ISSN 0026-2617, Microbiology, 2010, Vol. 79, No. 6, pp. 840–844. © Pleiades Publishing, Ltd., 2010.
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EXPERIMENTAL ARTICLES

New Data on the Distribution of Certain Psychrophilic Yeasts in Moscow Oblast

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Received March 4, 2010

Abstract—Several rare species and varieties of psychrophilic yeasts were isolated from the *Sphagnum* mosses and paludal vascular plants of Moscow oblast. Based on their 26S rDNA D1/D2 nucleotide sequences, they were assigned to the species *Sterigmatosporidium polymorphum, Rhodotorula psychrophenolica*, and *Aureobasidium pullulans* var. *subglaciale*. Thus, a new habitat of *S. polymorphum* was found and yeasts known previously only for the Alpine environments and the Arctic region have been isolated in the central regions of Russia for the first time.

Keywords: psychrophilic yeasts, *Sterigmatosporidium polymorphum, Rhodotorula psychrophenolica, Aureobasidium pullulans*, 26S rDNA.

DOI: 10.1134/S0026261710060172

After the first or several repeated isolations, rare species of microorganisms are often associated with the corresponding regions of isolation: the Arctic, Antarctica, high mountains, etc. However, sometimes these rare and "locally prevalent" species may also be found in similar, but geographically remote, natural habitats.

Plants and plant debris are the main natural habitat for most yeast species. Numerous and diverse yeast communities are formed on the surface of the living parts of plants [1, 2]. Due to their specific physiological properties and special vegetation conditions, *Sphagnum* mosses and paludal vascular plants are an interesting, but so far insufficiently studied, natural habitat for epiphytic yeasts. The role of *Sphagnum* mosses and paludal vascular plants in the formation of yeast communities consists in changing the nutritive regimen (lack of nitrogen and microelements and, in the case of *Sphagnum* mosses, considerable content of organic acids in the exudates) and low temperature in *Sphagnum* turf [3].

As a result of our year-round study of the epiphytic yeast community of *Sphagnum* mosses and paludal vascular plants, several species of psychrophilic (as judged by the maximal growth temperature [4]) yeasts—*Sterigmatosporidium polymorphum* Kraepelin et Schulze, *Rhodotorula psychrophenolica* Margesin et al., *Aureobasidium pullulans* var. *subglaciale* Zalar, de Hoog et Gunde-Cimerman—were isolated from a Moscow oblast ombrotrophic swamp.

Thus, a new habitat for rare yeast species was revealed in the course of this work and it is the first time the yeasts, whose distribution was previously noted only for Alpine environments and the Arctic region, to have been isolated in the central regions of Russia.

MATERIALS AND METHODS

Sphagnum mosses (*Sphagnum magellanicum* Brid., *Sph. angustifolium* (Russ.) C. Jens.) and paludal vascular plants (*Oxycoccus palustris* Pers., *Carex limosa* L., *Chamaedaphne calyculata* L., *Drosera rotundifolia* L.) were sampled every month from May 2008 to May 2009 from the Kurovskoe ombrotrophic swamp (Moscow oblast, Pushkin raion). Two parts of the plant were analyzed separately: the terraneous one and that submerged in the *Sphagnum* turf. Each part of the plant was sampled every month at three distant points of the swamp area in two replicates for each point spaced 5–10 m apart. Three sampled portions were taken from each sample and analyzed separately.

The yeast number and taxonomic composition were analyzed by the standard method of plating the glucose–peptone–yeast agar (GPYA) acidified with lactic acid (pH 4–4.5) to inhibit bacterial growth; to suppress the growth of mycelial fungi, cultivation at 10°C for one month was used [5]. The yeast cultures were identified by several morphological characteristics using manual [6] and by the results of analysis of the 26S (LSU) rDNA D1/D2 nucleotide sequences.

Amplification of the 26S rDNA D1/D2 region [7] was carried out according to the method described earlier [3] directly from the yeast biomass using the primers ITS1f (5'-CTTGGTCATTTAGAGGAAGTA), NL4 (5'-GGTCCGTGTTTCAAGACGG)

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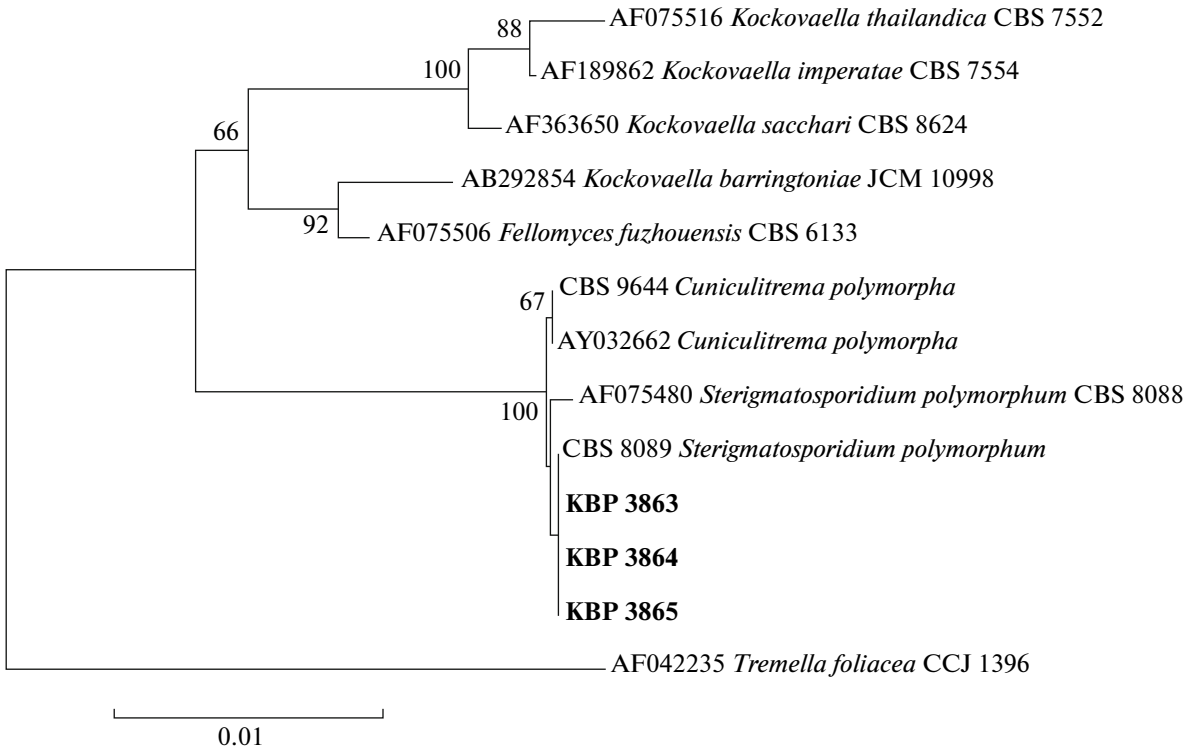

Fig. 1. Phylogenetic position of the isolated *Sterigmatosporidium polymorphum* strains. *Tremella foliacea* is an external group.

and the ScreenMix mixture for PCR (Evrogen, Moscow). The PCR product was purified with the BigDye XTerminator Purification Kit (Applied Biosystems, United States). The primer NL4 was used for sequencing. DNA sequencing was carried out using a BigDye Terminator V3.1 Cycle Sequencing Kit of reagents (Applied Biosystems, United States) with subsequent analysis of the reaction products on the Applied Biosystems 3130 × 1 Genetic Analyzer sequencer at NPO Syntol (Moscow). The MAFFT 6 [8] and MEGA 4 [9] software packages were used for phylogenetic analysis of the results obtained. The sequences for constructing the phylogenetic trees were used according to the data from the articles with descriptions from the GenBank NCBI (http://www. ncbi.nlm.nih.gov/) and CBS (http://www.cbs. knaw.nl/). The phylogenetic positions of the strains isolated (Figs. 1–3) were obtained using the neighbor-joining method. The numbers above the branching points indicate the taxon combination rate (>55%) for 1000 constructions. The scales show the number of substitutions per each nucleotide.

The strains studied are deposited in the Collection of Yeast Fungi of the Department of Soil Biology, Faculty of Soil Science, Moscow State University (KBP); the sequences obtained were submitted to GenBank NCBI.

RESULTS AND DISCUSSION

Nine strains of *Sterigmatosporidium polymorphum* forming several sterigmatoconidia on the cells were isolated in the course of our study. The temperature growth range for the strains was from 4–6 to 18–22°C. All the isolations were carried out only from submerged parts of the plants; on terraneous parts of plants and on plants under snow, such yeasts were not revealed. The predominance of these fungi in the epiphytic yeast community was observed only once, in January 2009, when *C. limosa* rootstock and the lower parts of the stems submerged in water under ice were analyzed. The isolation periods, the substrates, and the *S. polymorphum* abundance values for each substrate are presented in the table. The intermissions between the isolation periods and different substrates from which the isolations were made indicate a local pattern of distribution and the inconstancy of occurrence for the groupings of yeast species on the territory of the swamp area.

Phylogenetic analysis of the 26S rDNA D1/D2 nucleotide sequences was carried out for three strains (KBP 3864 (FN554715), KBP 3865 (FN554716), and KBP 3863 (FN554714)) (Fig. 1). The results showed the conspecificity of these strains with *S. polymorphum* CBS 8089. Cultivation on the media used for mycelium formation [5] did not reveal any teleomorphous structures in the cultures.

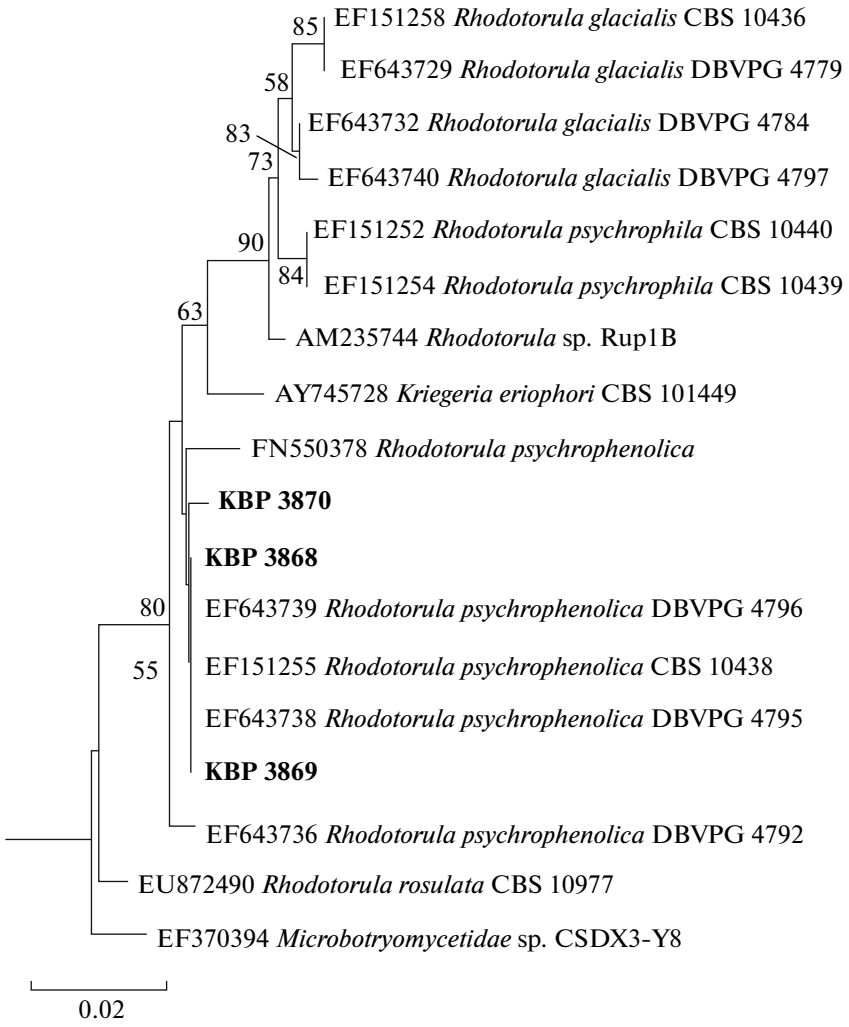

Fig. 2. Phylogenetic position of the isolated *Rhodotorula psychrophenolica* strains. *Rhodotorula minuta* CBS 319 (AF189945) is a hidden external group.

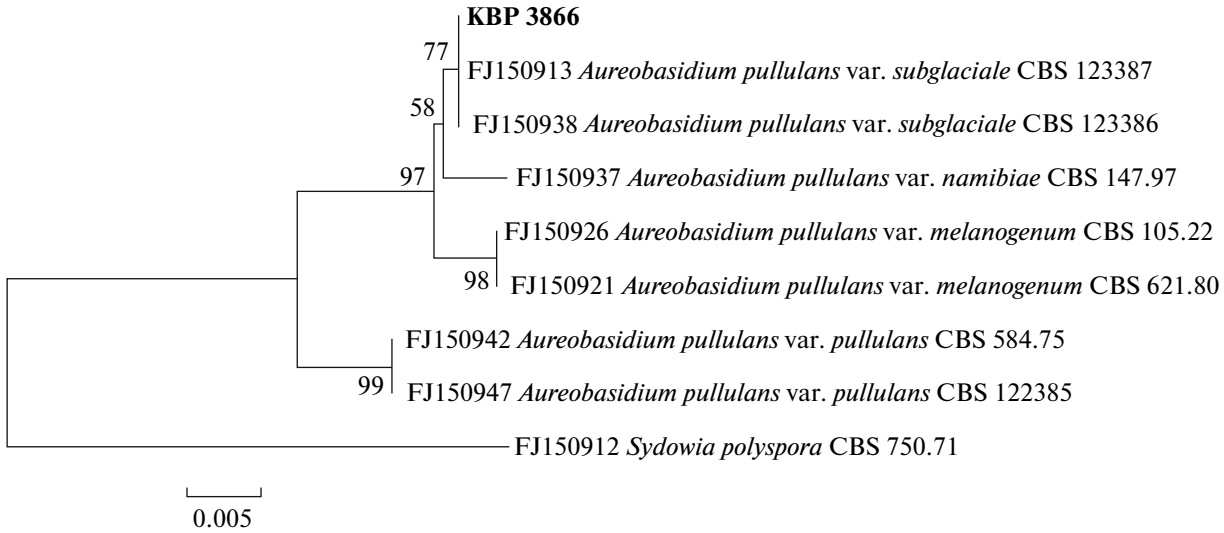

Fig. 3. Phylogenetic position of the isolated *Aureobasidium pullulans* var. *subglaciale* strains. *Sydowia polyspora* is an external group.

Isolation periods, substrates, and abundance values for the yeast species studied

Yeast species	Isolation period	Substrate	Abundance (%)/Rate of occurrence (%)
S. polymorphum	May 2008	*Sphagnum* spp. (lower part)	1.8/8.7
	November 2008	*C. calyculata* (lower part)	14.3/11.1
	January 2009	*C. limosa* (lower part)	98.3/88.9
R. psychrophenolica	April 2009	*Sphagnum* spp. (upper part)	10.2/29.3
	April 2009	*C. limosa* (upper part)	76.8/33.3
	April 2009	*O. palustris*	11.4/33.3
A. pullulans var. *subglaciale*	May 2008–May 2009	From all plants	16.8/30.7

S. polymorphum are dimorphic fungi within the order Tremellales forming several conidia (buds) on the stalk-cell at the yeast stage. The yeasts of this species were described in the 1970s after a single isolation from drenched pitwood planks in an ore mine (Germany) [6, 10, 11]. Yeasts similar to *S. polymorphum* for which a complete life cycle was revealed were isolated under investigation of the xylophage-associated microbiota. Based on the similarity of the morphological, physiological, and genotypic characteristics (the difference in the 26S rDNA D1/D2 region was two nucleotide pairs), it was proposed to consider it as a teleomorph of *S. polymorphum* named *Cuniculitrema polymorpha* [11]. To date, no information about new isolations of anamorph stage *C. polymorpha* has been available.

Importantly, both initially [10] and in the course of our investigation, *S. polymorphum* was isolated from natural objects in low-temperature aquatic environments. Teleomorphs were isolated from drier subjects: tree bark and the xylophage gallery [11].

One of the specific features of the yeast communities of paludal plants we noted earlier [3] is a relatively high share of nonpigmented dimorphic basidiomycetes, mainly from the class *Microbotryomycetes*. In April 2009, 12 strains of psychrophilic yeasts from the class *Microbotryomycetes* of the species *R. psychrophenolica* (strains KBP 3870 (FN555153), KBP 3869 (FN555152), and KBP 3868 (FN555151)) were isolated from the green parts of *Sphagnum* mosses and from certain paludal vascular plants (table) (Fig. 2). The temperature growth range for the strains isolated was from 4–6 to 15–18°C. All known earlier isolations of this yeast species were carried out only from the French, Austrian, and Italian Alps [12, 13].

An increase in the abundance of psychrophilic yeasts from the class *Microbotryomycetes*, in particular, the species *Leucosporidium scottii*, in *Sphagnum* turf was noted earlier and was associated with the constant low temperature of this habitat [14, 15].

So-called black yeasts were isolated quite frequently in the course of investigation of the yeast community of *Sphagnum* mosses and paludal vascular plants. Until recently, their taxonomy has mainly been based on the morphological characteristics [6] only and the isolation of varieties of certain species, in particular, *A. pullulans*, was difficult. A new revision that appeared recently [16] and is based primarily on phylogenetic analysis made it possible to assign the *A. pullulans* strains obtained in the course of this study to the variety *subglaciale* (KBP 3866 (FN554717)) (Fig. 3). The temperature growth range of the isolated strains was from 4–6 to 20–24°C. To date, all known isolations of this variety have been carried out from the island of Spitsbergen only [16].

According to its physiological characteristics, the variety of the species *A. pullulans* isolated in the course of this work belongs to the group of psychrophilic species. Moreover, the species *A. pullulans* is also known to be a typical epiphyte [2]. The combination of these two characteristics for the variety *subglaciale* isolated by us is illustrated clearly enough by the annual dynamics of this species on *Sphagnum* mosses (Fig. 4). This illustration demonstrates that it is from the surface (green) parts of the plants that this species is more frequently isolated and its maximum presence in the

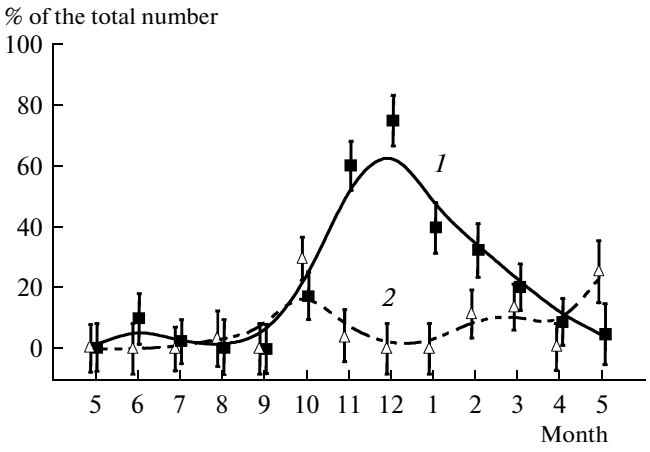

Fig. 4. Average monthly values of the relative abundance of *A. pullulans* var. *subglaciale* species on the *Sphagnum* moss samples. the green part (*1*) and the lower part of the plant without chlorophyll (*2*).

yeast community occurs precisely in the periods of lower temperatures.

Thus, as a result of our study, the previous isolations of *R. psychrophenolica* only as an Alpine species [12, 13] and of *A. pullulans* var. *subglaciale* as an arctic species [16] are supplemented by the isolations of these yeast species from the central part of Russia. Our investigation allows these species to be assigned for the first time to the group of epiphytic yeasts. The isolation of *S. polymorphum, R. psychrophenolica*, and *A. pullulans* var. *subglaciale* from *Sphagnum* mosses and paludal vascular plants from a Moscow oblast ombrotrophic swamp may give evidence of the continuity of the area of distribution for these species.

Sphagnum mosses and paludal vascular plants appear to be an interesting but, so far, poorly studied natural habitat, analysis of which may provide the answer to some of the problems of the ecology of yeast fungi.

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