	69.7	1.30	13.2
8	*Circinella muscae* F-659	35.9	28.8	64.1	6.9	57.2	1.92	14.7
9	*Circinella rigida* F-860	24.4	18.7	75.6	5.7	69.9	1.74	7.1
10	*Circinella naumovii* F-1250	26.7	18.7	73.3	3.6	69.7	1.33	9.5
11	*Mucor psychrophilus* F-1441	20.7	15.3	69.8	10.0	59.8	0.52	8.7
	Family Mycotyphaceae							
12	*Mycotypha indica* F-3498	32.3	24.7	66.4	5.8	60.6	1.66	11.2
13	*Mycotypha africana* F-1214	41.6	33.1	58.3	3.8	54.5	1.37	8.4
14	*Benjaminiella poitrasii* F-1353	24.9	16.4	70.3	3.7	66.6	0.78	7.3
	Family Pilobolaceae							
15	*Pilaira caucasica* F-1246	28.6	20.2	70.0	9.6	60.4	1.39	12.7
16	*Pilaira anomala* F-1322	18.9	12.5	81.2	6.2	75.0	0.81	12.5
	Family Radiomycetaceae							
17	*Radiomyces embreei* F-1352	19.7	17.1	80.2	11.8	68.4	1.68	14.2
18	*Radiomyces spectabilis* F-1354	22.9	17.6	77.1	4.4	72.7	1.65	9.4
	Family Synsephalastraceae							
19	*Synsephalastrum racemosum* F-438	17.0	15.2	82.9	6.5	76.4	2.06	21.3
20	*Synsephalastrum racemosum* F-623	20.1	18.9	79.8	6.3	73.5	2.00	10.1
21	*Synsephalastrum racemosum* F-1768	21.4	19.3	78.6	6.4	72.2	2.18	16.1
	Family Thamnidiaceae							
22	*Hesseltinella vesiculosa* F-1523	19.2	17.6	81.0	11.2	69.8	2.09	17.7
23	*Thamnidium elegans* F-696	37.2	29.2	62.7	5.6	57.1	1.54	17.1
24	*Thamnidium elegans* F-2497	32.5	22.3	67.6	3.8	63.8	1.84	13.6
25	*Pirella circinans* F-1722	31.3	25.9	68.2	8.8	59.4	1.06	12.9
26	*Fennellomyces linderi* F-1220	29.5	25.8	70.0	10.4	59.6	1.94	21.3
27	*Helicostylum elegans* F-1045	21.9	15.3	68.3	11.9	56.4	0.35	13.6
28	*Helicostylum pulchrum* F-1051	21.0	14.9	71.4	10.8	60.6	0.62	8.6
29	*Helicostylum pulchrum* F-1418	25.1	18.7	69.1	8.8	60.3	0.66	7.8
	Family Kickxellaceae							
30	*Linderina pennispora* F-1219	53.7	15.1	40.6	2.0	38.6	1.91	7.0

* The highest values detected in the screening are shown; C_{20} fatty acids were found in trace amounts in most of the strains.

Table 2. Intervals of variation in the content of major fatty acids in the species of the order Mucorales

Taxa	Fatty acids, % of fatty acid total					
	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	polyenoic C_{18}
Family Chaetocladiaceae						
genus *Chaetocladium*	traces–2.6	17.4–18.9	3.5–4.3	7.3–12.2	26.6–35.8	30.1–37.7
Family Mucoraceae*	traces–3.7	15.3–36.4	1.9–4.9	1.8–12.6	19.9–49.3	17.7–53.7
genus *Absidia*	traces–1.3	19.8–36.4	1.9–3.3	1.8–12.6	28.1–49.3	17.7–36.6
genus *Circinella*	2.2–3.7	18.0–28.8	2.6–4.9	3.6–6.9	19.9–36.1	22.5–53.7
Family Mycotyphaceae**	2.1–5.9	11.6–36.6	2.1–6.2	3.1–9.7	23.5–34.0	26.6–40.0
genus *Mycotypha*	2.1–2.3	24.3–36.6	3.6–6.2	3.8–9.7	23.5–28.4	26.6–40.0
Family Pilobolaceae						
genus *Pilaira*	4.9–8.5	12.5–20.2	1.3–2.0	6.2–9.6	21.4–23.7	40.7–53.7
Family Radiomycetaceae						
genus *Radiomyces*	traces	15.7–19.7	2.6–4.1	4.4–11.8	26.0–33.4	35.7–50.5
Family Synsephalastraceae						
genus *Synsephalastrum*	traces–0.6	10.6–19.9	1.2–2.1	6.3–8.7	44.9–54.8	21.6–26.8
Family Thamnidiaceae***	1.9–9.4	14.9–29.2	1.0–4.2	3.8–11.9	24.5–42.5	23.7–35.4
genus *Thamnidium*	6.0–9.4	22.3–29.2	1.9–4.2	3.8–5.6	27.4–33.5	24.2–37.4
genus *Helicostylum*	2.9–3.6	14.9–18.7	3.0–3.3	8.8–11.9	29.0–33.7	26.6–29.5

* Including the genus *Mucor.*
** Including the genus *Benjaminiella*.
*** Including the genera *Pirella*, *Fennellomyces*, and *Hesseltinella*.

Table 3. Composition and content of the major fatty acids in psychrotolerant zygomycetes

Growth and lipogenesis indicators	Strains						
	Chaetocladium			*Helicostylum*			*Mucor psychrophilus* F-1441
	brefeldii F-1047	*brefeldii* F-1112	*jonesii* F-1046	*elegans* F-1045	*pulchrum* F-1051	*pulchrum* F-1418	
Major fatty acids,* % of fatty acid total							
$C_{14:0}$	2.0	traces	2.6	3.6	2.9	3.1	2.5
$C_{16:0}$	17.4	18.9	18.3	15.3	14.9	18.7	15.3
$C_{16:1}$	3.5	4.0	4.3	3.0	3.2	3.3	2.9
$C_{18:0}$	10.4	7.3	12.2	11.9	10.8	8.8	10.0
$C_{18:1}$	28.3	35.8	26.6	29.0	31.1	33.7	33.2
$C_{18:2}$	18.1	15.4	15.5	12.4	13.0	11.1	12.5
$C_{18:3}$	19.6	14.7	20.5	15.0	16.5	15.5	14.1
$C_{20:0}$	traces	traces	traces	2.1	2.5	1.9	2.7
$C_{20:1}$	traces	1.9	traces	7.8	5.2	4.0	6.9
C_x	–	2.0	–	–	–	–	–

* $C_{12:0}$ and three unidentified fatty acid fractions, including C_x (presumably $C_{20:2}$), were found in trace amounts.

Figs. 1a, 1b). The content of the saturated $C_{18:0}$ acid fell between 1.8 and 12.2%. Unsaturated C_{18} acids were dominated by oleic acid, $C_{18:1}$, constituting one-third of the total fatty acids in most of the cultures, and even more in some of them. In *Syncephalastrum racemosum*, for instance, the oleic acid content exceeded 50% of the total fatty acids. Polyunsaturated fatty acids in all the studied species were represented by linoleic and linolenic acids, the latter identified with its C_{18}-isomer by gas-liquid chromatography with the standard (methyl ester of γ-linolenic acid).

Long-chain acids with twenty-carbon chains, including arachidonic acid, were found in most of the

Table 4. Fatty acids in two strains of *Absidia corymbifera*

Growth and lipogenesis indicators	Strains	
	A. corymbifera F-515	*A. corymbifera* F-965
Biomass, g/dl	1.88	1.30
Lipids, % of dry biomass	21.9	13.2
Fatty acids, % of fatty acid total		
$C_{14:0}$	traces	0.6
$C_{16:0}$	33.4	19.8
$C_{16:1}$	1.9	3.3
$C_{18:0}$	5.4	7.1
$C_{18:1}$	41.5	33.7
$C_{18:2}$	11.4	11.5
γ-$C_{18:3}$	6.3	24.5
(Total C_{18})/$C_{16:0}$	1.93	3.88
(Polyenoic C_{18})/$C_{18:1}$	0.43	1.08
$C_{18:2}/C_{18:1}$	0.27	0.34
$C_{18:3}/C_{18:2}$	0.53	2.09

Table 5. γ-Linolenic acid content in the lipids of different strains of *Absidia corymbifera*

Strains VKM	γ-Linolenic acid, % of fatty acid total	
	interval of variation	mean
F-515	6.3–10.7	9.40 ± 1.91
F-649	12.6–14.7	13.37 ± 1.27
F-965*	15.7–24.5	20.41 ± 3.12
F-1524	8.9–11.4	10.59 ± 0.84

* Arachidonic acid was found in trace amounts (below 2.4%).

cultures only as minor components. The psychrotolerant fungal species demonstrated higher levels of C_{20} acids (Table 3); for example, in *Helicostylum elegans* they totaled 9.9%. Interestingly, arachidonic acid was absent from most of the studied species or was present in trace or minor amounts (less than 2.4%), despite literature reports on high arachidonic acid content (4.3–24.3%) in the lipids of *Syncephalastrum racemosum*, *Absidia anomala*, and *Thamnidium elegans* [14].

Our results (Fig. 1a) indicate that zygomycetes of certain families actively produce essential polyunsaturated C_{18} fatty acids, which cannot be synthesized by mammals, including humans, and need to be ingested for their vital needs.

The dominant forms of C_{18} polyenoic acids differed between different species (Fig. 1b). The strains of the genus *Radiomyces* had linoleic acid content reaching 26.3–35.6% of the total fatty acids, whereas in the fatty acids of *Absidia* and *Pilaira*, the content of γ-linolenic acid exceeded 20%.

The fungi of the family Mucoraceae are widely held to be promising as highly active producers of γ-linolenic acid. We found the highest level of this acid in *Absidia corymbifera* F-965, a fungus of this family. The screening revealed a higher level of γ-linolenic acid in this strain than in *A. corymbifera* F-515 (Table 4), which, in turn, had higher contents of palmitic and oleic acids. The observed differences between these two strains in the contents of individual fatty acids reflects the lower intensity of the processes of the $C_{16:0}$ acid elongation and mono- and dienoic acid desaturation, especially catalyzed by δ-6-desaturase, in *A. corymbifera* F-515. These differences, however, may be related not only to lipid metabolism properties of the cultures, but also to their different physiological status. To account for these factors, we studied the fatty acid composition of these and two more strains of *A. corymbifera* in a time course experiment run for seven days. The results (Table 5) showed that *A. corymbifera* F-965 was the most active producer of γ-linolenic acid among the tested strains and that it could be regarded as a promising industrially important source of this compound. However, this form may be developed into an industrial strain only after careful examination of its health hazards in view of known pathogenic effects of this fungal species [15]. Another interesting feature of lipids from this zygomycete strain is the presence of lipid compounds not found in other fungi. In a study of the chemical structures of polar lipids from *A. corymbifera* F-965, Batrakov *et al*. [16] discovered novel nitrogen-containing phospholipids among the cellular lipids of this strain.

The fatty acid composition of other studied zygomycetes, including those representing still-unstudied taxa, show that, despite the fact that the fatty acid spectra of the fungi of the order Mucorales are virtually identical in all investigated strains, contents of some fatty acids vary significantly. Several highly active producers of essential fatty acids were discovered in our study; for example, intense synthesis of γ-linolenic acid was found not only in Mucoraceae zygomycetes, but also in the species from other families, as can be seen from Tables 1–6.

Drawing parallels with plant oils, which are classified by the content of unsaturated fatty acids prevailing in the fatty acid total [17, 18], we can assign fungal lipids to different types according to their prevalent fatty acids and treat them as analogs of the related plant oil types (Table 6). For instance, our earlier studies of zygomycetes of the genus *Cunninghamella* (*C. japonica*, *C. elegans*) revealed that the lipids produced by de novo synthesis contain 39–59% oleic acid [3] and thus correspond to the oleic type lipids; however, physiological influences can shift the lipid spectrum in the polyenoic (C_{18}) direction, with linoleic acid increasing to 18.3–18.6%,

Table 6. Major fatty acids in zygomycetes of the order Mucorales and in plant oils

Fatty acid source		Major fatty acids, % of fatty acid total					Oil type
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	
Plant oils							
Olive	1	(9.8–18.0)*		64.0–85.0	4.0–20.0	–	Oleic
	2	7.0–18.3	2.2–4.0	43.7–78.2	7.0–32.3	0.1–1.0	Oleic-linoleic
Sunflower	1	(8.0–12.0)*		25.0–35.0	55.0–72.0	–	Linoleic-oleic
	2	5.5–6.0	4.7–5.6	17.8–19.5	68.5–68.7	0.1–0.2	
Soybean	1	(12.0–15.0)*		6.0–11.0	65.0–80.0	8.0–14.0	Linoleic-oleic
	2	10.8	0.9	23.0–29.0	50.0–57.0	7.0–9.0	
Evening primrose	2	7.0–10.0	1.5–3.5	6.0–11.0	65.0–80.0	8.0–14.0	Linoleic-linolenic
Flax	1	(5.0–10.0)*		5.0–10.0	29.0–59.0	21.0–45.0	Linoleic-linolenic
	2	6.1–7.0	3.2–5.8	14.7–39.0	14.2–18.8	35.0–59.8	Linolenic-oleic
Zygomycete lipids**							
Family Syncephalastraceae genus *Syncephalastrum*		10.6–19.9	6.3–8.7	44.9–54.8	13.5–16.8	7.4–11.6	Oleic-linoleic
Family Mucoraceae genus *Circinella*		18.0–28.8	3.6–6.9	19.9–36.1	11.7–29.3	10.8–24.4	Oleic-linoleic
Family Radiomycetaceae genus *Radiomyces*		15.7–19.7	4.4–11.8	26.0–33.4	26.3–35.6	9.4–14.9	Linoleic-oleic
Family Mycotyphaceae genus *Mycotypha*		24.3–36.6	3.8–9.7	23.5–28.4	19.8–34.5	4.0–11.2	Linoleic-oleic
Family Thamnidiaceae genus *Helicostylum*		14.9–18.7	8.8–11.9	20.0–33.7	11.1–13.0	15.0–16.5	Oleic-linoleic
Family Chaetocladiaceae genus *Chaetocladium*		17.4–18.9	7.3–12.2	26.2–35.8	15.4–18.1	14.7–20.5	Oleic-linoleic
Family Pilobolaceae genus *Pilaira*		12.5–20.2	6.2–9.6	21.4–23.7	26.0–28.8	14.7–26.9	Linoleic-linoleic

Note: 1 denotes data from [17], and 2 denotes data from [18].
* Sum of two classes.
** Studied in this work.

Table 7. The highest yields of fatty acids in zygomycete cultures

Species	Fatty acids (mg/l)				
	$C_{18:2}$	$C_{18:3}$	total PUFA	$C_{18:1}$	total bioactive fatty acids
SyÒephalastrum racemosum	686.7	465.2	1151.9	2326.0	3477.9
Pilaira anomala	279.7	241.0	520.7	240.7	761.4
Pilaira caucasica	419.7	237.8	657.5	350.3	1007.8
Hesseltinella vesiculosa	446.5	708.0	1154.5	1428.0	2582.5
Circinella naumovii	371.4	309.3	680.7	202.8	883.5
Mycotypha indica	836.3	120.8	957.1	857.4	1814.5
Radiomyces spectabilis	505.6	271.4	777.0	350.5	1125.5

and linolenic acid, to 29.8–31.5% [19]. Lipids of the earlier-studied strains of *Blakeslea trispora* belong to the linoleic-oleic or oleic-linoleic type. Different strains of *B. trispora* vary in their oleic acid content from 17.4 to 32.2%, and in the linoleic acid content, from 16.8 to 42.7%. In this study, we showed that the lipids synthesized de novo in the species of the genera *Radiomyces* (family Radiomycetaceae) and *Circinella* (family Muc-

Table 8. Fatty acids in *Linderina pennispora* F-1219 (% of fatty acid total)

Fatty acids	Growth time, h	
	72	168
$C_{12:0}$	traces	traces
$C_{14:0}$	traces	traces
$C_{15:0}$	1.6	2.7
$C_{16:0}$	15.1	10.7
$C_{16:1}$	37.0	36.0
$C_{17:0}$	traces	traces
C_x	5.7	4.4
$C_{18:0}$	2.0	1.1
$C_{18:1}$	26.3	29.5
$C_{18:2}$	12.3	15.1
$C_{18:3}$	traces	0.6
$C_{22:0}$	traces	traces

oraceae) are dominated by oleic and linoleic acids. The lipids of the fungi of the family Pilobolaceae (genus *Pilaira*) can be placed in the linoleic-linolenic oil type with a high content of linolenic acid, and the lipids of the psychrotolerant fungi, such as *Helicostylum pulchrum* and *Chaetocladium jonesii* (families Thamnidiaceae and Chaetocladiaceae, respectively) belong to the oleic-linoleic type.

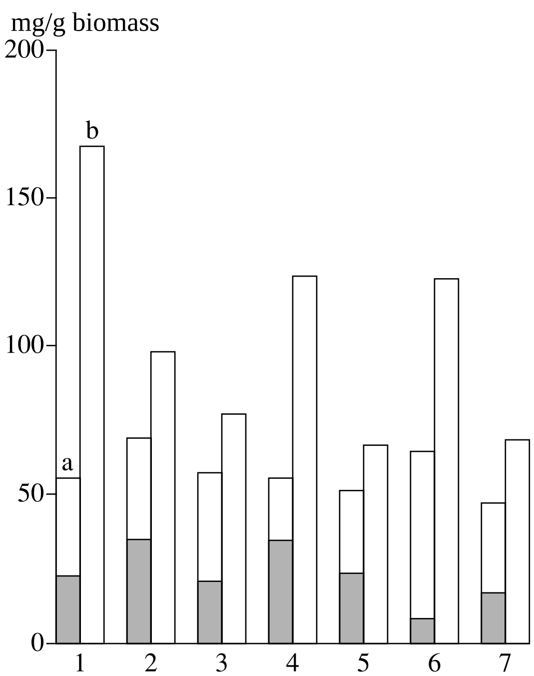

Fig. 2. The highest content of essential fatty acids in the biomass (mg/g) in the cultures of zygomycetes of different families: (a) total essential fatty acids (open bars, $C_{18:2}$; hatched bars, $C_{18:3}$) and (b) total bioactive fatty acids ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$). 1, *Syncephalastrum racemosum;* 2, *Pilaira anomala;* 3, *Pilaira caucasica;* 4, *Hesseltinella vesiculosa;* 5, *Circinella naumovii;* 6, *Mycotypha indica;* 7, *Radiomyces spectabilis.*

The data on the content of biologically active fatty acids (including linoleic, γ-linolenic and oleic acids) per one gram of fungal biomass (mg/g) are shown in Fig. 2, while Table 7 shows the data on the yield of these acids (mg/l). As can be seen from Fig. 2, the mycelia of the studied zygomycetes contained 66.3–167.2 mg/g of bioactive fatty acids; in the *Syncephalastrum racemosum* culture these acids constituted 16.7% of the dry weight. The largest amount of total essential fatty acids was found in *Pilaira anomala* (6.9% of the biomass); the highest values for individual essential acids in the fungal biomass were 56.5 mg/g for linoleic acid (*Mycotypha indica*), and 34.5 and 33.9 mg/g for linolenic acid (*Pilaira anomala* and *Hesseltinella vesiculosa*, respectively)

The highest achieved yields of fatty acids (Table 7) were as follows: 3477.9 mg/l of total unsaturated bioactive acids (*Syncephalastrum racemosum*), 1154.5 mg/l of total essential acids (*Hesseltinella vesiculosa*), 836.3 mg/l of linoleic acid (*Mycotypha indica*), and 708.0 mg/l of linolenic acid (*Hesseltinella vesiculosa*).

In addition to the fungi of the order Mucorales, we have studied the fatty acid spectrum of *Linderina pennispora* F-1219, a zygomycete of the family Kickxellaceae, which was earlier included in the order Mucorales but later singled out into its own order Kickxellales based on a complex of cultivation and morphological traits [20]. As can be seen from Tables 1 and 8, the lipids of *Linderina pennispora* are virtually identical in composition to the lipids of the fungi of the order Mucorales but differ from them in having a high content of short-chain fatty acids, which exceeds half of the total fatty acid content due to the abundance of the unsaturated palmitoleic acid ($C_{16:1}$), reaching 37% of the total fatty acids. We are not aware of any report on the high content of this acid in Mucorales fungi, although it may constitute about 20% in the lipids of the genera *Entomophthora* and *Conidiobolus* (order Entomophthoraceae) [3, 18].

This chemotaxonomic feature, the high palmitoleic acid content found by us in a species of the family Kickxellaceae and uncommon in the order Mucorales, is an additional reason to promote Kickxellaceae into a higher taxonomic rank and assign it to a separate order, Kickxellales [20]. Another chemotaxonomic difference was revealed by studies of sterol composition of zygomycetes. 22-Dihydroergosterol is the dominating sterol in fungi of the order Kickxellales, thereby distinguishing them from the fungi of the order Mucorales, in most of which ergosterol is the prevalent sterol form [7].

The new data on efficient synthesis of bioactive fatty acids by strains of several zygomycete taxa open new possibilities for use of these fungi in the biotechnological production of lipids necessary for the manufacture of drugs, dietary supplements and cosmetics.

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