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EXPERIMENTAL ARTICLES

The Prevalence of *cis*-9-Hexadecenoic Acid is a Specific Feature of the Fatty Acid Profile of Zygomycetes from the Order *Kickxellales*

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Abstract—The fatty acid profiles of zygomycetes from the family *Kickxellaceae* of the order *Kickxellales* were studied with reference to the species *Kicksella alabastrina* of the key genus *Kicksella* of the family and the species *Linderina pennispora.* When synthesized de novo, the lipids of these species show the prevalence of *cis*-9-hexadecenoic acid. This trait is stable and does not depend on cultivation conditions and can, therefore, be considered as a specific chemotaxonomic characteristic of fungi from the order *Kickxellales.* The fatty acid profiles of the two fungi under study are similar to that of sea buckthorn oil.

Key words: zygomycetes, order *Kickxellales*, family *Kickxellaceae*, fatty acids, *cis*-9-hexadecenoic acid.

The family *Kickxellaceae* of the class *Zygomycetes* comprises a separate order *Kickxellales*, which differs morphologically from another order of this class, *Mucorales* [1]. The order *Kickxellales* includes eight genera and 22 species [2], which are characterized by the formation of unicellular sporangioles located on sporocladia of a freakish form. Not all genera of this order produce zygospores. The recent 18S rDNA sequence analysis of representatives of the family *Kickxellaceae* confirmed that they form a separate cluster, which may have the status of an order [3]. Zygomycetes differ from most mucor fungi in that their major sterols are 22-dihydrosterols [4].

Our recent study showed that, unlike the fatty acids of mucor fungi, the fatty acids of the species *Linderina pennispora* (a representative of the order *Kickxellales*) are dominated by *cis*-9-hexadecenoic acid (palmitoleic acid, $C_{16:1}$) [5].

The aim of this work was to investigate the stability of this trait in two representatives of the order *Kickxellales* (*L. pennispora* and the species *Kicksella alabastrina* of the key genus *Kicksella* of the family *Kickxellaceae*) under varied cultivation conditions.

MATERIALS AND METHODS

Experiments were carried out with the zygomycetes *Kicksella alabastrina* VKM F-1104 and *Linderina pennispora* VKM F-1219, which were obtained from the All-Russia Collection of Microorganisms (VKM). The strains were maintained on malt extract agar.

To study the lipogenic activity and the fatty acid profiles of the strains, they were grown in nutrient media (11 variants) with the following basal composition (%): glucose, 4; KH_2PO_4, 0.14; $MgSO_4 \cdot 7H_2O$, 0.025; yeast extract, 0.1. The media were prepared with tap water. The initial pH of the media was 6.0–6.2. Medium 1 contained bactopeptone at a concentration corresponding to 0.12% nitrogen. Media 2 through 7 contained, respectively, $(NH_4)_2SO_4$, KNO_3, NH_4NO_3, asparagine, bactopeptone, and urea at a concentration of 0.042% nitrogen. Media 8 through 11 contained different concentrations of glucose (4 and 8%) and urea (0.1 and 0.05%).

The strains were grown in 250-ml Erlenmeyer flasks with 50 ml of the particular media either under stationary conditions or on a shaker (220 rpm).

In experiments with *L. pennispora* VKM F-1219, the material for inoculation was an aqueous suspension of fungal spores washed off from malt extract agar plates (3.5 B) incubated for 14 days. In experiments with *K. alabastrina* VKM F-1104, the material for inoculation was agar blocks (2 mm in diameter) added in an amount of 10–12 blocks per flask. The blocks were cut from the surface of agar plates incubated at 20°C for 14 days.

The dry weight of biomass was determined after drying it to a constant weight at 96°C. The fatty acid profiles and the content of lipids in the biomass were determined as described by Kates [6] and Konova *et al.* [5].

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Table 1. The growth, lipogenic activity, and fatty acid profiles of two zygomycetes from the order *Kickxellales* during submerged cultivation in medium 1

Parameter	Cultivation time, days					
	K. alabastrina F-1104	*L. pennispora* F-1219				
	14	1	2	3	4	7
Biomass, g dry wt/l	2.8	8.0	16.5	20.4	17.4	19.4
Lipids, % of dry biomass	7.8	4.02	5.17	4.52	3.54	4.23
Fatty acids, % of the total						
$C_{12:0}$	0.38	traces	1.43	traces	traces	traces
$C_{14:0}$	4.18	0.74	1.34	0.61	0.63	traces
$C_{15:0}$	2.58	3.88	4.08	2.83	3.07	1.99
$C_{16:1}$	45.05	26.16	24.97	27.98	28.25	26.66
$C_{17:0}$	11.74	16.95	15.94	11.95	12.69	16.21
$C_{18:1}$	3.94	11.43	13.20	20.97	21.30	21.45
$C_{18:2}$	14.27	24.77	18.48	17.57	14.82	13.39
$C_{18:3}$	–	–	–	–	–	–
$C_{20:0}$	–	traces	traces	traces	traces	traces
C_x	9.79	5.47	4.14	2.92	2.98	1.75

Note: *K. alabastrina* VKM F-1104 and *L. pennispora* VKM F-1219 were grown at 20 and 25–27°C, respectively. Some minor fatty acids (C_x) were not identified.

RESULTS AND DISCUSSION

K. alabastrina VKM F-1104 is a slowly growing micromycete. For this reason, the cultivation time of this strain was chosen to be 14 days, which corresponded to the stationary growth phase during submerged cultivation. By this time, the biomass reached 2.8 g dry wt/l, with a lipid content of no more than 7.8% (Table 1). Such a low lipid content suggests that *K. alabastrina* VKM F-1104 is not an oleagenous micromycete. The fatty acid profile of *K. alabastrina* VKM F-1104 was nearly the same as that of *L. pennispora* VKM F-1219. As in the case of *L. pennispora* VKM F-1219, the major fatty acid of *K. alabastrina* VKM F-1104 was *cis*-9-hexadecenoic acid, whose relative content reached 45.05% of the total fatty acids.

The study of the lipid composition of *K. alabastrina* VKM F-1104 in the course of its submerged cultivation (Table 1) showed that *cis*-9-hexadecenoic acid was prevalent both in the trophophase and in the stationary phase (idiophase). The maximum lipid content in the biomass did not exceed 4–5%, indicating that *K. alabastrina* VKM F-1104 is not an active lipid producer.

When *K. alabastrina* VKM F-1104 was grown in liquid media for 14 days under stationary conditions, the relative content of *cis*-9-hexadecenoic acid reached 32.8–36.8% of the total fatty acids, whereas the content of other individual fatty acids did not exceed 17–18%. The content of lipids in the biomass was 3.9%.

When *K. alabastrina* VKM F-1104 was grown on malt extract agar for 14 days under stationary conditions, the major fatty acids were $C_{16:1}$ (26.4%), $C_{16:0}$ (25.5%), $C_{18:1}$ (18.6%), and $C_{18:2}$ (22.8%), whereas the $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{17:0}$, and $C_{20:0}$ fatty acids were minor components (less than 2.8% each).

There is evidence that nitrogen sources and the proportion between carbon and nitrogen sources in the medium may influence the lipogenic activity and the fatty acid profiles of yeasts and mycelial fungi [7–11]. The experimental data presented in Table 2 show that the maximum biomass (5.4 g dry wt/l) was accumulated in the medium with ammonium nitrate as the nitrogen source. This was likely to be due to the utilization of both forms of nitrogen present in ammonium nitrate. The acidic and alkaline compounds ammonium sulfate and potassium nitrate maintained the growth of *K. alabastrina* VKM F-1104 less efficiently (4.6 and 1.6 g dry wt/l, respectively) than did ammonium nitrate, whereas the lipid content of the biomass was relatively high (12.7%) in both cases. This can be explained by a higher partition of glucose to lipid synthesis in the presence of less favorable nitrogen sources in the medium.

Organic nitrogen sources (bactopeptone and, in particular, asparagine and urea) were favorable for the accumulation of fungal biomass (up to 10.9 g dry wt/l). In this case, the lipid content of the biomass did not exceed the level typical of oleagenous cultures (13% of the biomass weight). The content of *cis*-9-hexadecenoic acid in the biomass grown in the presence of the organic nitrogen sources was somewhat higher than in

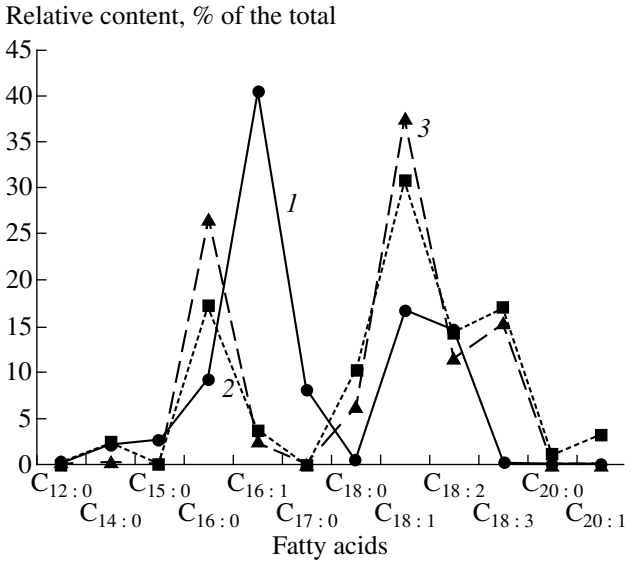
Table 2. The growth, lipogenic activity, and fatty acid profiles of *L. pennispora* VKM F-1219 cultivated on media 2 through 7 with different nitrogen sources at a concentration of 420 mg N/l

Nitrogen source	$(NH_4)_2SO_4$, no. 2		KNO_3, no. 3		NH_4NO_3, no. 4		Asparagine, no. 5		Bactopeptone, no. 6		Urea, no. 7	
Cultivation time, days	2	3	2	3	2	3	2	3	2	3	2	3
Biomass, g dry wt/l	4.1	4.6	1.5	1.6	4.5	5.4	10.2	10.9	6.3	7.0	9.2	10.9
Lipids, % of dry biomass	6.5	10.7	10.2	12.7	7.4	5.8	4.4	4.7	8.0	9.1	8.2	9.8
Fatty acids, % of the total												
$C_{12:0}$	traces	traces	1.79	traces	traces	traces	traces	traces	traces	traces	traces	traces
$C_{14:0}$	1.87	0.78	2.09	0.63	traces	0.73	traces	traces	1.85	traces	2.82	1.54
$C_{15:0}$	4.54	4.10	1.51	2.10	3.04	3.16	2.35	1.85	2.33	2.14	2.35	2.37
$C_{16:0}$	12.24	12.18	25.0	15.20	16.34	17.10	18.33	13.63	21.23	17.08	19.20	17.78
$C_{16:1}$	38.30	37.64	19.95	31.20	32.60	32.43	43.33	36.90	29.43	31.51	42.12	36.55
$C_{17:0}$	4.13	4.34	3.71	5.04	5.02	4.53	3.14	3.22	4.91	4.30	2.79	2.34
$C_{18:0}$	2.02	1.82	4.20	2.30	2.04	2.07	1.21	1.88	2.68	2.54	2.62	2.08
$C_{18:1}$	14.9	14.14	30.36	31.20	23.95	22.75	19.83	26.96	25.98	30.32	14.54	24.07
$C_{18:2}$	21.99	24.03	11.35	12.30	17.98	17.21	11.80	15.55	9.88	12.10	13.55	13.27
$C_{18:3}$	–	–	–	–	–	–	–	–	–	–	–	–
$C_{20:0}$	–	–	–	–	–	–	–	–	–	–	–	–

Note: Fatty acids also contained unidentified fractions, whose retention times were higher than those of $C_{15:0}$ and $C_{16:1}$.

the case of mineral nitrogen sources and varied from 29.4 to 42.1%.

Varying the proportions of carbon and nitrogen in the medium (Table 3) showed that a concentration of nitrogen equal to 210 mg/l is sufficient to produce biomass in an amount of more than 7 g dry wt/l. At all the carbon-to-nitrogen ratios studied, the lipid content of the biomass did not exceed 7%. The maximum relative content of *cis*-9-hexadecenoic acid (more than 50% of the total fatty acids) was observed at C/N ratios equal to 78 and 156.



The fatty acid profiles of (*1*) micromycetes from the order *Kickxellales* and (*2*) psychrotolerant and (*3*) thermotolerant strains from the order *Mucorales*.

It should be noted that *cis*-9-hexadecenoic acid is typically a minor fatty acid in eukaryotic organisms, whereas its relative content in the fatty acids of the yeast *Saccharomyces cerevisiae* may reach 52% [12]. In mycelial eukaryotes, the maximum content of *cis*-9-hexadecenoic acid (14–20% of the total) was observed in zygomycetes from the genera *Entomophthora* and *Conidiobolus* of the order *Entomophthorales* [9, 12]. To the best of our knowledge, the stable prevalence of *cis*-9-hexadecenoic acid in the fatty acid profile of zygomycetes from the order *Kickxellales* has not yet been reported.

Among plant oils, sea buckthorn oil is known for a high content of *cis*-9-hexadecenoic acid, which, together with some other fatty acids, carotenoids, and α-tocopherol, is likely to be responsible for the pharmacological effect of this oil [13, 14]. According to our estimations, the contents of *cis*-9-hexadecenoic acid in the fatty acids of sea buckthorn oil and the *Kickxellales* fungi are close (28.0 ± 4.6 and 30.6 ± 6.5%, respectively). Thus, the lipids of *Kickxellales* fungi are an analogue of sea buckthorn oil.

The figure shows the fatty acid profiles of fungi from the orders *Mucorales* and *Kickxellales* (the data of

Table 3. The growth, lipogenic activity, and fatty acid profiles of *L. pennispora* VKM F-1219 cultivated on media 8 through 11 at different carbon-to-nitrogen ratios

Medium	No. 8		No. 9		No. 10		No. 11	
Cultivation time, days	3	4	3	4	3	4	3	4
Biomass, g dry wt/l	6.2	6.9	7.3	7.4	7.1	7.2	6.4	7.4
Lipids, % of dry biomass	5.1	4.7	4.3	6.4	6.9	6.1	5.6	5.7
Fatty acids, % of the total								
$C_{12:0}$	traces	traces	traces	traces	traces	traces	traces	traces
$C_{14:0}$	traces	traces	traces	traces	1.25	traces	traces	0.52
$C_{15:0}$	1.71	1.73	1.33	2.31	1.37	1.54	2.32	1.68
$C_{16:0}$	13.56	14.49	12.60	13.75	15.45	13.24	15.78	15.38
$C_{16:1}$	45.60	41.82	52.46	41.39	35.24	42.84	50.98	42.67
$C_{17:0}$	2.53	2.75	2.75	2.35	2.42	2.30	2.06	2.14
$C_{18:0}$	traces	1.37	traces	traces	3.49	1.44	traces	1.11
$C_{18:1}$	17.81	19.00	12.25	15.58	25.76	27.68	19.41	26.36
$C_{18:2}$	18.70	18.83	18.60	23.72	15.00	10.96	9.43	10.14
$C_{18:3}$	–	–	–	–	–	–	–	–
$C_{20:0}$	–	–	–	–	–	–	–	–

Note: Medium 8 contained 4% glucose and 420 mg N/l (C/N = 39); medium 9 contained 8% glucose and 420 mg N/l (C/N = 78); medium 10 contained 4% glucose and 210 mg N/l (C/N = 78); medium 11 contained 8% glucose and 210 mg N/l (C/N = 156). Fatty acids also contained unidentified fractions, whose retention times were higher than those of $C_{15:0}$ and $C_{16:1}$.

this and the previous work [5]). The order *Mucorales* is represented by psychrotolerant fungi of the genus *Helicostylum* (three strains) and *Chaetocladium* (three strains) and four thermotolerant strains of the genus *Absidia.* As can be seen from this figure, the fungi of the order *Kickxellales* are characterized by the prevalence of *cis*-9-hexadecenoic acid, whereas the fungi of the order *Mucorales* are characterized by the prevalence of *cis*-6,9,12-octadecatrienoic (γ-linolenic) acid.

The prevalence of *cis*-9-hexadecenoic acid in the fatty acid profiles of the zygomycetes *L. pennispora* and *K. alabastrina* is observed under different cultivation conditions and can, therefore, be considered a valuable chemotaxonomic characteristic of representatives of the order *Kickxellales.* The presence of *cis*-6,9,12-octadecatrienoic acid is typical of fungi from the order *Mucorales* [5] and may be used as their characteristic [15].

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