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EXPERIMENTAL ARTICLES

Lipid Synthesis by *Geomyces pannorum* under the Impact of Stress Factors

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Abstract—Lipogenic activity and fatty acid composition of two strains of *Geomyces pannorum* were studied in the course of fungal growth. The strains were isolated from an Arctic cryopeg lens (VKM FW-2241) and from Central Russia (VKM F-3808). The adaptive reactions in both strains towards the temperature decreasing to 2°C involved intensification of the fatty acid desaturation. The degree of lipid unsaturation increased mainly due to a higher amount of α-linolenic acid (α-$C_{18:3}$) especially in the case of strain VKM FW-2241. Elevated NaCl concentration in the medium enhanced the level of linoleic acid ($C_{18:2}$) which apparently played a specific role in osmoprotection. Strain VKM FW-2241 was more tolerant to increased salinity than strain VKM F-3808. Almost complete inhibition of the growth of strains VKM F-3808 and VKM FW-2241 occurred at salinity of 10 and 20%, respectively; however, the viability of the strains was not affected. Under the combined effect of high salinity and hypothermia, the ratio between $C_{18:2}$ and α-$C_{18:3}$ acids was intermediate, indicating that these acids were involved in two adaptation mechanisms. The inhibition of fungal growth under stress was found to result in lipid overproduction. An increased pool of energy-rich lipids in fungi possibly contributes to their strategy of cell survival.

Key words: Geomyces pannorum, lipids, fatty acids, stress factors, cryopeg.

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Survival of the microorganisms inhabiting the ecotopes with stress conditions depends on their capability for adaptation to environmental signals. The adaptation processes involve genetic mechanisms, synthesis of specific proteins, enzyme variability, and intracellular accumulation of protective carbohydrates, mainly trehalose and polyols, as well as such known osmoprotectants as proline, betaine, and certain amino acids. Lipids, especially those located in the cell membranes, also play a significant role in the adaptation processes [1–3].

Variations in the membrane fluidity due to a change in the molecular structure of the fatty acids in intracellular lipids in response to external signals play a key role in the adaptation of microorganisms. According to recent literature, the ratios between individual fatty acid fractions as well as the amount of polyunsaturated fatty acids are of great importance for the systemic response for both prokaryotic and eukaryotic microorganisms to stress. For instance, a temperature shift from the optimum caused a change in the chain length of fatty acids and the degree of their branching in prokaryotic microorganisms (e.g., in a number of bacteria) and modified the level of fatty acid unsaturation and the proportion of polyunsaturated fatty acids in eukaryotic microorganisms, particularly in fungi and oomycetes [4–6].

There is information that the growth of fungi under hypothermic conditions was associated with the production of unusual lipids, such as the betaine lipid (diacylglyceryltrimethylhomoserine) [7] and stearidonic acid [8].

Thus, both the variety of known fatty acids and detection of new molecular structures of lipid compounds in microorganisms [9–13] are indicative of their great physiological significance, although many questions still remain unclear.

The aim of this work was to study the lipogenic activity and characterize the lipids produced de novo by *Geomyces pannorum.* These everytopic micromycetes are frequently encountered at different depths in Arctic and Antarctic permafrost grounds, especially in cold saline solutions (cryopegs) [14, 15]; their physiological peculiarities and adaptive potential have therefore attracted great interest from researchers. Information available in literature concerning lipid synthesis by sev-

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eral strains of this fungal species is scant and scarce. Therefore, it is of interest to compare lipid synthesis in the strains isolated from different environments including natural specific ecotopes characterized by hypothermia, increased salinity, and hypoxia, as well as those isolated from the usual econiches of Central Russia.

MATERIALS AND METHODS

The studies on lipid synthesis under different cultivation conditions were carried out with two strains of *Geomyces pannorum*, VKM F-3808 isolated from the hair of a vole *Clethrionomys glareolus* in the Tver oblast, Russia, and VKM FW-2241 isolated from cryopeg saline water (borehole 15/99, depth of 21.0 m, age of about 100–120 thousand years, Kolyma lowland, region of Lake Yakutskoe, Russia).

Submerged cultivation of the fungi was performed in 100-ml flasks with 20 ml of Czapek medium on a shaker (180 rpm). A spore suspension of a 7-day culture was used as an inoculum (5%, vol/vol). Cultivation conditions were varied by lowering the temperature to 2°C and by increasing the salinity to 10 or 20% of NaCl. The culture growth was monitored by measuring biomass weight. In comparative studies on the effect of stress factors, the cultivation time varied from 10 to 30 days.

The lipids were analyzed according to Kates [16] by extraction of biomass sequentially with chloroform–methanol mixtures (2 : 1 and 1 : 2, vol/vol). The percentage of lipids was calculated per dry biomass.

Fatty acid methyl esters (FAMEs) were obtained by acid methanolysis of isolated lipids and analyzed on a model 3700 gas-liquid chromatograph (Russia) equipped with a flame-ionization detector and a glass column (1 m × 3 mm) packed with 17% diethyleneglycol succinate (DEGS) on Chromosorb WAW-DMCS-HP (80–100 mesh) under the isothermal regime (the column and injector temperatures were 180 and 250°C, respectively). Argon was used as a carrier gas at a flow rate of 50 ml/min.

FAMEs were identified by comparing their retention times with those of the standards. The content of individual fatty acids in the mixture was determined by the triangulation method and expressed as a percentage of the total. The unsaturation index and iodine number were calculated from the obtained data on the content of individual unsaturated fatty acids.

RESULTS AND DISCUSSION

To study the type of lipids produced by *G. pannorum* strains, the fungi were cultivated in Czapek medium at 20°C for ten days. Table 1 shows the mean values of biomass and lipid contents; the figure demonstrates time courses for the growth and lipogenic activity of the fungi.

Table 1. Parameters of growth and lipid synthesis at 20°C in *G. pannorum* strains isolated from different ecotopes

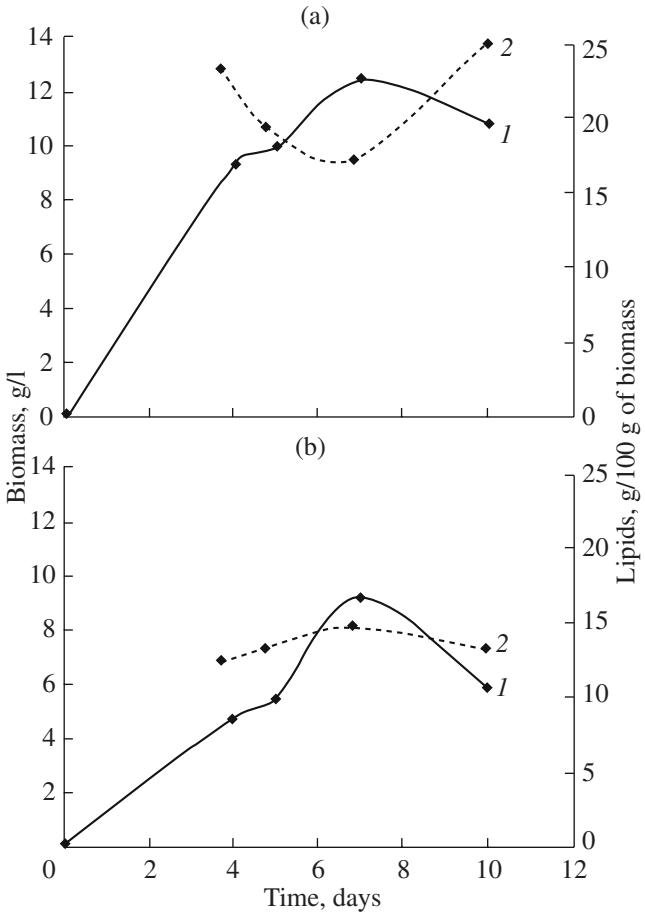
Parameters	Strains	
	F-3808	FW-2241
Biomass, g/l	10.58 ± 2.13	6.38 ± 3.14
Lipids, g/100 g of biomass	21.35 ± 5.55	13.42 ± 1.53
Fatty acids, % of the total fatty acids*		
$C_{12:0}$	0.32 ± 1.00	–
$C_{14:0}$	0.62 ± 0.49	0.36 ± 0.41
$C_{16:0}$	16.71 ± 2.96	18.29 ± 3.84
$C_{16:1}$	1.52 ± 0.32	2.10 ± 0.72
$C_{18:0}$	4.16 ± 1.48	3.94 ± 0.65
$C_{18:1}$	41.45 ± 3.61	35.47 ± 6.91
$C_{18:2}$	33.16 ± 6.37	36.06 ± 4.09
$C_{18:3}$	1.63 ± 0.70	2.42 ± 1.12
Unsaturation index	1.14 ± 0.08	1.17 ± 0.04

* Trace amounts of fatty acids, which were identified preliminary as $C_{15:0}$, $C_{17:0}$, $C_{20:0}$, $C_{22:0}$, were found in both strains.

It was shown that strain VKM F-3808 differed from strain VKM FW-2241 by more active growth (biomass up to 12.4 g/l after 7 days of cultivation) and pronounced lipogenic activity; the lipid pool reached 25% of biomass even under conditions not optimized for lipid overproduction.

The Arctic strain *G. pannorum* VKM FW-2241 was characterized by slow growth; biomass accumulation on the fifth day of cultivation was about 50% of that of strain VKM F-3808. By the 5th and 7th day of growth, the biomass accumulation by strain VKM F-3808 reached 80% of the maximum level; only 20% was produced in the following two days. In the case of strain VKM FW-2241, these parameters were 60 and 40%, respectively; a change in the growth phases for the latter strain was also retarded. A slower growth of strain VKM FW-2241 was not accompanied by an increase in lipogenic activity; the lipid pool was below 15% of biomass.

No considerable changes in the composition of individual fatty acids were revealed in the course of growth for the studied strains. The lipids included C_{12}-C_{18} fatty acids with the predominance of unsaturated C_{18} acids, mainly oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids; this is typical of many mycelial fungi, e.g., representatives of the genus *Penicillium*. In the fraction of saturated fatty acids, palmitic acid ($C_{16:0}$) prevailed, whereas the content of stearic acid ($C_{18:0}$) was about 4%. In some experiments, the amount of polyenes in the lipid pool (mg/g) slightly increased in strain VKM FW-2241; this may be



Parameters of growth (*1*) and lipid synthesis (*2*) in the course of cultivation of strains VKM F-3808 (a) and VKM FW-2241 (b).

associated with the predominance of phospholipids, whereas in strain VKM F-3808, a higher lipid content was accompanied by a greater proportion of a more saturated lipid fraction, triacylglycerols.

Thus, the fatty acid profiles for the studied *G. pannorum* strains were similar to those found in other anamorphic fungi.

The aim of the subsequent experiments was to compare lipogenic activity and fatty acid composition of *G. pannorum* strains isolated from the ecotopes with different physicochemical characteristics and to study the effect of temperature and increased salinity on these parameters.

Comparative data on the individual and combined effects of hypothermia and high salinity on the growth and lipogenic activity of the studied strains and on the composition of the certain groups and individual fractions of the main fatty acids are presented in Tables 2 and 3, respectively.

At a decreased cultivation temperature, the growth of the strain from Central Russia decreased by a greater extent than that of the Arctic strain; by the end of exponential growth, biomass of these strains was 35 and 23% lower, respectively. The cultivation temperature had no effect on the lipogenic activity of both strains; the lipid pool of strain VKM FW-2241 remained considerably lower than that of strain VKM F-3808 at both temperatures.

Although no alteration in the fatty acid spectra for both strains was revealed at a lowered temperature, the content of certain groups and individual fractions of fatty acids changed markedly (Table 3); in particular, in both strains, the level of short-chain fatty acids (C_{12}–C_{16}), mainly saturated fatty acids, which are the products of the synthetic stage of fatty acid biosynthesis, decreased almost twofold.

The next stage in lipid synthesis includes reactions of elongation and desaturation of short-chain fatty acids involving elongases and desaturases; induction of these processes at a lowered temperature is typical of fungi. When the cultivation temperature was decreased from 20 to 2°C, the sum of long-chain fatty acids increased, as well as the amount of polyunsaturated fatty acids which increased from 260.9 to 313.2 and from 288.6 to 443.2 mg/g in strains VKM F-3808 and VKM FW-2241, respectively.

It was revealed that an increase in the level of lipid unsaturation in response to hypothermia occurred only due to enhanced synthesis of linolenic acid; its percent-

Table 2. Effect of stress factors on the growth parameters and lipogenic activity of *G. pannorum* strains VKM F-3808 and VKM FW-2241

Parameters	Strains VKM											
	F-3808						FW-2241					
Cultivation temperature, °C	20			2			20			2		
NaCl concentration in the medium, %	0	10	20	0	10	20	0	10	20	0	10	20
Biomass, g/l	10.58	0.22	0.17	6.89	1.71	0.40	6.38	3.21	0.29	4.91	3.96	0.41
Lipids, g/100 g of biomass	21.35	84.20	38.24	20.90	14.42	23.35	13.42	10.23	18.62	11.12	11.45	28.92

Note: In Tables 2 and 3, the control variant means cultivation of fungi without stress impact (salt concentration, 0%; temperature, 20°C).

Table 3. Effect of stress factors on the content of certain groups and individual fractions of fatty acids in lipids of *G. pannorum* strains VKM F-3808 and VKM FW-2241

Parameters	Strains											
	F-3808						FW-2241					
Cultivation temperature, °C	20			2			20			2		
NaCl concentration in the medium, %	0	10	20	0	10	20	0	10	20	0	10	20
Fatty acids, % of the total fatty acids												
The sum of short-chain acids (C_{12}–C_{16})	19.22	17.83	23.20	11.24	11.67	22.17	21.48	20.81	19.79	13.77	13.96	24.44
The sum of long-chain acids (C_{18})	80.40	80.16	76.80	88.75	88.33	77.83	77.88	79.19	80.21	86.22	86.04	75.56
$C_{18:0}$	4.16	7.69	7.06	2.48	4.14	4.45	3.94	2.48	4.48	3.05	2.81	4.27
The sum of unsaturated fatty acids (C_{18})	76.24	72.46	69.74	86.27	84.20	73.38	73.94	76.70	75.73	82.82	83.22	71.29
$C_{18:1}$	41.45	42.60	38.29	44.51	39.72	45.62	35.47	31.41	40.86	23.73	27.42	38.60
The sum of polyunsaturated fatty acids (C_{18})	34.79	29.86	31.45	41.76	44.47	27.76	38.48	45.29	34.87	59.09	55.80	32.70
$C_{18:2}$	33.16	29.07	30.95	30.55	38.79	26.78	36.06	44.49	33.54	32.45	49.55	31.84
$C_{18:3}$	1.63	0.79	0.50	11.21	5.68	0.99	2.42	0.80	1.33	26.64	6.25	0.86

age of the total fatty acids increased by seven and eleven times in strains VKM F-3808 and VKM FW-2241, respectively, that is indicative of intense desaturation for oleic and linoleic acids under these conditions.

It is possible that a higher increase in the level of linolenic acid in strain VKM FW2241 as compared with strain VKM F-3808 can be due to a lower content of triacylglycerols in the former strain; however, low cultivation temperature undoubtedly had a stimulatory effect on the synthesis of linolenic acid in both strains.

An increase in lipid unsaturation was more evident when the iodine number of lipids was calculated; lowering the temperature from 20 to 2°C increased this index from 94.0 to 102.2 in strain VKM F-3808 and from 121.4 to 147.7 in strain VKM FW-2241; these changes certainly affected the physicochemical properties of fungal lipids and, consequently, the cell response to stress.

The natural ecotope of strain VKM FW-2241 was characterized by high salinity (170–300 mg NaCl/l) [15], therefore, it was of particular importance to study the lipid synthesis in *G. pannorum* strains grown in media with different concentrations of sodium chloride at aforementioned temperatures.

It was revealed that in all variants, high salinity had a negative effect on the growth of both strains, although to a different extent. An increase in NaCl concentration to 20% inhibited growth of both strains independently of temperature; however, the strains retained viability and high lipogenic activity; the lipid pool in the biomass of strains VKM F-3808 and VKM FW-2241 was 38.2 % and 18.6 %, respectively.

At 10% NaCl, the growth of strain VKM F-3808 was almost completely inhibited at both temperatures; however, the culture retained viability and its lipogenic activity increased to such an extent that the lipid content of the biomass reached 84.2%.

Strain VKM FW-2241 isolated from a cryopeg cold saline lens was more tolerant to extreme NaCl concentrations than strain VKM F-3800. At 20°C, an increase in NaCl concentration to 10% resulted in a biomass decrease from 6.4 to 3.2 g/l by the end of the exponential phase and had no effect on the lipid pool, whereas the lowering of the cultivation temperature to 2°C decreased the biomass yield by 20%.

As seen from Table 3, under conditions of cell survival (20% NaCl) at both temperatures, the strains were characterized by increased lipogenic activity and reduced desaturation activity; the level of polyenes remained unchanged or decreased as compared to the control.

In strain VKM F-3808 grown in the medium with 10% NaCl at 20°C, an increase in the lipid production was accompanied by elongation of the fatty acids produced at the first stage of lipid synthesis (C_{16}) to long-chain C_{18} acids; the content of polyunsaturated fatty acids decreased, and the levels of saturated and monoenoic C_{18} acids increased (their sum reached 50.3%); these findings correlated with the aforementioned increase in the lipid pool, which is usually more saturated than the membrane lipids.

In strain VKM FW-2241 grown at 20°C and 10% NaCl, the content of unsaturated and polyunsaturated fatty acids increased mainly at the expense of intense synthesis of linoleic acid, which is a specific response of this strain to the salt stress.

Thus, the study of lipid synthesis in *G. pannorum* strains isolated from different ecotopes and cultivated under hypothermia, high salinity, or their combined impact revealed that both strains exhibited adaptive reactions of the same type, although the level of their tolerance to the stress effect was different.

The phenomenon of an increase in the content of polyunsaturated fatty acids observed in fungi and other eukaryotic microorganisms, which involves oxygen-dependent desaturases, is considered to be a general adaptive response of the cells to impact of stress. We revealed that in the studied strains of *G. pannorum* grown under hypothermic conditions, an increase in the unsaturation index (from 1.14–1.17 to 1.40–1.70) occurred exclusively at the expense of intense synthesis of linolenic acid and active desaturation of oleic and linoleic acids; this process was the most active in strain VKM FW-2241. There are data in literature that an increased level of polyunsaturated fatty acids in the cells of thermotolerant yeasts and lactobacilli was associated with a decrease in the concentration of oxygen and its toxic radicals [6, 17].

We revealed that in the Arctic strain VKM FW-2241, which was more tolerant to high salinity, the adaptive reaction involved an increase in the linoleic acid content for the total of fatty acids up to 333.7 mg/g.

Like the other fungi, the studied strains of *G. pannorum* were characterized by a higher content of linoleic acid in the lipids compared to linolenic acid (Table 3). However, under the impact of stress the $C_{18:2}/C_{18:3}$ ratio changed significantly in both strains in a similar manner. In strain VKM FW-2241, this ratio was 15 in the absence of stress, sharply decreased (12-fold) under hypothermia, and increased almost fourfold under the salinity stress; the combined impact of two stress factors decreased this ratio twofold as compared to the control. Thus, the combined effect of two stress factors influenced the lipid metabolism in an intermediate way as compared to the action of individual factors. The observed variations in the content of linoleic and linolenic acids under hypothermia and high salinity are indicative of their involvement in the adaptive response of fungi to stress.

The specific role of linoleic acid in the adaptation of growing cultures to high salinity under hypothermic conditions is doubtless and is not restricted to its participation in the regulation of membrane fluidity. For example, an interrelation between the physiological adaptation of cells to high salinity and to the aeration level was observed in yeasts under combined impact of these stress factors [18]. Such metabolites of linoleic acid as epoxides, as well as enzymes of the fatty acid oxygenation (lipoxygenase and others) may be also involved in the adaptation processes [6, 17]. Moreover, variations in fatty acid composition can influence the transport of osmoprotectors (glycine betaine) as it was shown in *Lactococcus lactis* [5]. Thus, physiological and biochemical bases of the life strategy under stress are more complicated than any single physiological reaction.

It should be noted that under almost complete inhibition of fungal growth, cells retained viability and were capable of lipid overproduction; the lipid pool contained not only functionally important polar lipids of cell membranes but also large amounts of energy-rich triacylglycerols. The revealed increased lipogenic activity of *G. pannorum* strains appeared to be involved in the survival strategy.

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