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Yeast Resources in Natural Habitats at Polar Circle Latitude

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Summary

Yeast communities in 8 types of natural habitats in typical biotopes of the White Sea coastal zone at the polar circle latitude are described. Approximately 60 species among 780 isolated yeast strains were identified. The greatest diversity was revealed between the groupings of yeasts inhabiting soils and various parts of living plants and those associated with insect habitats. Basidiomycetous species were prevalent in the former case while ascomycetes predominated in the latter.

Keywords: yeasts, biodiversity, microbial resources

Introduction

Yeast natural biodiversity is of interest not only to yeast ecology and taxonomy, but to global microbial biotechnology as well. In order to estimate the role of the geographic factors in the distribution of yeasts in terrestrial ecosystems it is best to compare yeast populations in the main natural substrates which are present in all geographical zones. The investigation of various yeast habitats in a single geographical area produces source materials for inventory of the yeast resources in that region and creates the possibility for purposeful search and isolation of the most interesting yeast species for science and new technology. The present research is the continuation of our long-term study of yeast communities in soils and other habitats in various geographical zones of the former USSR *(1)*.

Material and Methods

More then 100 samples of various natural substrates, mainly of wild berries, were collected within the period of 3 years (1992–1994) in late summer and early autumn in the neighbourhood of the Biological Station of Moscow State University, located on the coast of the White Sea in the eastern part of the Kindo peninsula in the Kandalaksha gulf region. The predominant vegetation there are coniferous forests with birch groves, meadows and marshes in the coastal zone.

The dilution plate method with mechanical disintegration of substrates was used. Plating was done on acidified malt agar (pH = 3.5) and plates were incubated at 20–25 °C for 1–2 weeks and at 5 °C for 3–4 weeks. The species identification was carried out by standard methods *(2,3)* with the use of modern manuals and computer programs *(4,5)*. The frequency of each species was calculated separately for every habitat as a percentage of samples containing that particular species from the total number of analyzed samples of that habitat.

Results

Many more yeast isolates were obtained when plates were incubated at 5 °C than at 20–25 °C. More than 780 strains were isolated from various habitats in the main natural biotopes on the White Sea shore. The vast majority of isolates were identified and approximately 60 species of 19 genera were recognized (Table 1). Some of them were eurytopic ones, such as *Aureobasidium pullulans, Cryptococcus laurentii, Trichosporon pullulans, Debaryomyces hansenii* and 2 species of *Cystofilobasidium*. Nearly half of all species were isolated only from one or two samples and some of them were stenotopic which are known to be specific for definite habitats. Basidiomycetous yeasts were prevalent and constituted 66% of all identified species. Not all strains could be identified and we consider some of them to be new for science. Some strains were not »true« yeasts but rather the anamorphs of pathogenic or saprotrophic fungi of the genera *Taphrina, Ustilago, Tremella*, and

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Table 1. The frequency (%) of yeast species in various habitats

Species	Berries	Flowers	Littoral plants	Forest plants	Soils	Anthills	Fungi	Frass
Ascomycetous yeasts								
Candida apicola (Hajsig) Meyer *et* Yarrow	2							
Candida bimundalis Boldin *et al.*								100
Candida freyschusii Buckley *et* van Uden								100
Candida japonica Sugiyama *et* Goto							25	
Candida kruisii (Kratochvilova *et* Ondrusova) Meyer *et* Yarrow							25	
Candida krusei (Castellani) Barkhout	4							
Candida molishiana (Zikes) Meyer *et* Yarrow	2							
Candida pseudointermedia Nakase *et al.*			7					
Candida quercitrusa (van Uden et do CarmoSousa) Meyer *et* Phaff	2							
Candida sake (Saito *et* Ota) van Uden *et* Buckley	4							
Candida santamariae Montrocher	4		7					
Candida sp. 1	4		7					
Candida sp. 2			7					
Debaryomyces hansenii (Zop) Lodder et Kreger-van Rij	5		13	29	29	25	25	
Debaryomyces polymorphus (Klocker) Price *et* Phaff						100		
Metschnikowia pulcherrima Pitt *et* Miller	5	17						
Metschnikowia reukaufii Pitt *et* Miller	2	33						
Pichia spartinae Ahearn *et al.*			7					
Basidiomycetous yeasts								
Bullera armeniaca Buhagiar *et al.*	2							
Cryptococcus albidus (Saito) Skinner	18		13	43			25	
Cryptococcus flavus (Saito) Phaff *et* Fell	21			14				
Cryptococcus gastricus Reiersol *et* di Menna	2							
Cryptococcus heveanensis (Groenewege) Baptist *et* Kurtzman				29				
Cryptococcus hungaricus (Zsolt) Phaff *et* Fell	4							
Cryptococcus laurentii (Kufferath) Skinner	61	100	87	43	43	25	25	
Cryptococcus luteolus (Saito) Skinner	2							
Cryptococcus magnus (Lodder *et* Kregervan Rij) Baptist *et* Kurtzman	2							
Cryptococcus podzolicus (Bab'eva *et* Reshetova) Golubev					14			
Cryptococcus skinneri Phaff *et* do Carmo Sousa	2							
Cryptococcus terricolus Pedersen	5				43			
Cryptococcus sp.	7							
Cystofilobasidium capitatum (Fell *et al.*) Obervinkler *et* Bandoni	14	33	20	29				
Cystofilobasidium infirmo-miniatum (Fell *et al.*) Hamamoto *et al.*	12	33	33	14	14			
Cystofilobasidium sp.	2							
Leucosporidium antarcticum Fell *et al.*	2							
Leucosporidium scottii Fell *et al.*	2							
Mrakia sp.	2							
Rhodotorula aurantiaca (Saito) Lodder	5	17						
Rhodotorula buffonii (Ramirez) Roeijmans *et al.*	2							
Rhodotorula fujisanensis (Soneda) Johnson *et* Phaff	5						25	
Rhodotorula glutinis (Fresenius) Harrison	4	17				25		
Rhodotorula hylophila (van der Walt *et al.*) Miranda *et* Weijman	2							
Rhodotorula minuta (Saito) Harrison	2					25		
Rhodotorula mucilaginosa (Jorgensen) Harrison	2						25	
Rhodotorula muscorum (di Menna) von Arx *et* Weijman	2		20					
Rhodotorula sp. 1	9		20					
Rhodotorula sp. 2						25		
Rhodotorula sp. 3							75	
Sporobolomyces roseus Kluyver *et* van Niel		67	27	14				
Sporobolomyces salmonicolor (Fisher *et* Brebeck) Kluyver *et* van Niel			7					
Trichosporon cutaneum (de Beurm *et al.*) Ota	4				29			
Trichosporon pullulans (Lindner) Diddens *et* Lodder	2	33			43		25	
Yeast-like fungi								
Auerobasidium pullulans (Arnaud) de Bary	+	+	+	+	+			
Holtermannia corniformis	2							
Taphrina carnea	7			29				
Taphrina robinsoniana	19							
Tilletiopsis spp.	4					25		
Tremella aurantia Fries	2							
Tremella encephala Fries	14					25		
Tremella sp.	7							
Number of samples	57	6	15	7	18	4	9	1
Number of species	46	10	15	10	8	8	9	2

Holtermannia. *Aureobasidium pullulans* was not isolated in pure culture so its proportion among other yeast species was not estimated.

All samples were divided into 8 groups on the basis of their peculiarities as habitats for yeasts: various parts of plants (leaves, berries, flowers, thallus of algae), soils, fruit bodies of some macromycetes, and samples from insect habitats such as anthills and pine frass. The descriptions of yeast communities in each of these habitats are given below.

Berries

Sixty samples of berries were analyzed, which are typical for North Russian forests and were collected in coniferous forests and marshes. They belong to 12 plant species from 5 families: *Vaccinaceae* (bilberry, great bilberry, cowberry, cranberry, bog cranberry), *Rosaceae* (ashberry, cloudberry, raspberry, stone berry), *Empetraceae* (crowberry), *Ericaceae* (bear's whortlberry), and *Cornaceae* (dwarf cornel).

Forty five yeast species were identified among 610 strains of yeasts isolated from berries; 33 of them were of basidiomycetous affinity while 12 were ascomycetes. As a rule black yeasts belonging to the genus *Aureobasidium (A. pullulans?)* constitute approximately 50% of the colonies on a plate. The diversity of yeast species ranged from 3 on bear's whortlberry to 18 on berries on dwarf cornel. *Cryptococcus laurentii* constituted 60% of the isolates among all yeast strains. Ascomycetous yeasts were represented by 6 anamorph species of the genus *Candida* and 2 species of *Metschnikowia (M. pulcherrima* and *M. reukaufii)*. The strains we identified as anamorphs of the genus *Taphrina* were quite different from other yeasts. In streak culture they had flat dry growth with grayish-red or grayish-yellow pigmentation uncommon for the true yeasts. Old cultures were very heterogenous in size and form of cells. Chlamidospores were also common for them. Species identification was carried out by key *(6)*. Two anamorphs presumably *Taphrina robinsoniana* and *T. carnea* were recognized. No species with vigorous fermentative activity such as *Saccharomyces, Torulaspora, Pichia* or *Hanseniaspora* were found on berries in that northern region as it was in the case of similar habitats located at other latitudes *(7)*. Some strains were obligate psychrophilic with maximum growth temperature 20 °C. The latter are supposed to be unknown species of genus *Mrakia*.

Flowers

Six samples of flowers which belong to 3 plant species, namely four of *Aster tripolium*, one of *Dianthus deltoides*, and one of *Chamomilla suaveolens* were analyzed. Nine species of yeasts were isolated from these samples; *Cryptococcus laurentii* was found in all of them and *Sporobolomyces roseus* in 4 of 6. *Metschnikowia pulcherrima* and *M. reukaufii* were isolated from some samples of flowers and of berries. The last two yeast species were absent from all other habitats of the region. A similar yeast composition was found in various flowers of some regions in Canada and California *(7)*. The effect of geography on the species sets found there was minimal as well as in our study.

Vegetation of littoral zone

Supralittoral vegetation was represented in the study by 3 species of typical plants, namely four samples of *Cochlearia arctica*, five of *Plantago maritima*, and four of *Salicornia europea*. Two samples of marine algae, *Fucus vesiculosus* and *Ascophyllus nodosum*, were also analyzed. Thirteen species of yeasts were isolated from these samples. *Cryptococcus laurentii* and two species of *Cystofilobasidium (C. capitatum* and *C. infirmo-miniatum)* were found to be predominant. The anamorphic species *Candida santamariae* and the salt-tolerant teleomorphic *Pichia spartinae* were isolated only from this habitat and should be considered as indicator species. Common *Sporobolomyces roseus* and some species of *Rhodotorula* represent yeasts of basidiomycetous affinity. One strain of the latter genus was not identified as belongig to some described species and is supposed to be a new one.

Green parts of some forest trees and grasses

Pine needles of three *Picea abies*, leaves of two *Betula pubescens*, and two *Equisetum arvense* specimens were examined. Ten species of yeasts were isolated from these substrates. The »black yeast«, *Aureobasidium pullulans*, was found to be an absolute dominant species. *Cryptococcus laurentii* and *Cr. albidus* were abundant too. Numerous strains of *Taphrina* spp. were isolated from leaves of *Betula pubescens* heavily infected by rust. The same yeast-like spp. were isolated from half of the berries samples in forest ecosystems of the region.

Soils

Seven soil samples from coniferous forest, meadows, marshes, and rocks were examined. Only 7 species of yeasts were identified. *Cryptococcus terricolus* and *Cr. laurentii* as well as *Trichosporon pullulans* and *Tr. cutaneum* were the most common species. *Cr. podzolicus* was found in only one soil sample although this species frequently occurs in coniferous forest soils of mid-latitudes of Russia *(1)*. *Lipomyces* spp. were not revealed in the present study although we isolated *L. starkeyi* from one sample of podzolic soil on this territory earlier *(8)*. We suppose, that this region is the northern geographical boundary of the last two soil yeasts.

Anthills

There are many anthills of *Formica rufa* in the forests of the examined area. We have analyzed the substrates of 5 nests. Four of them were inhabited while one old hill was vacated by the insects. Yeasts were found in the inhabited hills only and their population was very specific. It was represented generally by two species of *Debaryomyces. D. polymorphus* was found in all samples and in great numbers. *D. hansenii* was obtained from one anthill. Other yeasts represented by well known epiphytous species, namely *Rhodotorula glutinis, R. minuta*, and *Cryptococcus laurentii*, were rare. Unusual isolates were *Tremella encephala* (anamorph), *Tilletiopsis* sp., and *Rhodotorula* sp. 2.

Fungi: fruit bodies

Nine samples of some fungal fruiting bodies (*Pezizales* and *Aphyllophorales*) were analyzed and 9 species of

yeasts were isolated. A specific one was *Candida kruisii* which was isolated originally elsewhere from *Boletus purpureus (9)*. Among the yeast species isolated from fungi, *Rhodotorula fujisanensis* is of special interest, as we have repeatedly isolated this yeast from various fungi in other regions of Russia. Other isolates include three *Cryptococcus* species (*C. albidus, C. laurentii*, and *C. podzolicus)* as well as some common species of *Trichosporon* and *Rhodotorula* which should be considered as contaminants brought from soil or plants.

Frass of bark beetles

One sample of frass taken from pine bark was examined. Two ascomycetous species *Candida bimundalis* (anamorpha of *Pichia bimundalis*) and *C. freyschussii* were the only inhabitants of this substratum. These species are known to be associated with bark beetles on coniferous trees in Europe and Asia *(4)*. At the polar circle latitude these species of yeasts were found for the first time.

Discussion

As was shown earlier *(1)* yeast complexes corresponding to three main types of substrates in terrestrial ecosystems – green parts of plants, dead plant remnants (litter, peat), and soil mineral horizons – are represented by different sets of yeast species in different geographical zones from tundra to desert. These species sets include both ubiquitous eurytopic yeasts and stenotopic ones. The latter are usually associated with specific types of substrates mainly in insect habitats. These »loci« are very interesting for studies of yeast diversity in nature.

Comparative analysis of yeast populations in various habitats within a single limited geographical area revealed differences or similarities in the composition of yeast communities associated with natural substrates. In the present research the greatest difference was observed between yeast communities in soils and on various parts of living plants or in the habitats of insects (Fig. 1). The ratio of ascomycetous yeasts and their specific composition increases in the following order: soil → plant parts → fungi → anthills → frass (Fig. 2). In general, yeasts

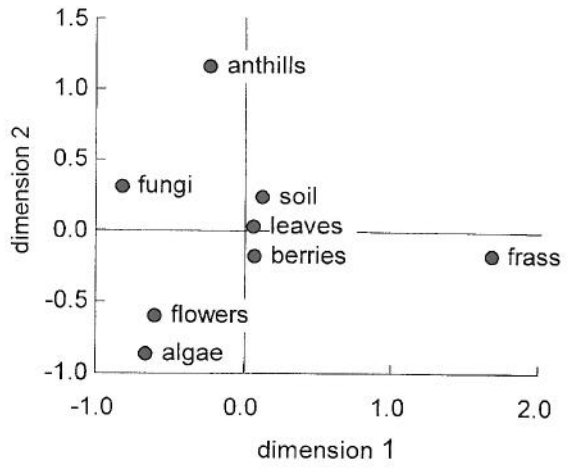

Fig. 1. Results of multidimensional scaling of different habitats on the basis of composition of yeast communities

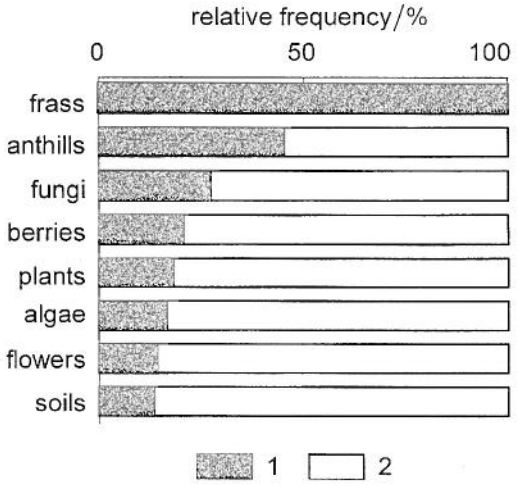

Fig. 2. The ratio between yeast species of ascomycetous (1) and basidiomycetous (2) affinity in different habitats

communities in the examines geographical region differ from those located at other latitudes by the following features:

- low density and diversity of ballistosporogenous yeasts;
- low ratio of yeasts with fermenting activity in such habitats as berries;
- absence or very limited distribution of soil yeasts typical for this habitat in other geographical regions.

Conclusion

The present study is the first description of yeast communities in various natural habitats of the Russian polar circle region. Geographical limits for some yeasts were detected. The data obtained are compared with similar characteristics of other geographical regions. Such studies are an ecological foundation for purposeful search and isolation of industrially important yeasts essential for development of new branches of microbial biotechnology.

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Izvori kvasaca u prirodnim staništima polarnog kruga

Sažetak

Opisane su zajednice kvasaca iz osam tipova prirodnih staništa u tipičnim biotopima primorja Bijeloga mora, na geografskoj širini polarnog kruga. Identificirano je približno 60 vrsta između 780 izoliranih sojeva kvasaca. Najveće razlike uočene su između kvasaca iz tla i raznih dijelova živih biljaka te kvasaca povezanih sa staništima insekata. U prvih su prevladavale vrste basidiomiceta, a u drugih askomiceti.