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SHORT COMMUNICATIONS

Growth of the Fungus *Geomyces pannorum* under Anaerobiosis

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Fungi of the genus *Geomyces* are eurytopic organisms with a broad species distribution area. It is reasonable to study the adaptive capabilities of fungal cultures in members of the same microbial species existing under drastically different environmental conditions. In our case, these are cryopegs of the Kolyma lowland and the soil of Central Russia. Cryopegs are supercooled saturated salt solutions characterized by a constant temperature (from –10 to –15°C) and total mineralization of 60–300 g/l [1, 2]. Thus, water remains unfrozen and is under anaerobic conditions (*Eh* from –56 to –240 mV) [3]. The metabolic status of mycelial fungi under deep anaerobiosis combined with the low temperature and high salinity of the environment remains unknown; it is, however, highly important to determine the mechanism responsible for the preservation of the viability of fungal organisms under such extreme conditions.

The goal of the present work was investigation of the metabolic capabilities of two *Geomyces pannorum* strains, specifically, of their ability to grow and form the fermentation products in the absence of oxygen in the media with different redox potentials (*Eh*). The objects of research were the strain VKM FW-2241 isolated from cryopeg water in permafrost deposits of the Arctic [4] and the strain VKM F-3808 isolated from the hair of bank vole *Cletrionomys glareolus* (Tver oblast, Russia). The partial 18S rRNA gene sequences of both strains are deposited in GenBank under accession numbers AY873966 and AY873968, respectively.

The strains under study were grown on a modified Czapek medium [5]. In the experiments with different reducing agents, ammonium sulfate was added to the medium (N-Czapek medium) instead of the same amount of $NaNO_3$ and pH of the medium was adjusted to 7.0. The prepared medium was boiled and dispensed under the current of oxygen-free nitrogen; inoculations were carried out anaerobically [6]. Cultivation was performed in 12.5-ml Hungate tubes with 10 ml of the medium and in round-bottom flasks with 120 ml of the medium, equipped with two ports (for the electrode and for sampling). Sampling was carried out with a syringe through a butyl rubber stopper.

Eh of the medium was measured by a platinum electrode combined with an Ag/AgCl reference electrode (Ingold PT-4800-M5, Stainbach). The reference electrode had a potential of +210 mV, which was taken into account when calculating *Eh* of the medium. The following chemical compounds were used as reducing agents for the media: sodium thioglycollate, 0.5%; cysteine hydrochloride, 0.25%; ascorbic acid, 1%; and sodium sulfide, 2.5%; pH in the 3 M NaOH solutions was adjusted to neutral values. The cultures were incubated for 1 month at 6 and 16°C.

The biomass was assayed by the gravimetric method. Acetate and oxygen were assayed by gas chromatography. Lactate was assayed enzymatically [7].

Initially, the capacity of the cultures under study for growth in the presence of different concentrations of reducing agents in the medium (the mixture of cysteine hydrochloride and sodium sulfide) was tested. It was established that the rate of culture growth in air did not depend on reducer concentration (0.5–3.0% vol/vol) and was close to the control value. Culture growth was not observed under anaerobic conditions (in the atmosphere of oxygen-free nitrogen) in an anaerostat on solid medium for 30 days. In the test tubes with liquid medium prepared without bubbling with nitrogen, fungal growth was always observed, although its intensity decreased with increasing reducer content in the medium. When the temperature decreased to 6°C, fungal growth slowed down but was observed in all experimental variants. The measurement of oxygen concentrations in the gas phase showed them to be no more than 2%. In the variants with the medium prepared in the current of oxygen-free nitrogen without a reducer, the indicator also showed the presence of oxygen (0.8–2.0%) and weak culture growth was observed. Thus, the fungi cultivated under microaerophilic conditions showed a weak but sustained growth decreasing on addition of reducer.

The next stage of the work was testing of *G. pannorum* strains VKM FW-2241 and VKM F-3808 in the

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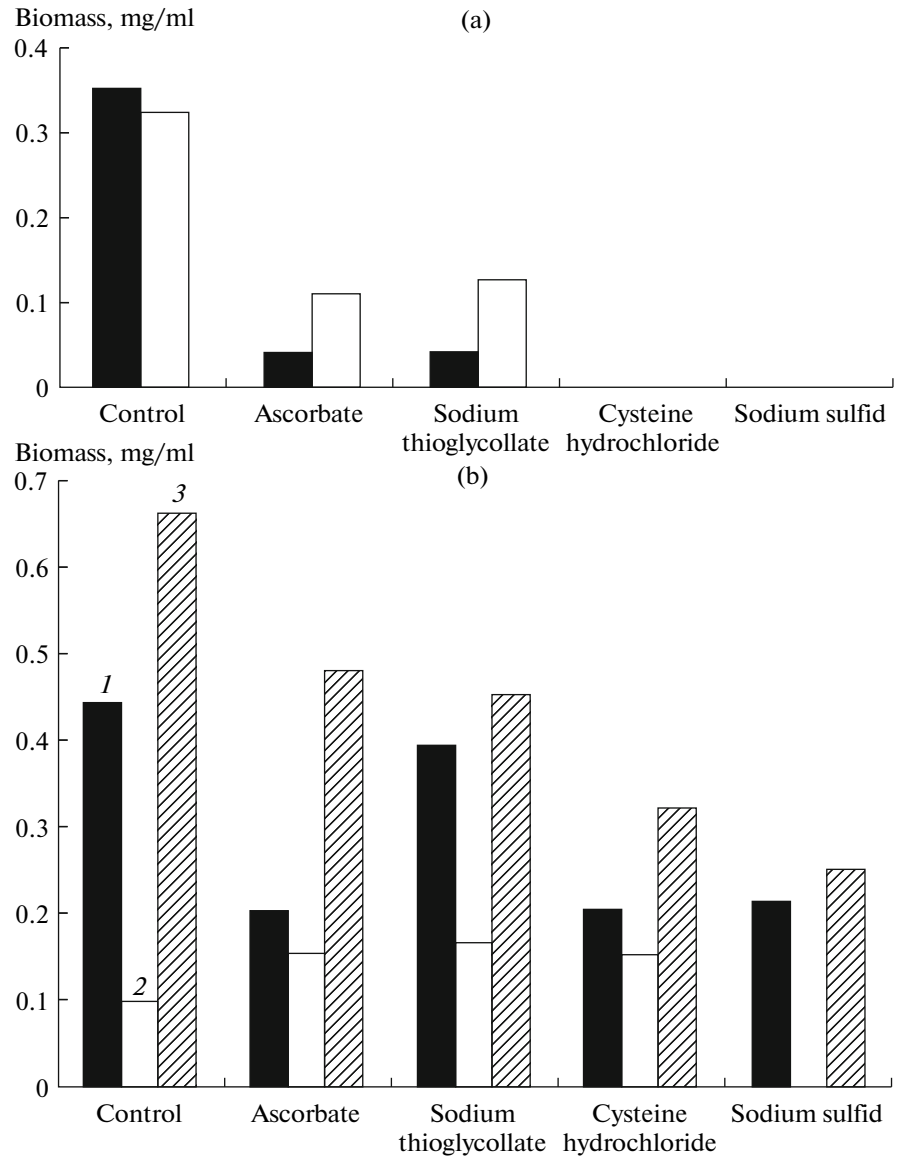

Biomass formation by strains F-3808 (a) and FW-2241 (b) under different temperature and redox conditions: at 6°C (*1*), at 16°C (*2*), and at 6°C and 10% NaCl in the cultivation medium (*3*).

presence of reducers creating different levels of redox potential in the medium: sodium thioglycollate, cysteine hydrochloride, ascorbic acid, and sodium sulfide. N-Czapek medium prepared without access to oxygen was used as a control. The absence of oxygen in the gas phase was confirmed by chromatographic analysis. *Eh* values for the above reducers varied during the growth within the following ranges: +10 to −15 mV for the control, 0 to −30 mV for ascorbic acid, −98 to −118 mV for sodium thioglycollate, −195 to −235 mV for cysteine hydrochloride, and −275 to −295 mV for sodium sulfide.

The presented experimental results show (figure) that growth of the strains at different *Eh* values of the medium was characterized by formation of different quantities of biomass. The strain VKM FW-2241 (figure, b) grew at a lower redox potential (down to −280 mV), which was especially evident at a lower temperature, compared to the strain F-3808 (figure, a) (to −108 mV). The maximal biomass accumulated in the control at *Eh* +5 mV.

Fungal growth under different redox conditions at 6 and 16°C was accompanied by accumulation in the culture liquid of acetate and lactate as fermentation products (table). Evaluation of experimental data demonstrated that in the case of strain VKM FW-2241 (isolated from a cryopeg), under all redox conditions (*Eh* of the medium from +5 to −280 mV) adaptation to anaerobiosis resulted in increased formation of metabolic products at lower cultivation temperatures.

Acetate and lactate formation by the strains of *Geomyces pannorum* in the presence of different reducers

Reducer	Content of metabolites, mM			
	Strain VKM F-3808		Strain VKM FW-2241*	
	Lactate	Acetate	Lactate	Acetate
Incubation temperature 6°C				
Control	1.93	0	2.74/1.22	0/0.8
Ascorbic acid	2.89	0	4.55/1.98	1.30/1.1
Sodium thioglycollate	1.38	0.3	3.60/5.76	1.00/1.94
Cysteine hydrochloride	0	0	3.58/5.1	0/2.22
Sodium sulfide	0	0	1.65/1.76	0/1.04
Incubation temperature 16°C				
Control	4.30	0	2.30	0
Ascorbic acid	2.91	0.92	2.30	1.02
Sodium thioglycollate	1.93	0	1.51	0.34
Cysteine hydrochloride	0	0	0.75	0
Sodium sulfide	0	0	0	0

* The denominator contains the values of metabolite content during cultivation in the presence of 10% NaCl.

Typically, the highest production of metabolites was observed with ascorbic acid as a reducer. During cultivation of the strain VKM F-3808, only lactate formation was observed, except for the variants with sodium thioglycollate (incubation at 6°C) and ascorbic acid (incubation at 16°C).

Further evidence of the fact that the absence of oxygen does not result in the death of micromycetes may be aerobic plating on petri dishes of the fungal cultures which have sustained anaerobic conditions: growth was absent only when the inoculum had been grown in the medium with sodium sulfide as a reducer. Incubation of micromycetes at *Eh* from +5 to −215 mV merely stimulated subsequent growth under the conditions of unlimited oxygen supply.

In order to estimate the effect of decreased the redox potential of the medium at low temperatures in combination with high salinity of the medium on the preservation of viability of the fungi, the strain VKM FW-2241 (a typical representative of cryopeg microbiota) was cultivated at a decreased redox potential in a liquid medium containing 10% NaCl in the presence of sodium thioglycollate (*Eh* of −90 to −128 mV), cysteine hydrochloride (*Eh* of −205 to −215 mV), and sodium sulfide (*Eh* of −285 to −290 mV). The culture grown aerobically on the Czapek medium with the same salt concentration was used as a control.

The results show (table) that, rather than inhibiting growth, the introduction of NaCl into the medium to a certain extent stimulated development of strain VKM FW-2241 under both aerobic and anaerobic conditions. The saline medium had a particularly notable effect on the amount of metabolites formed: the quantities of lactate and acetate at *Eh* below −90 mV increased by 1.5–2.5 times compared to the control. The metabolites were accumulated in the maximum amount during growth on the medium with sodium thioglycollate. Since strain VKM F-3808 grows very poorly in media with high concentration of salt (10% NaCl) [8], its development under the lower redox potential of the medium in combination with high salinity was not studied.

Thus, the study of the capability of the mesophilic (VKM F-3808) and psychrotolerant (VKM FW-2241) strains of *Geomyces* sp. fungi to develop at low temperatures and different *Eh* values demonstrated that both strains were able to grow in the absence of oxygen after a single cultivation in the microaerophilic medium (2% O_2). At the same time, while for strain VKM FW-2241 had simply to "remember" its habitat (cryopegs), strain VKM F-3808 had to adapt its metabolism to the conditions of anaerobiosis and to the ability to grow at a low temperature (6°C). Both strains could grow on the media within a wide *Eh* range with the optima of +5 mV at 6°C and −108 mV at 16°C. In both strains, fermentation was accompanied by accumulation of acetate and lactate.

Our experiments give fundamental evidence for the fact that the natural conditions of cryopegs do not prevent development of the fungi of the genus *Geomyces*. It was established for the first time that, under conditions of low temperatures, high salinity of the medium, and the absence of oxygen, their metabolism becomes more effective, providing survival and competitiveness of the fungi of this genus under extreme conditions.

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