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EXPERIMENTAL ARTICLES

Yeast Diversity in Hydromorphic Soils with Reference to a Grass–Sphagnum Wetland in Western Siberia and a Hummocky Tundra Region at Cape Barrow (Alaska)

A. V. Polyakova*, I. Yu. Chernov, and N. S. Panikov***

**Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117811 Russia*
***Institute of Soil Science of the Moscow State University and Russian Academy of Sciences,*
Vorob'evy gory, Moscow, 119899 Russia

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Abstract—The microbiological analysis of 78 samples taken from a high bog in Western Siberia and from a tundra wetland soil in Alaska showed the presence of 23 yeast species belonging to the genera *Bullera, Candida, Cryptococcus, Debaryomyces, Hanseniaspora, Metschnikowia, Mrakia, Pichia, Rhodotorula, Saccharomyces, Sporobolomyces, Torulaspora*, and *Trichosporon.* Peat samples from the high bog were dominated by eurytopic anamorphic basidiomycetous species, such as *Rhodotorula mucilaginosa* and *Sporobolomyces roseus,* and by the ascomycetous yeasts *Candida* spp. and *Debaryomyces hansenii.* These samples also contained two rare ascomycetous species (*Candida paludigena* and *Schizoblastosporion starkeyi-henricii*), which so far have been found only in taiga wetland soils. The wetland Alaskan soil was dominated by one yeast species (*Cryptococcus gilvescens*), which is a typical inhabitant of tundra soils. Therefore, geographic factors may serve for a more reliable prediction of yeast diversity in soils than the physicochemical or ecotopic parameters of these soils.

Key words: yeasts, oligotrophic and mesotrophic bogs, peat, abundance.

Yeast fungi are ubiquitous in soils and on the surface of plants in virtually all geographic zones. The geographic aspects of yeast ecology are presently known for a wide range of zonal soils [1]. However, little is known about the yeast diversity of intrazonal soils, such as vast wetlands in the tundra and forest areas of Eurasia and North America.

The yeast microflora of taiga bogs has not been sufficiently studies. The stenobiont yeast species typical of boreal marshy soils have not yet been found in other habitats. The adaptive capacity of peatland soil yeasts was discussed by Bab'eva and Blagodatskaya [2]. The investigation of yeast diversity in the bogs and peatlands of the central part of European Russia [3] was not extended. To the best of our knowledge, there is no data in the literature concerning yeast ecology in the vast and diverse wetlands of western Siberia, which themselves are dominated by upland sphagnum oligotrophic and mesotrophic bogs. This type of habitats is distinguished by a severe deficiency of essential mineral components, a redundancy of carbon sources, acidity (pH 3–5), a low buffer capacity, low temperatures, as well as anoxic and stationary conditions, which are favorable for the accumulation of toxic plant metabolites. These features make sphagnum peatlands extreme biotopes, where microbial activity is suppressed (peats are formed exactly for this reason) and where specific microorganisms, primarily methanotrophs, are selected [4, 5].

According to the data of Bab'eva and Chernov [1], tundra peats and moss cover are characterized by the maximum abundance and diversity of yeast species. The adaptive capacity of yeasts in these habitats is characterized by psychrophily and the outbreaks of yeast growth over short favorable summer periods [6]. Moderate acidophily is typical of the yeasts that inhabit sphagnum bogs in the boreal zone. The ability of tundra yeasts to grow under low osmotic pressures is less evident than other adaptive capacities of these yeasts.

The aim of the present study was to characterize the abundance and diversity of yeasts in northern wetlands with reference to those around two field stations: the Plotnikovo station located in the Tomsk region in western Siberia, which is dominated by oligotrophic and mesotrophic taiga bogs [7, 8], and the Barrow station (Alaska) located in the typical tundra region with a severe continental climate, tending to become warmer in the last decade [9, 10].

MATERIALS AND METHODS

The Plotnikovo field station with geographic coordinates of 57° N and 82° E is located close to the center of the Bakchar bog at the watershed between the Bak-

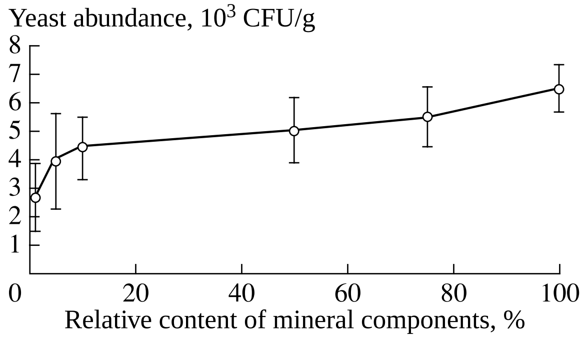

Fig. 1. Dependence of the number of detected yeasts on the relative content of mineral components in the synthetic nutrient medium.

char and Iksa Rivers in the western zone of the Tomsk region. The Bakchar bog of the so-called Narym type is part of the Great Vasyugan wetland, which itself is dominated by swampy pools and oligotrophic vegetation, while mesotrophic and eutrophic vegetation is scarce. Samples were collected in August 1998, in May through July 1999, and in July 2000 beneath the following plant associations:

(1) a suppressed pine (*Pinus silvestris*) forest along the bog periphery with the *Ledum polustre, Oxycoccus microcarpus, Andromeda polifolia,* and *Chamaedaphne calyculata* shrubs and the *Sphagnum fuscum* cover;

(2) an *Eriophorum vaginatum, Sphagnum angustifolium,* and *Sph. magellanicum* association in the hummocky region of the bog;

(3) a sedge–sphagnum association dominated by *Carex rostrata, C. limosa, Sph. majus*, and *Sphagnum* sp.;

(4) an association of *Menyanthes trifoliata* and *Sphagnum* sp. in the bog region with swampy pools 5 to 10 m in diameter;

(5) an association of the horsetail *Equisetum fluviatile* and the sedge *C. rostrata* with the *Sph. majus* cover between peat ridges up to 50 cm in height overgrown by bushes and *Betula nana.*

Climatic conditions at the Plotnikovo station are characterized by an average July temperature of 16.8°C, summer precipitations of 352 mm, the number of days with average daily temperatures above zero equal to 186 per year, and a vegetation period of 190 days.

The Barrow station with geographic coordinates of 71° 18′ N and 156° 40′ E is located 2 km south of the Arctic Ocean coast. Climatic conditions at the Barrow station are characterized by an average July temperature of 2.5°C, summer precipitations of 36 mm, the number of days with average daily temperatures above zero equal to 250 per year, and a vegetation period of about 90 days. The samples of peaty gley soil were collected in September 1998 and in September 1999 in a wet hummocky tundra region overgrown by *E. vaginatum.*

In total, 78 peaty soil samples taken from 9 soil horizons were analyzed. Samples from the Bakchar bog, taken from depths of 50–200 cm using a peat sampler manufactured at the Kuopio University (Finland), were analyzed immediately after the sampling. Samples at the Barrow station were taken from depths ranging from the soil surface to the upper permafrost surface located at a depth of 30 cm and stored at –18°C until used for analysis.

The abundance and the taxonomic composition of yeasts were determined by plating peaty soil samples onto a wort agar acidified to pH 3.5 with lactic acid. In preliminary experiments, the samples were also plated onto a basal synthetic medium containing (g/l) NH_4SO_4, 0.4; KH_2PO_4, 0.8; $MgSO_4$, 0.1; CaCl2, 0.02; glucose, 1.5; agar, 20; biotin, 10^{-6}; and a mixture of microelements, 5×10^{-5}. The concentration of all mineral components of this medium was proportionally varied from 1 to 100%, leaving the agar and glucose concentrations unchanged.

The plates were incubated in a refrigerator at 6°C for two weeks. Colonial morphotypes were differentiated by examining the grown yeast colonies with a binocular lens. Some representatives of each morphotype were isolated in pure cultures and identified based on their morphological and physiological characteristics [11, 12]. Most of the isolated yeast strains were easily identified based only on their morphological characteristics. At the same time, the representatives of the anamorphic genera *Candida, Rhodotorula*, and *Cryptococcus* could be identified only using the complete range of physiological characteristics. Some yeast isolates were identified to a generic level. For each of the soil samples analyzed, the total number of yeasts and the relative abundances of yeast species were determined.

RESULTS AND DISCUSSION

Dependence of the number of identified yeast species on the composition of test nutrient media. Generally, yeasts are isolated from natural habitats using the acidified wort agar (AWA), which by itself can provide for the nutritional requirements of all presently known yeast species [11, 12]. However, as was the case with methanotrophs [4, 5], the growth of some yeasts of authochthonous communities adapted to extremely low osmotic pressures could be inhibited by some mineral components of the test nutrient medium. To verify this supposition, the same peat sample was plated onto AWA (as the control) and synthetic agar media containing equal amounts of glucose and agar but different amounts of mineral components. As can be seen from the data presented in Fig. 1, the isolated yeasts were not oligotrophic, since the number of enumerated yeast species increased with increasing the

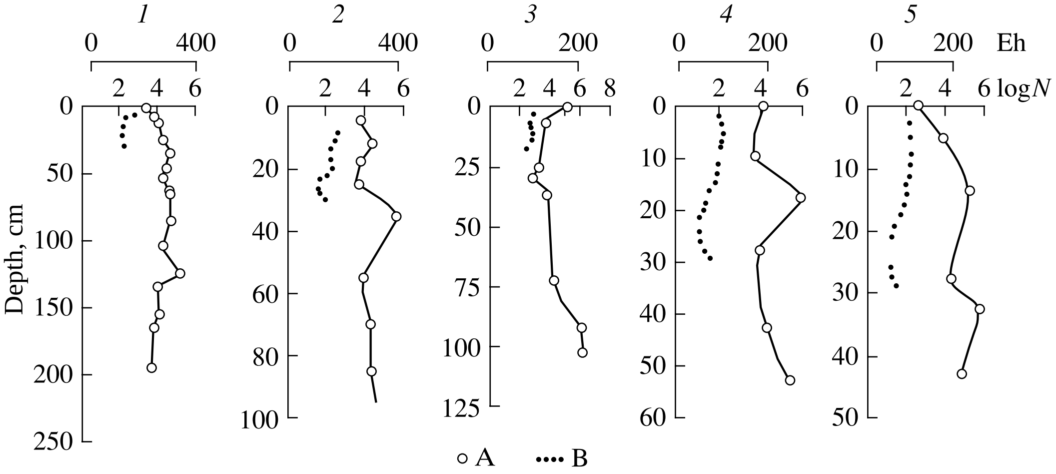

Fig. 2. Vertical distribution of yeasts in the peat soil of the Bakchar bog beneath various plant associations designated by figures *1–5* (for their description, see the *Materials and Methods* section).

concentration of the mineral components of the synthetic growth medium. The number of yeast colonies grown on the medium with the maximum mineral content was close to the number of colonies grown on AWA (the colonies were enumerated on the 5th and 10th days of incubation at room temperature).

Taking into account these results, the attempts to use synthetic mineral media for the enumeration of yeasts were no longer undertaken. The nature of the stimulating effect of salts on the colonial growth of yeasts remains unknown, since, even though low concentrations of N, P, Fe, Mg and other essential elements are limiting to yeasts, small yeast colonies still would grow. The activation of resting microbial forms by high salt concentrations, as was observed by Panikov and Evdokimova for *Penicillium funiculosum* [13], is also unlikely, inasmuch as the salt concentrations used in our experiments were not stressful. The results of these experiments, in which different salt concentrations were used, are of interest from an ecological point of view. It is evident that the growth of yeasts in freshwater sphagnum bogs is limited by available mineral sources. In other words, yeasts in acidic sphagnum bogs act as ecological patients, whose tolerance is close to a threshold value. In this property, such yeasts considerably differ from acidophilic methanotrophs and moderate acidophiles, such as the *Bejerinkia* and *Rhodopseudomonas* yeasts, which themselves are well adapted to growth in unbuffered diluted media [4, 6].

Vertical variation in the total number of yeasts in relation to the type of vegetation. The total number of yeasts in all samples examined varied from 10^2 to 1.4×10^6 CFU/g dry peat, averaging 8.6×10^4 CFU/g dry peat with a standard deviation of 12 CFU/g dry peat. It is evident that yeast abundance in the peaty soils studied is higher than in the mineral horizons of other soils, where yeast abundance usually does not exceed 10^4 CFU/g soil [1]. The total number of yeasts in the upper 30-cm layer of the bog soils calculated per 1 cm^3 averaged $(1.0 \pm 0.2) \times 10^3$ CFU/cm^3. Such yeast abundance is also higher than that typical of soddy podzolic soils in spruce forests (about 2.6×10^2 CFU/cm^3 soil).

Depending on the depth of soil horizons and the type of vegetation, yeast abundance varied within several orders of magnitude (Fig. 2). Unlike yeast abundance in forest soils, where it peaks in the upper horizons [3, 14], yeast abundance in the bog soils was higher in the middle and deeper horizons.

We failed to reveal any statistically significant relation of yeast abundance to ecotopic parameters, particularly to the redox potential (Fig. 2). Nor could we record any increase in the content of fermentative yeasts in anoxic soil horizons (Table 1). The overwhelming majority of yeasts isolated from the peat soils were strictly aerobic; however, their numbers in the oxic and anoxic soil horizons were essentially the same. Presumably, the growth of yeasts in peat soils weakly depends on the degree of aeration, and respiring yeasts can easily survive anoxic conditions. Furthermore, it is likely that toxic metabolites, which commonly accumulate in anoxic environments, do not significantly affect bog yeasts.

The number of yeasts in the tundra boggy soil of Cape Barrow varied from 10^4–10^5 CFU/g soil in the surface horizons to less than 10^2 CFU/g soil (the sensitivity threshold of the method) at depths exceeding 20 cm (Fig. 3).

The taxonomic composition of yeasts in the Bakchar bog. On the whole, we succeeded in obtaining 23 yeast isolates belonging to 14 genera (Table 2). Twenty of these isolates were identified to the species level, while three (*Candida* and *Pichia* spp.) did not correspond to any of the known species of these genera.

The yeast communities of peats from the Bakchar bog and Alaska considerably differed in their taxonomic structure. The Bakchar bog peat was dominated

Table 1. Vertical profile of fermentative yeasts in the boggy soil

Depth, cm	Fermentative yeasts, %
0–10	12.85
10–20	15.10
20–30	16.61
30–40	18.67
50–100	17.87
<100	4.33
Average for all horizons	15.81

by basidiomycetous yeasts, such as *Cryptococcus albidus, C. laurentii, Rhodotorula mucilaginosa, Sporobolomyces roseus,* and *Trichosporon pullulans.* In their adaptation characteristics, these yeasts represent phytobionts and saprobionts residing on living and dead plants and in the organogenic horizons of boreal soils [1]. The most abundant species were the pigmented epiphytic yeasts *Rh. mucilaginosa* and *Sp. roseus,* whose relative content in the total yeast population varied from 40 to 70%, depending on the soil horizon. About half of all yeast species isolated from the Bakchar bog peat represented ascomycetous yeasts of the genera *Candida*, *Debaryomyces*, *Hanseniaspora*, *Metschnikowia, Pichia, Saccharomyces,* and *Torulaspora.* The most abundant ascomycetes were asporogenous yeasts of the genus *Candida.* Some strains of these yeasts did not correspond to any of the known species of this genus and, therefore, may represent new species. Other ascomycetous species, such as *Debaryomyces hansenii, Hanseniaspora uvarum, Metschnikowia pulcherrima, Saccharomyces paradoxus,* and *Candida paludigena* were earlier detected in peats of the central part of Russia [3].

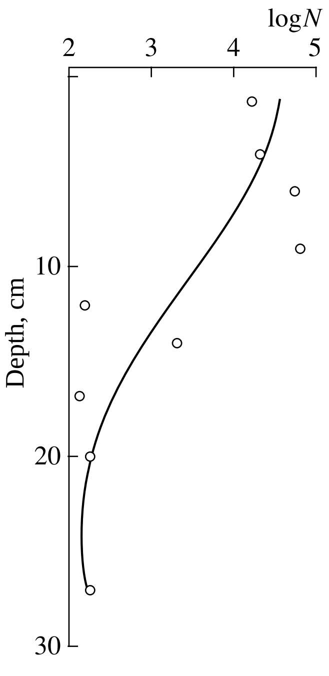

Fig. 3. Vertical distribution of yeasts in the boggy tundra soil of Cape Barrow overgrown by *Eriophorum vaginatum.*

Noteworthy is a rare species of ascomycetous anamorphic yeasts with a specific monopolar type of budding, *Schizoblastosporion starkeyi-henricii.* All presently known representatives of this rare species were isolated from the peats of oligotrophic bogs [2]. Analysis showed that there is an apparent relationship between the autecological characteristics of this species and its habitat.

Most of the ascomycetous yeasts isolated during this study are typical saccharolytics capable of utilizing mono- and disaccharides with the production of ethanol. Such yeasts are rarely isolated from mineral soil horizons, since their typical habitats (tree sap, juicy fruits, the intestinal tract of some invertebrates, etc.) are rich in easily metabolizable organic substances [1]. The presence of these yeasts in the upper peat horizons can be explained by the fact that they occur there in a state of dormancy or the survival of unfavorable conditions. Alternatively, they may utilize some sugars released in trace amounts as a result of the slow decomposition of the peat lignocelluloses.

The taxonomic composition of yeasts in peats varied depending on the depth of sampling and the type of vegetation; however, statistically significant differences were not established. Analysis by the method of the major components of mutual species occurrence (Fig. 4) showed that the both types of yeast communities studied were dominated by similar species and did not contain any exclusive species. The overlapping of data points corresponding to the peat samples taken from the Bakchar bog (but not from Alaska) indicated that there is no hiatus between different regions of this bog ecosystem.

The species composition of yeasts in the tundra soil. The yeast communities of the peat soil samples from Cape Barrow greatly differed from those of the Bakchar bog. First of all, ascomycetous yeasts were absent, which by itself is typical of soils in the climatic pessimum, including the zones of tundra, mountains, and subtropic deserts [15, 16]. All samples of the tundra soils were dominated by representatives of the genus *Cryptococcus.* In their morphological and physiological characteristics, these yeasts were most close to the species *C. gilvescens,* which dominates in all types of soils in the Taimyr tundra, including gleisolic, soddy, and boggy soils [17], as well as in the upper course of the Kolyma River [18]. In other climatic zones, the yeast *C. gilvescens* was never detected. Other basidiomycetous yeasts, such as *C. albidus* and *Rh. mucilaginosa,* are eurytopic species, which can be found in any types of soil.

Table 2. Relative abundance of soil yeast species in relation to the type of vegetation

Yeast species	Bakchar bog					Barrow
	1	2	3	4	5	
Debaryomyces hansenii Saez	1.3	6.4	2.6	17.3		
Hanseniaspora uvarum Shehata *et al.*				5.1		
Metschnikowia pulÒherrima Pitt et Miller				1.7		
Saccharomyces paradoxus Meyen et Hansen		8.8				
Candida paludigena Golubev et Blagodatskaya	5.8					
Candida sake van Uden et Buckley			<0.1			
Candida spp.	4.9	20.0	1.5	6.6	20.8	
Pichia capsulata Kurtzman		1.9				
Pichia jadinii Kurtzman				0.6		
Pichia spp.					4.9	
Torulaspora sp.		<0.1				
Shizoblastosporion starkeyi-henricii Ciferri				1.3		
Bullera punicea Nakase et Suzuki			6.0			
Cryptococcus albidus Skinner	15.7	5.3	7.5	20.4	9.1	4.7
Cryptococcus gilvescens Chernov et Bab'eva						52.9
Cryptococcus hungaricus Phaff et Fell			0.5			
Cryptococcus laurentii Skinner		1.1	2.2			
Cryptococcus sp.	0.46	0.2				
Mrakia frigida Yamada et Komagata		8.6				
Rhodotorula glutinis Harrison			0.5	6.3		
Rhodotorula mucilaginosa Harrison	31.1	35.4	24.8	18.0	18.2	42.3
Rhodotorula sp. 1			0.1			
Rhodotorula sp. 2	0.1		1.0			
Sporobolomyces roseus Kluyver et C.B.Niel	40.7	11.3	47.4	22.5	46.8	
Trichosporon pullulans Diddens et Lodder			4.6			
Number of soil samples	18	20	17	8	6	9
Number of species detected in given samples	8	11	13	10	5	3

Note: For the description of the type of vegetation, see the *Materials* and *Methods* section.

To conclude, the yeast communities of the high Bakchar peat bog in western Siberia and of the boggy tundra soils in Alaska differ considerably in taxonomic composition. The yeast communities of the Bakchar bog are dominated by eurytopic basidiomycetous yeasts, which can also be found in forest soils, and by ascomycetous yeasts, among which some species (as *C. paludigena* and *Schizoblastosporion starkeyi-henricii*) are restricted to the peat soils of the taiga region. The last circumstance distinguishes boreal boggy soils from automorphic forest soils (in the latter soils, ascomycetous yeasts, except lipomycetes, are sporadically encountered). The yeast population of the Alaskan tundra is dominated by one species, *C. gilvescens*, which in itself is a typical yeast species of tundra soils, even nonboggy ones.

The two north boggy ecosystems studied are characterized by several common features: anoxia, peat formation due to the slow decomposition of organic matter, a low mineralization, acidity (the acidity of the Bakchar bog is higher than that of the Alaskan soils), low temperatures, and long severe winter (winter at Barrow is more severe than winter in western Siberia). Low microbial activity in the Bakchar bog is likely due to a deficiency of essential mineral components, whereas low microbial activity in the Alaskan soils is primarily due to low temperatures. The yeast communities of the Bakchar bog differ from those of the Alaskan tundra even greater than from those of dry (automorphic) soils located at the same latitude. At the same time, the yeast communities of analogous habitats in North America, as well as in Siberia, are similar. Therefore, geographic factors (more specifically, latitude-dependent zonal

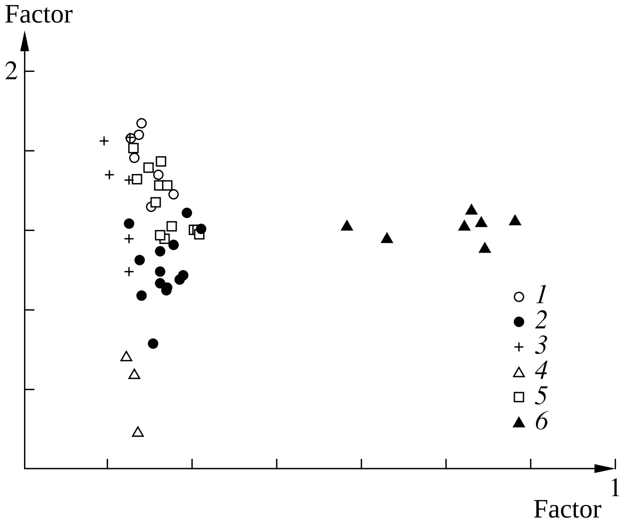

Fig. 4. The analysis of yeast communities by the method of the major components of mutual species occurrence. Dark triangles (*6*) refer to the tundra soil of Cape Barrow. The other data points (*1*–*5*) refer to different sites of the Bakchar bog (for their description, see the *Materials and Methods* section).

regularities) may serve for a more reliable prediction of yeast diversity in soils than the physicochemical or ecotopic characteristics of these soils may serve.

ACKNOWLEDGMENTS

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REFERENCES

1. Bab’eva, I.P. and Chernov, I.Yu., Geographical Aspects of Yeast Ecology, *Physiol. Gen. Biol. Rev.*, 1995, vol. 9, no. 3, pp. 1–54.
2. Bab’eva, I.P. and Blagodatskaya, V.M., The Physiological and Ecological Characterization of the Yeast *Schizoblastosporion starkeyi-henricii*, *Mikrobiologiya*, 1972, vol. 41, no. 1, pp. 99–104.
3. Golubev, V.I., Blagodatskaya, V.M., Manukyan, A.R., and Liss, O.L., Yeast Microflora of Peats, *Izv. Akad. Nauk SSSR, Ser. Biol.*, 1981, no. 2, pp. 181–187.
4. Dedysh, S.N., Liesak, W., Khmelenina, V.N., Suzina, N.E., Trotsenko, Yu.A., Semrau, J.D., Abing, A.M., Panikov, N.S., and Tiedje, J.M., *Methylocella palustris* gen. nov., sp. nov., a New Methane-oxidizing Acidophilic Bacterium from Peat Bogs Representing a Novel Sub-Type of Serine-Pathway Methanotrophs, *Int. J. Syst. Bacteriol.*, 2000, vol. 50, pp. 955–969.
5. Dedysh, S.N., Panikov, N.S., Liesak, W., Groskopf, R., Zhou, J., and Tiedje, J.M., Isolation of the Elusive Acidophilic Methanotrophs from Northern Peat Wetlands, *Science*, 1998, vol. 282, no. 5387, pp. 281–284.
6. Panikov, N.S., *Kinetika rosta mikroorganizmov: Obshchie zakonomernosti i ekologicheskie prilozheniya* (Kinetics of Microbial Growth: General Laws and Ecological Applications), Moscow: Nauka, 1992.
7. Panikov, N.S., Fluxes of CO_2 and CH_4 in High-Latitude Wetlands: Measuring, Modelling, and Predicting Response to Climate Change, *Polar Research*, 1999, vol. 18, no. 2, pp. 237–244.
8. Panikov, N.S., Sizova, M.V., Zelenev, V.V., Machov, G.A., Naumov, A.V., and Gadzhiev, I.M., Methane and Carbon Dioxide Emission from Several Vasyugan Wetlands: Spatial and Temporal Flux Variations, *Ecol. Chem.*, 1995, vol. 4, no. 1, pp. 13–23.
9. Oechel, W.C., Hastings, S.T., Vourlitis, G., Jenkins, M., Riechers, G., and Grulke, N., Recent Change of Arctic Tundra Ecosystems from a Net Carbon Dioxide Sink to a Source, *Nature*, 1993, vol. 361, pp. 520–523.
10. Oechel, W.C., Vourlitis, G.L., Hastings, S.J., Zulueta, R.C., Hinzman, L., and Kane, D., Acclimation of Ecosystem CO_2 Exchange in the Alaskan Arctic in Response to Decade Climate Warming, *Nature*, 2000, vol. 406, pp. 978–980.
11. Barnett, J.A., Payne, R.W., and Yarrow, D.A., *A Guide to Identifying and Classifying Yeasts*, Cambridge: Cambridge Univ., 1991.
12. *The Yeasts: A Taxonomic Study*, Kurtzman, C.P. and Fell, J.W., Eds., Amsterdam: Elsevier, 1998.
13. Panikov, N.S. and Evdokimova, N.V., Effect of Mineral Salts on the Growth and Amylase Biosynthesis in a Batch *Penicillium funiculosum* Culture, *Prikl. Biokhim. Mikrobiol.*, 1989, vol. 25, no. 1, pp. 84–90.
14. Golovchenko, A.V., Polyanskaya, L.M., Dobrovol’skaya, T.G., Vasil’eva, L.V., Chernov, I.Yu., and Zvyagintsev, D.G., The Spatial Distribution and Structure of Microbial Complexes in Swamp–Forest Ecosystems, *Pochvovedenie*, 1993, no. 10, pp. 78–89.
15. Bab’eva, I.P. and Reshetova, I.S., Yeasts in Pamir Soils, *Vestn. Mosk. Univ., Ser. Biol. Pochvoved.*, 1971, no. 2, pp. 80–86.
16. Chernov, I.Yu., Synekological Analysis of Yeast Groups in Taimyr Tundra, *Ekologiya*, 1985, no. 1, pp. 54–60.
17. Chernov, I.Yu. and Bab’eva, I.P., A New Yeast Species of the Genus *Cryptococcus* Isolated from Tundra Soil, *Mikrobiologiya*, 1988, vol. 57, no. 6, pp. 1031–1034.
18. Golubev, V.I., Yeasts of the Arctic West Siberian Tundra, *Izv. Akad. Nauk SSSR, Ser. Biol.*, 1986, no. 4, pp. 609–612.