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EXPERIMENTAL ARTICLES

Halo- and Psychrotolerant *Geomyces* Fungi from Arctic Cryopegs and Marine Deposits

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Abstract—Comparative characterization of *Geomyces* isolates was performed. The isolates were obtained from Arctic cryopegs and the surrounding ancient marine deposits, from nonsaline permafrost soils, and from temperate environments. Microbiological (cultural and morphological) and molecular criteria were used to confirm the identification of the isolates as *Geomyces pannorum.* The isolates from cryopegs and surrounding marine deposits were shown to differ from those obtained from nonsaline soils and temperate environments in their ability to grow at negative temperatures (–2°C) under increased salt concentration (10%). The results are discussed in relation to the possible inheritance of the adaptive characteristics acquired in specific environments.

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Key words: *Geomyces pannorum*, permafrost soils, cryopegs.

Cryopegs are bodies of highly mineralized water with subzero temperature which can exist for a geologically significant time. We have studied the Arctic cryopeg lenses of the Kolyma lowland (shore of the East Siberian Sea, the vicinity of Lake Yakutskoe, depths of 12–30 m; Fig. 1a). They are confined to the mQII marine horizon of 20 m thickness, which was accumulated in a marine coastal lagoon. After the regression of the Polar Ocean at the end of the mid Pleistocene (100–120 ka ago), the water-saturated bottom sediments were transferred to the subaerial state and froze. In the course of freezing, the salts moved from the sediments into water; thus brines were formed, which did not freeze at subzero temperatures. Cryopegs are a unique hydrological system with subzero temperature (fluctuations from –9 to –11°C) and high salinity (170–300 g/l), which have been isolated from external factors throughout their geological history [1].

It is known that viability is not impaired by the low temperatures of the frozen strata, when the rates of biochemical reactions and biological processes decrease drastically. The interest in studies of the microbial communities of cryopegs is caused by the importance both to general biology and to astrobiology of the preservation of viable organisms in aqueous media for geological intervals of time.

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The total numbers of microorganisms inhabiting cryopegs are the same as in present-day water bodies. The metabolic activity of this psychrophilic, halophilic–halotolerant microbial prokaryotic community under subzero temperatures and high salinity has been confirmed [1]. Psychrotolerant microorganisms of the genera *Clostridium* and *Psychrobacter*, including new species, have been revealed among the cryopeg bacteria [2]. We have demonstrated the existence of a halo- and psychrotolerant community of mycelial fungi in cryopegs [3]. The micromycetes obtained from this habitat belong to 12 taxa, mostly of anamorphic fungi. Micromycetes of the species *Geomyces pannorum* occupy a special position among the isolated fungi. According to our data, they are better adapted to the cryopeg environment, although they are neither truly halophilic nor truly psychrophilic; they constitute 75% of all the isolates obtained. These organisms were isolated on media with increased NaCl content (up to 20%) [4]. *Geomyces pannorum* and *Geomyces vinaceus* are widespread not only in the Arctic, but in the Antarctic region as well. For example, they were revealed in eight out of the ten soil samples collected in the vicinity of the Ross Sea, Antarctica [5]. Fungi of the genus *Geomyces* were found in the vicinity of the Potter peninsula in the Antarctic [6]. The study of the adaptive capabilities of these fungi is therefore important in order to determine whether they grow actively in the extreme conditions of cryopegs or simply survive there. The strains of *Geomyces pannorum* differ

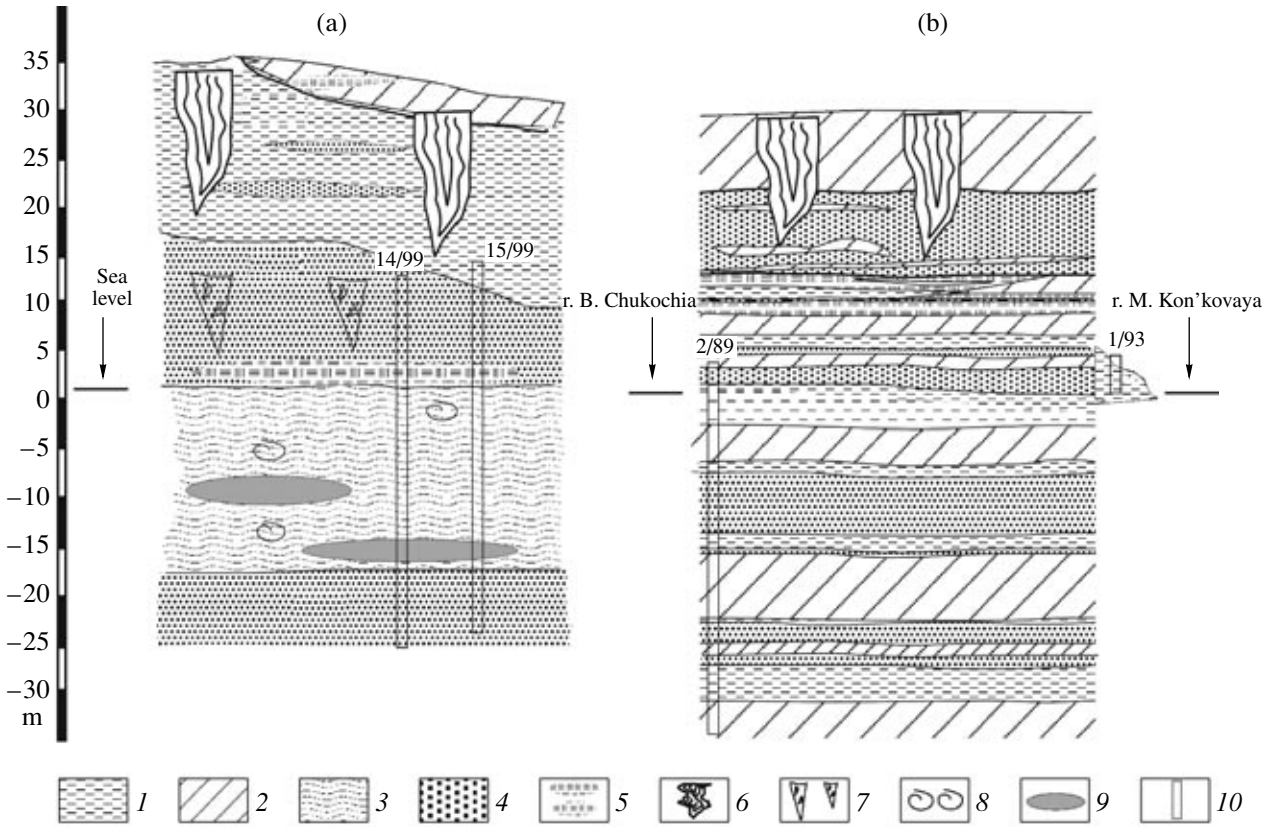

Fig. 1. Geological section of the late Cenozoic deposits: (a) East Siberian Sea shoreline; (b) Bolshaya Chukochia and Malaya Kon'kovaya interfluve. (*1*) Sandy loam; (*2*) loam; (*3*) sand and loam layers; (*4*) sand; (*5*) peaty soil; (*6*) polygonal ice wedges; (*7*) grounded ice wedges; (*8*) marine fauna; (*9*) cryopeg lenses; (*10*) boreholes.

not only in their cultural and morphological characteristics and response to temperature, but in their ability to survive in a variety of ecological niches. Such variability calls for the use of molecular approaches in order to determine the similarities and differences between the cultures isolated from various sources.

In the present article, growth of *Geomyces* fungi under extreme temperature and osmotic conditions was investigated. Strains isolated from various biotopes and exhibiting substantial cultural and morphological differences were compared on the basis of the results of molecular genetic analysis.

MATERIALS AND METHODS

The effect of temperature and NaCl concentration on growth of *Geomyces* fungi was determined for cultures isolated from various environments. Four strains were obtained from cryopegs and two from the surrounding marine permafrost deposits. Four strains were used for comparison: two were isolated from samples collected in Central Russia and two from the Kolyma lowland permafrost nonsaline Holocene (borehole 1/93) and late Pliocene (borehole 2/89) Arctic deposits (Fig. 1b, Table 1).

For the study of the growth of the *Geomyces* isolates obtained from cryopegs and marine deposits at low temperatures and increased salt concentrations, plates of Czapek medium (with or without 10% NaCl) were inoculated in triplicate with spore suspensions (ca. 10^6 spores/ml). The diameters of 12 colonies in the course of growth were determined for each strain. Statistical analysis of the results was performed. The cultivation temperatures were –2, 0, 4, and 26°C. Spore germination was assessed microscopically (×160–400). The formation of a growth tube 2 μm or longer was recorded. The duration of these experiments was 30 to 80 days, depending on the incubation conditions.

The cultural and morphological characteristics of all the strains were determined on Czapek medium and malt agar (MA) after 14 days of incubation according to the regular procedure [7].

The total DNA was isolated from the fungal mycelium by the Cenis method [8]. The evolutionally labile ITS1 and ITS2 sequences and the evolutionally conservative 5.8S rRNA gene sequences were determined. For the PCR amplification of the investigated rRNA operon fragment, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers were used. PCR amplification was performed with a GeneAmp PCR System 2700

Table 1. *Geomyces pannorum* strains used in this study

VKM strain designation	GenBank accession no.*	Location and source of isolation	
BKM F-3808	AY873968	Central Russia	Fur of a *Cletrionomus glareolus* field mouse, Krutitsy, Staritsa rigion, Tver oblast, Russia
FW-1103	DQ189226	Central Russia	Soddy podzolic soil, potato field, Zvenigorod, Moscow oblast, Russia
FW-751	DQ189227	Nonsaline permafrost tidal deposits, 5–8 ka	Borehole 1/93 (depth 4.0 m), flood plain of M. Kon'kovaya river, Kolyma lowland, Russia (69°23′ N, 158°28′ E)
FW-969	DQ189225	Nonsaline permafrost lacustrine-alluvial deposits, Olerian suite, 1.8–2.0 Ma	Borehole 2/89, basin of Chukochia river, Kolyma lowland, Russia (69°29′ N, 156°59′ E)
FW-857	DQ189229	Cryopegs, 100–120 ka	Borehole 14/99, Lake Yakutskoe, Kolyma lowland, Russia (69°59′ N, 159°30′ E)
FW-2236	DQ189228	Cryopegs, 100–120 ka	Borehole 15/99, Lake Yakutskoe, Kolyma lowland, Russia (69°59′ N, 159°30′ E)
FW-2238	AY873965	Cryopegs, 100–120 ka	
FW-2241	AY873966	Cryopegs, 100–120 ka	
FW-2260	DQ189224	Saline marine permafrost deposits, Kon'kovskaya suite, 100–120 ka	Borehole 14/99 (depth 14.32–15.46 m), Lake Yakutskoe, Kolyma lowland, Russia (69°59′ N, 159°30′ E)
FW-2264	AY873967		

* GenBank. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=nucleotide.

(Applied Biosystems, United States) in 25 μl of the mixture containing tenfold PCR buffer (Fermentas, Lithuania), 2.5 μl; 25 mM $MgCl_2$, 1.5 μl; a mixture of four deoxyribonucleoside triphoshpates (2.5 mM each), 2.5 μl; ITS1 and ITS4 primers, 1 μl each; genomic DNA, 0.025 μg; and *Taq* DNA polymerase (Fermentas, Lithuania), 0.5 U. After preliminary denaturation (94°C, 3 min), 30 amplification cycles were performed under the following conditions: denaturing (94°C, 30 s), annealing (55°C, 30 s), and synthesis (72°C, 80 s).

The nucleotide sequence of the amplified rRNA operon fragment was determined using a CEQ2000XL automatic DNA sequencer (Beckman Coulter, United States) according to the manufacturer's recommendations.

The related nucleotide sequences were recovered from the GenBank database using the BLASTn software package. The ClustalX software package [9] was used to align the nucleotide sequences. The matrix of evolutionary distances was constructed using the Jukes and Cantor algorithm [10]. The phylogenetic tree was constructed using the TREECON software package [11].

RESULTS AND DISCUSSION

In spite of substantial morphological differences (Table 2), all the strains obtained from different environments were assigned, on the basis of their conidiogenesis type (formation of thallic arthroconidia) and similar micromorphological features [7], to the species *Geomyces pannorum* in our previous publication.

Comparative analysis of the evolutionally labile and evolutionally conservative sequences of various fragments of rRNA genes is widely used in mycology for organism identification at the intraspecific, species, and higher levels [12]. This approach was applied to the estimation of the genetic relatedness of the isolates obtained from cryopegs, from environmental samples collected in Central Russia, and from nonsaline permafrost deposits. The evolutionally labile ITS1 and ITS2 sequences and the evolutionally conservative 5.8S rRNA gene sequences were determined for the obtained *Geomyces pannorum* cultures (Table 1). The research on various fungal taxa (genera *Penicillium*, *Colletotrichum*, *Phytophthora*) revealed that ITS1 and ITS2 rDNA regions could be used to determine the taxonomic and phylogenetic relationships between the species within the genera. These regions are believed to vary only slightly within species, while their variation between the species of a genus is significant. They are therefore widely used for the rapid identification of fungal species by restriction analysis of the amplified ITS1-5.8S-ITS2 rDNA region [13]. For example, the analysis of the restriction profiles of the ITS1-5.8S-ITS2 rDNA region (RFLP analysis) of 139 strains of 20 *Chrysosporium* species (this genus is closely related to *Geomyces*) revealed that for nine species the interstrain

Table 2. Cultural and morphological characteristics of the strains

Strain no. (FW-)	Colony diameter after 14 days		Color	Radial grooves	Colony margin	Reverse	Conidia
	Czapek	MA					
Species description [7]	No data	2.5–4.0	White, pale yellow to dark gray, dark yellow, or shades of brown	Defined	Defined, regular, sometimes serrated	Creamy, yellowish to yellow-brown and red-brown, brown in the center	Subhyaline, smooth-walled, ovoid, cuneiform, slightly clavate, 3–6 × 2–4 μm, scar 1–1.5 μm
3808*	1.95	2.37	Pale yellow	Defined	2–3 mm, defined, regular, submerged	Yellow-brown	Subhyaline, smooth-walled, clavate, 2.7 × 2.1 μm, scar 0.9 μm
1103	1.35	1.58	Pale yellow with a shade of pink	Defined	1 mm, defined, regular, submerged	Dark red-brown	Subhyaline, smooth-walled, clavate, 3.3 × 2.5 μm, scar 1.0–1.1 μm
751	1.06	1.68	Gray with pink sectors	Defined	1–2 mm, defined, regular, submerged	Dark red-brown	Subhyaline, smooth-walled, regular, clavate, 2.9 × 2.3 μm, scar 0.9 μm
969	1.55	1.95	Grayish	Defined	4 mm, defined, regular	Yellow-brown	Subhyaline, smooth-walled, cuneiform, 2.5 × 2.1 μm, scar 0.9 μ]m
2264	0.64	1.28	Grayish	Not defined	1 mm, defined, regular, submerged	Dark gray with a shade of brown	Subhyaline, smooth-walled, highly variable in shape, often curved, 4.4 × 3.1 μm, scar 1.5 μm
2238	0.75	1.52	Grayish	Not defined	1 mm, defined, regular, submerged	Dark gray with a shade of brown	Subhyaline, smooth-walled, cuneiform, 3.7 × 3.0 μm, scar 1.2 μm
2241	0.63	1.45	Grayish	Not defined	1 mm, defined, regular, submerged	Dark gray with a shade of brown	Subhyaline, smooth-walled, clavate, 3.6 × 3.0 μm, scar 1.2 μm
2236	0.54	1.35	Pale yellow with a shade of pink	Defined	1 mm, defined, regular, submerged	Red-brown	Subhyaline, smooth-walled, clavate, 2.8 × 2.0 μm, scar 0.9 μm
2260	0.93	1.45	Pale yellow with a shade of pink, red-brown pigment in the medium	Defined	1 mm, defined, regular, submerged	Red-brown	Subhyaline, smooth-walled, clavate, 3.7 × 2.9 μm, scar 1.3 μm
857	0.35	1.13	Pale yellow with a shade of pink	Defined	1 mm, defined, regular, submerged	Red-brown	Subhyaline, with a clearly visible dark wall, smooth-walled, clavate, 4.2 × 3.1 μm, scar 1.5 μm

* VKM F-3808. All the other strains designated (FW-).

variability within species was higher than the interspecific variability [14].

The DNA fragments sequenced in the course of the present work included 18S rRNA genes (fragment), ITS1, 5.8S rRNA, ITS2, and 28S rRNA (fragment). For the six strains from cryopegs and marine deposits, the two from nonsaline permafrost soils, and strain FW-1103 from Central Russia, the length of the sequenced fragments was 566 nucleotides. For the culture VKM F-3808, the length of the sequenced fragments was 568 nucleotides. Molecular analysis revealed identical nucleotide sequences of the sequenced fragment in the following strains: FW-2238, FW-2241, and FW-2264; FW-857 and FW-2236; FW-751 and FW-1103. The culture FW-969 was the closest to VKM F-3808 (four nucleotide substitutions in ITS1 region); FW-751 and FW-1103 were the most remote (15 nucleotide substitutions in the ITS1 and ITS2 regions).

The average data on the growth of the investigated strains on Czapek medium at positive temperatures (0, 4, and 26°C) are presented in Fig. 2. All the strains were capable of growth in this temperature range. Both the strains from Central Russia and from nonsaline deposits exhibited higher growth rates at 26°C than at 4°C; in the latter case this difference was less pronounced. On the contrary, the strains from cryopegs and marine deposits exhibited higher growth rates at 4°C. A general decrease in growth rates occurred at 0°C. However, while the colony size at 0°C was 1.9-fold less than at 26°C for the isolates from cryopegs and marine deposits, it was 2.9–3.7-fold less for the isolates from other environments.

Other findings also indicate better adaptation of the cryopeg strains to growth at low temperatures. In our experiments, cultivation of *G. pannorum* at –2°C resulted in spore germination beginning 2–3 weeks after inoculation; by the 80th day, the colonies of FW-2238 and FW-2241 reached 5.7 and 4.65 mm in diameter, respectively. For the strains isolated from Central Russia and from nonsaline permafrost deposits, the formation of growth tubes was first detected only 45 days after inoculation. They were formed from 5–20% of the spores; their length was 10–40 μm (Fig. 3).

Our experiments on the cultivation of *G. pannorum* on media with 10% NaCl have demonstrated that the strains isolated from Central Russia and from nonsaline permafrost deposits were less adapted to high salt concentrations than those isolated from cryopegs. Under this NaCl concentration, their spores did not germinate over the whole course of the incubation period.

All the investigated strains from cryopegs and marine deposits were resistant to high NaCl concentrations [4]. On the 30th day of incubation at 4 and 26°C, these cultures formed either microcolonies (300–800 μm in diameter) or macrocolonies (ca. 0.5 cm). Growth at 26°C was two times slower than at 4°C.

The experiments on *G. pannorum* growth at lower temperatures on media with 10% NaCl required prolonged incubation. The dynamics of growth-tube formation for the strains from cryopegs and marine deposits in the course of an 80-day experiment at 0°C is presented in Table 3. The formation of the growth tubes commenced on the 30th day. In some strains, the length of the growth tubes reached 150–300 μm by the 80th day. The spores of the other group of strains did not germinate within this period. On the media without additional salt, all the cultures formed colonies after 45 days of incubation; their diameter was 1.0–2.2 cm for the strains from cryopegs and marine deposits and 1.1–1.5 cm for those isolated from Central Russia and from nonsaline permafrost deposits.

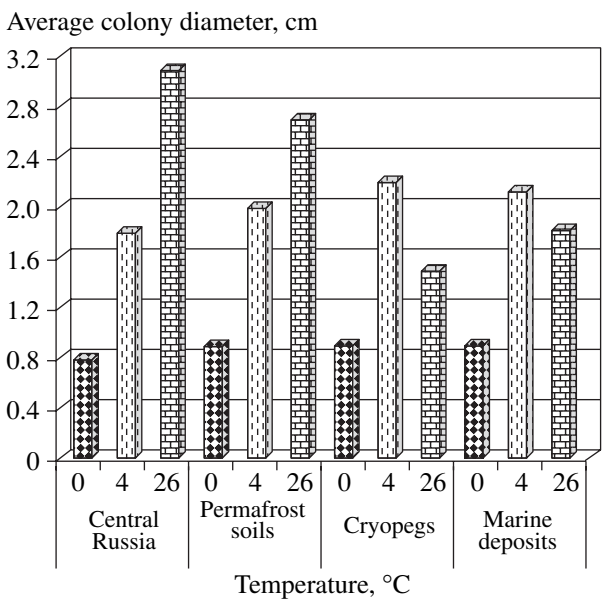

Fig. 2. Medium values of the colonies diameter of the *Geomyces* strains obtained from various environments (Czapek medium, experiment duration, 30 days).

In the case of strains FW-2241 and FW-2238, growth tubes were formed after two months of incubation at –2°C on media with 10% NaCl; the spores of all the investigated cultures germinated to some degree by the 80th day of incubation (Table 3). The strains obtained from Central Russia and from nonsaline permafrost deposits did not form growth tubes in the course of the experiment.

These results demonstrate that *Geomyces* fungi from the halo- and psychrophilic community of cold brines can both survive and grow under extreme conditions. According to our data, these fungi predominate in cryopegs and can be isolated from the majority of samples. Our experiments have demonstrated that the growth optimum of the strains isolated from cryopegs and marine permafrost deposits shifted to lower temperatures. Moreover, all these strains grew at subzero temperatures. Under these conditions, their growth rates are hundreds of times higher than those of the strains from other environments (Fig. 3).

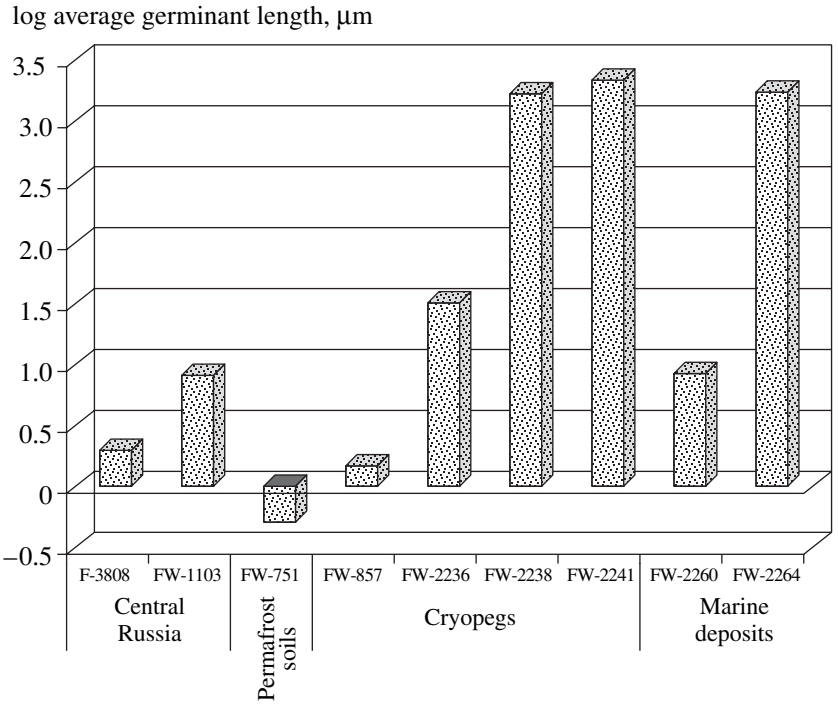

Fig. 3. Spore germination (average germinant length) by *Geomyces* isolates at –2°C (Czapek medium, experiment duration, 45 days).

The published data concerning the *G. pannorum* strain isolated from Antarctic liverwort moss (67°34′ S, 68°08′ W) confirm its growth at –2°C with a radial rate of 0.05 mm/day [15]. This finding is in accordance with our results on the Kolyma strains. For example, the growth rate of strains FW-2241 and FW-2238 under these conditions was ca. 0.04 mm/day. Italian researchers working with the frozen soils of Franz Josef Land have isolated fungi belonging to 21 genera; they stated that only four of these, including *Geomyces*, could exhibit growth, however weak, all year round. The majority of the fungal population of arctic soils is capable of growth only during a brief time interval of increased temperature; they survive the remaining part of the year in resting stages [16]. The biochemical mechanisms of fungal resistance to low temperatures are known. For example, *Geomyces* fungi have been shown to change the composition and the total content of fatty acids depending on growth temperature [17]. The amount of polyunsaturated fatty acids and the total unsaturation index increased when these fungi were incubated at 5°C [18].

Fungi are known to exist in hypersaline solutions; the diversity of fungi isolated from hypersaline biotopes is quite high [19]. The investigated *Geomyces* isolates from cryopegs were capable of growth on media with increased NaCl content (10%) under low positive and negative temperatures. Our data demonstrated spore germination after 60-day incubation of strains FW-2241 and FW-2238 at –2°C. These cultures were isolated from cryopegs on media with 5–20% NaCl. Germination of the spores of all the strains isolated from cryopeg brines and marine deposits occurred by the 80th day of incubation on salt-containing media at subzero temperatures. No spore germination occurred under these incubation conditions in the cultures of the strains of the other group, i.e., those isolated from Central Russia and from nonsaline permafrost deposits.

Our research has demonstrated that *G. pannorum* cultures isolated from different environments can exhibit significant morphological differences (Table 2) and physiological peculiarities, including the ability to grow under various temperatures, depending on the source of isolation. Therefore the species identification of newly obtained isolates can be difficult. This may be the case with other extremophilic fungi as well. Comparative molecular genetic analysis of the ITS1-5.8S-ITS2 rDNA fragment was therefore applied in order to determine the relations between the investigated strains. In spite of the morphological and physiological differences between the strains, no genetic differences between the isolates were revealed (including the organisms from ancient Pliocene deposits) (Fig. 4). The range of variation between the nucleotide sequences of the ITS1-5.8S-ITS2 rDNA fragment did not exceed the intraspecific one. Thus, all the cultures can be assigned to the species *G. pannorum*.

The present work not only confirms the ability of *Geomyces* fungi to grow at negative temperatures, but also expands our knowledge of the ecological possibilities of this genus. The adaptive capabilities to grow

Table 3. Spore germination of *Geomyces* strains on Czapek medium with 10% NaCl at 0 and –2°C

Strains	Germination	Temperature			
		0°C			–2°C
		30 days	45 days	80 days	80 days*
FW-857	% germinated spores	5	10	10	5
	Germ tube length, μm	5	10	15	5
FW-2236	% germinated spores	90	100	100	3
	Germ tube length, μm	35	55	80	5
FW-2241	% germinated spores	80	100	100	80
	Germ tube length, μm	20	30	150	30
FW-2238	% germinated spores	50	90	100	80
	Germ tube length, μm	15	35	150	20
FW-2260	% germinated spores	5	20	30	3
	Germ tube length, μm	5	15	15	5
FW-2264	% germinated spores	20	50	80	60
	Germ tube length, μm	30	50	350	10

* Spore germination on the 30th and 45th day was not detected under experimental conditions.

under the combined effect of negative temperature and high salinity were demonstrated.

The available information concerning microbial cross-resistance to a combination of various stressing agents indicates that cryopeg fungi may have other adaptive capabilities. Since high salt concentrations result in both osmotic and oxidative stresses, these fungi are probably adapted to growth under microaerophilic conditions when the effect of the latter stressor is decreased [20]. Further research is required to confirm or reject this hypothesis. Moreover, the maintenance in VKM of cultures isolated from cryopegs will promote research in this direction.

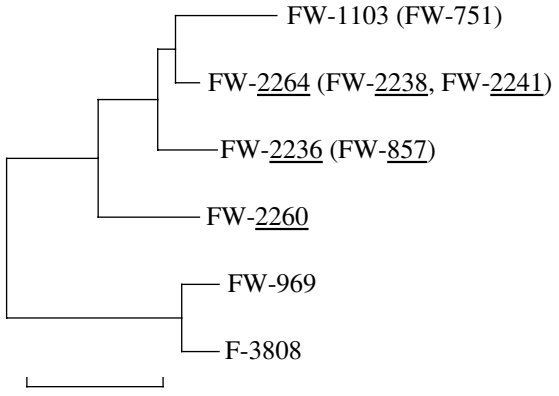

Fig. 4. Phylogenetic tree of the *Geomyces* strains isolated from cryopegs and surrounding marine deposits (underlined), from Middle Russia, and from nonsaline permafrost deposits constructed on the basis of nucleotide sequences of rRNA operon fragments. The scale bar corresponds to one nucleotide substitution per 100 nucleotides.

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REFERENCES

1. Gilichinsky, D., Rivkina, E., Shcherbakova, V., Laurinavichuis, K., and Tiedje, J., Supercooled Water Brines within Permafrost – An Unknown Ecological Niche for Microorganisms: a Model for Astrobiology, *Astrobiology,* 2003, vol. 3, no. 2, pp. 331–341.
2. Shcherbakova, V.A., Chuvilskaya, N.A., Rivkina, E.M., Pecheritsyna, S.A., Laurinavichius, K.S., Suzina, N.E., Osipov, G.A., Lysenko, A.M., Gilichinsky, D.A., and Akimenko, V.K., Novel Psychrophilic Anaerobic Spore-Forming Bacterium from the Overcooled Water Brine in Permafrost: Description *Clostridium algoriphilum* sp. nov., *Extremophiles*, 2005, vol. 9, no. 3, pp. 239–246.
3. Ozerskaya, S.M., Ivanushkina, N.E., Kochkina, G.A., Fattakhova, R.N., and Gilichinsky, D.A., Mycelial Fungi in Cryopegs, *Int. J. Astrobiol*, 2005, vol. 3, no. 4, pp. 327–331.
4. Gilichinsky, D., Rivkina, E., Bakermans, C., Shcherbakova, V., Petrovskaya, L., Ozerskaya, S., Ivanushkina, N., Kochkina, G., Laurinavichuis, K., Pecheritsina, S., Fat-

takhova, R., and Tiedje, J., Biodiversity of Cryopegs in Permafrost, *FEMS Microbial Ecol.*, 2005, vol. 53, pp. 117–128.

5. Marshall, W.A., Aerial Transport of Keratinaceous Substrate and Distribution of the Fungus *Geomyces pannorum* in Antarctic Soils, *Microb. Ecol.*, 1998, vol. 6, pp. 212–219.
6. Comerio, R.M. and McCormack, W., Some Micromycetes Isolated from Spoiled Food and Soil in Argentine Antarctica, *Rev. Iberoam. Mycol.*, 2004, vol. 21, pp. 128–134.
7. Van Oorschot, C.A.N., A Revision of *Chrysosporium* and Allied Genera, *Studies in Mycology*, 1980, vol. 20, pp. 1–89.
8. Cenis, J.L., Rapid Extraction of Fungal DNA for PCR Amplification, *Nucleic Acids Res.*, 1992, vol. 20, p. 2380.
9. Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G., The CLUSTAL_X Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools, *Nucleic Acids Res.*, 1997, vol. 25, pp. 4876–4882.
10. Jukes, T.H and Cantor, C.R, Evolution of Protein Molecules, *Mammalian protein metabolism*, Munro, H.N., Ed., New York: Academic, 1969, pp. 21–132.
11. Van de Peer, Y. and De Wachter, R., TREECON for Windows: a Software Package for the Construction and Drawing of Evolutionary Trees for the Microsoft Windows Environment, *Comput. Applic. Biosci*, 1994, vol. 10, pp. 569–570.
12. Edel, V., *Use of PCR and RFLP in Fungal Systematics, Chemical Fungal Taxonomy*, Frisvad J.C., Bridge P.D., and Arora D.K, Eds., New York: Marcel Dekker, 1998, pp. 51–76.
13. Edel, V., Polymerase Chain Reaction in Mycology: An Overview, *Applications of PCR in Mycology*, Bridge P.D., Arora, D.K., Reddy, C.A., and Elander, R.P., Eds., Cambridge: Cambridge Univer. Press, 1998, pp. 1–20.
14. Vidal, P. and Guarro, J., Identification and Phylogeny of *Chrysosporium* Species Using RFLP of the PCR-ITS Region, *Studies in Mycology*, 2002, vol. 47, pp. 189–199.
15. Hughes, K.A., Lawley, B., and Newsham, K.K., Solar UV-B Radiation Inhibits the Growth of Antarctic Terrestrial Fungi, *Appl. Environ. Microbiol.*, 2003, vol. 69, no. 3, pp. 1488–1491.
16. Bergero, R., Girlanda, M., Varese, G.C., Intili, D., and Luppi, A.M., Psychrooligotrophic Fungi from Arctic Soils of Franz Joseph Land, *Polar biology*, 1999, vol. 21, no. 6, pp. 361–368.
17. Finotti, E., Paolino, C., Lancia, B., and Mercantini, R., Metabolic Differences Between Two Antarctic Strains of *Geomyces pannorum, Curr. Microbiol.*, 1996, vol. 32, no. 1, pp. 7–10.
18. Weinstein, R.N., Montiel, P.O., and Johnstone, K., Influence of Growth Temperature on Lipid and Soluble Carbohydrate Synthesis by Fungi Isolated from Fellfield Soil in the Maritime Antarctic, *Mycologia*, 2000, vol. 92, no. 2, pp. 222–229.
19. Grishkan, I., Nero, E., and Wasser, S.P., Soil Micromycete in the Hypersaline Dead Sea Costal Area, Israel, *Mycol. Progr.*, 2003, vol. 2, no. 1, pp. 19–28.
20. Plakunov, V.K., Geidebrekht, O.V., and Shelemekh, O.V., Microorganisms under Multistress: hfrm or Good?, in *Trudy Instituta Mikrobiologii im. S.N. Vinogradskogo. Vyp. 12* (Proc. Winogradsky Inst. Microbiol., vol. 12), Moscow: Nauka, 2004, pp. 361–375.