MYCOLOGY

Fungi in Bottom Sediments of the Barents and Kara Seas

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Abstract⎯The mycobiota of bottom sediments at depths of 128−472 m was investigated in Barents and Kara sea areas remote from the shore. The species composition and fungal abundance, that is, the number of fungal colony-forming units (CFUs), were determined in 5 samples from the Kara Sea and in 14 samples of the Barents Sea. For the first time for the Arctic seas, the fungal biomass was determined in 12 samples of the bottom sediments from the Barents Sea. It was found that fungal abundance in the bottom sediments of the both seas did not exceed 13 CFUs per 1 g of dry substrate weight. In total, only 58 colonies of filamentous fungi belonging to 22 morphotypes, 8 of which were sterile, were isolated from all the samples. No more than six morphotypes were contained in 1 g of dried substrate; they were mostly species of the genus *Cladosporium* and sterile isolates. The study of the fungal biomass detected both spores and fungal mycelium in the bottom sediments. The total biomass was extremely low and ranged from 0.1 to 0.620 mg/g of the studied substrate. Small spores (with a diameter less than 3 μm) absolutely predominated (from 88 to 99.7% of the biomass).

Keywords: marine fungi, bottom sediments, diversity, biomass, Arctic, Barents Sea, Kara Sea **DOI:** 10.1134/S1063074017050029

INTRODUCTION

Traditionally, mycobiota of marine soils and sediments are studied using culture methods [1, 23]. When these methods are used, fungi are isolated from sediments of tidal marshes [1, 14] down to depths over 4000 m [19], from the seas of equatorial regions [19, 28] to temperate [3, 9, 22], subarctic [2, 14], and Arctic [1, 18] latitudes. The common trend of modern studies of the diversity of mycobiota in various marine ecotopes is the addition of culture methods to the molecular approach. This is mostly connected with the molecular identification of isolated cultures; other approaches are used less often. In particular, the methods of metagenomics revealed the presence of fungi in various bottom sediments, including the deep-sea zone [24]. Direct microscopy is very rarely used in the study of sea substrates. Only two works are known; one of these is devoted to the study of the mycobiota of seashore marshes of the White Sea [14] and the other deals with fungi in the deep-sea sediments of the central Indian Ocean [19]. Both articles noted the presence of fungal mycelium, but the exact values of the fungal biomass were given only in the first paper.

In general, despite the development of marine mycology and the rise of interest in Arctic research, there is still very little information on the fungi of the Arctic seas. Therefore, Artemchuk [1] gave information only on the generic composition of fungi isolated from the intertidal substrate of the Dal'nezelenetskaya

Bay of the Barents Sea. For micromycetes of the Arctic bottom sediments, one study was devoted to the diversity of mycobiota in some areas of the western and central parts of the Kara Sea [18]. The aim of this paper is to study the mycelial fungi of bottom material from the remote regions of the Arctic Ocean with the use of culture and direct microscopic methods.

MATERIALS AND METHODS

The material for the study was sampled in bottom sediments in 2014 in late August in the Kara Sea and in early October in the Barents Sea (Table 1). In the Kara Sea, the samples were collected in the northeastern part at a distance of 350–400 km from the coast, while in the Barents Sea samples were collected in the northern part at a distance of 220–300 km from the shore (see Fig. 1). These areas of the Barents and Kara seas are similar according to a complex of the environmental conditions (constant temperature of about 0°C, oceanic salinity, high silting, great depths, and remoteness from the shore). In total, we performed studies at 14 stations in the Barents Sea and at five stations in the Kara Sea. Sediments were raised to the ship with the use of a bottom grab sampler. From the soils, $2-3$ samples of 1 cm³ were taken with a cut syringe at a distance of 10–15 cm; the material from one dredger was placed in one envelope. The envelopes with samples were dried and processed within

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Station	Date of sampling	Depth, m	Soil					
The Kara Sea								
$KS-01$	August 25, 2014	193	Mud					
$KS-02$	August 25, 2014	193	$\pmb{\mathcal{H}}$					
$KS-03$	August 26, 2014	128	$\pmb{\cdot}$					
$KS-04$	August 27, 2014	472	Mud, clay					
$KS-05$	August 27, 2014	472	$\pmb{\cdot}$					
The Barents Sea								
BS-01	October 5, 2014	259	Mud, sand					
$BS-02*$	October 6, 2014	180	$\pmb{\prime}$					
$BS-03*$	October 6, 2014	265	$\boldsymbol{\prime}$					
$BS-04*$	October 6, 2014	259	$\pmb{\cdots}$					
$BS-05*$	October 6, 2014	269	$\pmb{\mathcal{H}}$					
BS-06*	October 8, 2014	241	$\boldsymbol{\prime}$					
$BS-07*$	October 8, 2014	218	$\pmb{\cdot}$					
$BS-08*$	October 9, 2014	203	$\boldsymbol{\prime}$					
BS-09*	October 9, 2014	308	$\pmb{\mathcal{H}}$					
$BS-10*$	October 9, 2014	304	$\pmb{\cdots}$					
$BS-11*$	October 9, 2014	297	$\boldsymbol{\prime}$					
$BS-12*$	October 9, 2014	276	$^{\prime\prime}$					
$BS-13*$	October 8, 2014	242	$\pmb{\cdots}$					
$BS-14$	October 8, 2014	308	$\pmb{\mathcal{H}}$					

Table 1. The characteristics of the collected samples

* Samples with a calculated biomass. KS, the Kara Sea; BS, the Barents Sea.

45 days. The total weight of the dry material was $1.0-2.4$ g per sample.

For seeding, 1 g of dry sample was used. The weighted sample was mixed with sterile water to obtain the total volume up to 10 mL; 1 mL of the resulting suspension was placed on the surface of the medium in a Petri dish and spread with a spatula. For each variant, we used ten dishes with Malt Extract Agar media (wort with 0.03% total sugar, 34‰ sea salt, and amikacin at 500 mg/0.5 L medium). The dishes with inoculations were incubated for 2 months at the temperature of 4°C and then pure cultures were isolated from the formed colonies. Fungi were identified in pure cultures by their morphological-culture features using the corresponding manuals and publications [10, 20, 26, 30, 31]. Synonymy was checked with Index Fungorum database (www.indexfungorum.org). The concept of "colony-forming units" (CFUs) was used for estimating the number of fungi; the number of colonies formed was indicated per 1 g of dry material.

The structure of the fungal biomass was determined using the method of luminescence microscopy. The length of fungal hyphae and the volume of spores were measured according to Bloem et al. [17]. The number of fungal propagules was recorded by luminescence in calcofluor white preparations bound to chitin of the cell walls of fungi [12] using a Zeiss Axioskop 2plus fluorescent microscope (Germany). The spore abundance and the size of the mycelium were calculated for the content of fungal biomass (mg dry biomass/g of absolutely dry soil), taking the fact that that the density was 0.837 g/cm³ for spores and 0.628 g/cm³ for mycelium into account [11].

RESULTS AND DISCUSSION

The Number and Diversity of Fungi

Of the samples, 58 colonies of mycelial fungi were isolated, including 24 colonies from the Kara Sea samples and 34 colonies from samples of the Barents Sea (Table 2). We found from 1 to 13 colonies in the samples of the Kara Sea and from 0 to 5 colonies in the samples of the Barents Sea per 1 g of dry material. The samples from the Barents Sea showed no correlation between the number of isolated fungi and the location of the sampling points and the depth of sampling. In the Kara Sea, most fungi were found in a relatively shallow station located at a depth of 128 m (13 colonies), while the lowest fungal abundance was found in the deepest station (1 colony, a depth of 472 m). On average, somewhat more fungi were isolated from the

		Barents Sea		Kara Sea		Total	
Morphotype	SN	CN	SN	CN	SN	CN	
Zygomycota							
Umbelopsis isabellina (Oudem.) W. Gams 2003		$\boldsymbol{0}$	1	1	1	1	
Ascomycota							
Acremonium murorum (Corda) W. Gams 1971	1	1	$\mathbf{0}$	$\mathbf{0}$			
Alternaria alternata (Fr.) Keissl. 1912	1	1	θ	θ	1		
Aspergillus flavus Link 1809	θ	θ	1	1	1		
Cadophora fastigiata Lagerb. et Melin 1927	1	1	$\mathbf{0}$	$\mathbf{0}$	1		
Cladosporium cladosporioides (Fresen.) G.A. de Vries 1952	4	7	$\overline{4}$	7	8	14	
Cladosporium cf. fusiforme Zalar, de Hoog et Gunde-Cim. 2007	2	3	θ	$\mathbf{0}$	2	3	
Cladosporium cf. psychrotolerans Zalar, De Hoog et Gunde-Cim. 2007	2	3	1	3	3	6	
Cladosporium sphaerospermum Penzig 1882	$\overline{4}$	6	θ	θ	4	6	
Penicillium madriti G. Sm. 1961	1	2	θ	θ	1	2	
Penicillium megasporum Orpurt et Fennell 1955	$\bf{0}$	$\boldsymbol{0}$	1	1	1		
Sarocladium strictum (W. Gams) Summerb. 2011	2	4	θ	θ	2		
Scopulariopsis brumptii Salv.-Duval 1935	θ	$\overline{0}$	1	1	1	1	
Ulocladium chartarum (Preuss) E.G. Simmons 1967	2	2	1	1	3	3	
Non-spore-bearing cultures							
Sterile Type 1	$\mathbf{0}$	θ	1	1	1		
Sterile Type 2	$\bf{0}$	$\mathbf{0}$	1	1	1		
Sterile Type 3	Ω	θ	1	2	1	2	
Sterile Type 4.1	2	2	$\mathbf{0}$	$\mathbf{0}$	2	2	
Sterile Type 4.2	θ	θ	1	4	1		
Sterile Type 5	1	1	$\mathbf{0}$	$\mathbf{0}$	1		
Sterile Type 6	1		θ	θ	1		
Sterile Type 7	θ	θ	1	1	1		
TOTAL	12	34	5	24	17	58	

Table 2. The species composition and fungal abundance in the studied sediments

SN, the number of samples with this morphotype; CN, the total number of colonies of this morphotype. TOTAL gives the total number of fungal colonies isolated from all the samples (sample dry weight $= 1$ g).

Kara Sea samples, although the difference in abundance cannot be considered fundamental: there were mostly single numbers, and in two samples, dozens, of colonies per gram of substrate.

The values we determined are extremely low, since the number of fungi in most of the previously studied marine sediments was tens–hundreds CFUs per 1 g (or 1 cm^3) of substrate: in the bottom grounds of the subarctic White Sea [2], in some areas of the Japan and Okhotsk seas [5, 22] and the Black Sea [3], as well as in the coastal waters of the Indian Ocean [28]. Moreover, the values of the fungal abundance we obtained are 2–4 orders of magnitude lower than are known for soils in the tundra zone bordering the arctic seas [5, 6]. Such extremely low levels of fungi were previously shown only for the deep-sea sediments of the central Indian Ocean [19], as well as for sediments of relatively deep areas of the Kara Sea remote from the mainland [18]. This investigation has shown that the abundance of fungi in remote areas of the Kara Sea is lower than in the coastal and especially in the estuary waters. In our case, the low abundance of fungi seems to be associated with the great distance from the mainland and with an insignificant input of fungal propagules with terrigenous effluents, as well as with the extreme severity of environmental conditions in the studied ecotopes. Although this was not the goal of the present work, we noted that the number of yeast in these sediments was insignificant: 36 colonies were isolated in total, of which 31 colonies were isolated from the Barents Sea sediments. However, the relative abundance of yeast fungi in the investigated samples was high: they accounted for almost half of all the isolated colonies in most samples from the Barents Sea, while in some samples they prevailed. Previously, such a high relative abundance of yeast was not observed in marine sediments. This can probably be explained in this case by the particular extremity of the conditions, if we consider yeast forms as one of the possible adaptations to conditions of constant cold, low oxygen content, high salinity and increased hydrostatic pressure [27].

Considering the low number of species, we found a quite diverse composition of fungi. We classified all the isolated cultures as 22 morphotypes: 8 were sterile, and 14 had sporulation. In total, representatives of 10 genera were found: 1 species from the phylum Zygomycota (*Umbelopsis isabellina*) and nine genera with 13 species: anamorphs of Ascomycetes. We detected from zero to six morphotypes per 1 g of the investigated substrate, but in most cases, we found one to two morphotypes. The genus *Cladosporium* Link was the most diverse, as it was represented by four species; the genus *Penicillium* Link included two species and other genera were identified with only one species each. Isolates obtained from sediments of the both seas were assigned to three species: *Ulocladium chartarum* (3 colonies from two samples from the Barents Sea, with 1 colony in each and 1 colony in a sample from the Kara Sea), *Cladosporium cladosporioides* (14 colonies in total, in four samples from the Barents Sea and in four samples from the Kara Sea), and some isolates that were morphologically similar to the species *Cladosporium psychrotolerans* (3 colonies in two samples from the Barents Sea and 3 colonies in one sample from the Kara Sea). All other species were isolated from samples of only one of the studied seas. We obtained 13 sterile isolates: 9 colonies in four samples from the Kara Sea and 4 colonies in four samples from the Barents Sea (Table 2). Light-colored sterile forms predominated in samples of the both seas.

Finding a relatively large number of sterile mycelia is common in research of marine fungal cultures. They are always isolated and in some cases are even dominants. As an example, non-spore-bearing cultures prevailed in deep-sea sediments of the Indian Ocean [19] and in the bottom sediments of the Kara Sea [18], i.e., in the most extreme (deep-water and cold) zones of the areas of the World Ocean that have been studied thus far. A high abundance of sterile forms is generally characteristic of various extreme habitats, including cold water [27]. Species of the genus *Cladosporium*, especially *C. cladosporioides* and *C. sphaerospermum*, are the fungi that most commonly occur in the world [20]. There is evidence that they are common in extremely cold habitats, such as soils of highlands, as well as Arctic and Antarctic regions [21, 27]. As well, it has been noted that *C. cladosporioides* can grow even at -10° C [25]. At least one of these species is isolated in almost any study of marine mycobiota cultures, but the predominance of species of this genus has not been shown for marine ecotopes.

Our finding of fungal isolates that are morphologically similar to other two species of the genus *Cladosporium* was very interesting: *C. fusiforme* and *C. psy-*

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chrotolerans. Both species are close to *C. sphaerospermum*; their distribution is thus far little known, but both are associated with various hypersaline habitats [31]. *C. fusiforme* may occur somewhat more widely than *C. psychrotolerans*. The species *U. chartarum,* which is detected in the sediments of the both seas, is also cosmopolitan and common in various ecosystems, including marine ecosystems [20, 30]. Other species were represented mainly by single colonies in individual samples. The ecology of these species is extremely diverse: some species are typical of cold regions and others are usual inhabitants of hot climate environments. As an example, the only observed zygomycete *U. isabellina* is common in soils of cold and moderately cold regions and is known to be psychrotolerant and oligotroph [21]; *Cadophora fastigiata* is a soil saprotroph or xylotroph, which is also more common in the middle and polar latitudes [20, 21]. At the same time, *Aspergillus flavus* is a cosmopolitan species that is most widely distributed on different substrates in tropical and subtropical regions; *Scopulariopsis brumptii* also prefers warm and hot climate zones [20]. Of the two *Cephalosporium*-like fungi, *Sarocladium strictum* is the most common soil species of this group, and *Gliomastix murorum* is often mentioned in studies on marine mycology [14, 18, 20].

It was unexpected that such territorial psychrotolerant components as *Pseudogymnoascus pannorum* or species of the genus *Tolypocladium*, and species of the genus *Penicillium* were absent from the studied mycobiota. We observed an abundant presence of *P. pannorum* and *Tolypocladium* spp. in the bottom sediments of the cold-water White Sea [2] and Kara Sea [18]. The same species are common on the coasts of the Arctic seas [5, 6] and in other extremely cold ecosystems [21]. However, these micromycetes were absent in this case, while the genus *Penicillium* was represented by three isolates of two fairly rare species. Among the fungi we identified, there were no obligatory marine species [23], unless they were sterile isolates.

The Biomass of the Fungi

The total biomass of fungi ranged from 0.100 to 0.620 (an average of 0.330) mg/g of substrate (Table 3). The length of the mycelium was from 0.8 to 11.1 m; the spore number in 1 g of soil varied from 9.3×10^3 to 3.5×10^5 units/g of dry substrate. Until now, for marine ecotopes the only data on the biomass of the mycelium (excluding spores) in the bottom sediments and in the intertidal sediments and soils of the White Sea were known from a publication by Sogonov and Marfenina [14], who gave the value for the mycelium biomass of approximately 200 mg/g, i.e., 3–4 orders of magnitude higher than in the bottom sediments we investigated. Similar values of fungal biomass are known only for such extreme habitats as Arctic deserts [16] and various ecotopes of Antarctica [7]. The values we obtained are 4–6 orders of magnitude lower than

Station	Mycelium		Spore biomass					
	biomass	length, m	diameter, µm		Total spore biomass	Total biomass		
			2	3	5			
$BS-02$	0.014	11.1	0.073	0.260	0.051	0.384 ± 0.03	0.398 ± 0.04	
$BS-03$	0.011	8.7	0.099	0.280	0.051	0.430 ± 0.04	0.441 ± 0.04	
$BS-04$	0.004	3.2	0.094	0.186	0.017	0.297 ± 0.02	0.301 ± 0.03	
$BS-05$	0.007	5.6	0.116	0.175	0.051	0.342 ± 0.03	0.349 ± 0.04	
$BS-06$	0.012	9.5	0.069	0.019	Ω	0.088	0.100	
$BS-07$	0.005	4.4	0.049	0.153	0.068	0.270 ± 0.03	0.275 ± 0.04	
$BS-08$	0.003	2.4	0.076	0.157	0.068	0.301 ± 0.03	0.304 ± 0.03	
$BS-09$	0.007	5.6	0.110	0.349	0.153	0.613 ± 0.06	0.620 ± 0.06	
$BS-10$	0.010	7.9	0.023	0.073	0.005	0.101 ± 0.01	0.111 ± 0.01	
$BS-11$	0.010	7.9	0.104	0.164	0.136	0.404 ± 0.04	0.414 ± 0.04	
$BS-12$	0.002	1.6	0.059	0.153	0.136	0.348 ± 0.03	0.350 ± 0.03	
$BS-13$	0.001	0.8	0.089	0.142	0.068	0.299 ± 0.03	0.300 ± 0.03	

Table 3. The biomass of the mycelium and spores of fungi in the bottom sediments of the Barents Sea

The biomass of the mycelium and spores in mg/g of sample weight. BS, the Barents Sea.

all known values obtained for soils of terrestrial biotopes. As an example, in the soils of seaside meadows of the White Sea coast, the biomass of the mycelium (without spores) reaches 2000 mg/g of soil, while it is even higher in forest soils [14]. If the length of the mycelium is calculated, one gram of the upper horizons of undisturbed podzolic soils of Karelia includes from 780 to 1050 m of mycelium; the length of myce-

Fig. 1. The areas of bottom sediment sampling for the study of mycobiota in the Barents and Kara seas.

lium even in the Bf mineral horizon of these soils reached several hundred meters [13], while 393 m/g of soils was found of the Taimyr Peninsula in the arid conditions of the Arctic [29]. Only extremely limited data are available on the number of spores in natural soils and sediments. The values that we obtained are 3 to 5 orders of magnitude lower than those known from the literature. As an example, in peat soils of the Western Siberia, the number of fungal spores ranges from 1.0×10^7 to 2.1×10^8 spores/g of substrate [4, 15].

Another peculiarity of the investigated soils is that most of their fungal biomass is represented by spores (from 88 to 99.7%) rather than by mycelium (Table 3). Such a predominance of spores in biomass was previously shown by Nikitin et al. only for biotopes of Antarctica [8]. The published data refer to terrestrial ecotopes. The percentage of the mycelium in the biomass of some soils of temperate latitudes ranges from 70 to 99% [15]. Thus, the number of spores in the bottom sediments of the studied Arctic seas is relatively low and a majority of fungal spores are inactive, that is, in the resting state. Apparently, low temperatures, low oxygen content, salinity, and other factors hinder their development.

We also revealed some peculiarities in the morphology of the fungal structures. In particular, the mycelium was mostly thin (about 3 μm thick). Some mycelia had arthritic or blastic conidiogenesis, which are also characteristic of yeast-like fungi. We did not find the buckles on the mycelium that occur in some basidiomycetes. Each of the samples contained artifacts in the form of rod-shaped structures 3 μm in length and 1−1.5 μm in width, which are presumably fungal spores (conidia). The total biomass of such structures in each sample was insignificant (from 0.001 to 0.005 mg/g of substrate). In general, the spores (from 61 to 100%) were small $($ 3 \mu m); large spores ($>5 \mu m$) were very rare. The fungal biomass was the largest in the samples where the mycelium was not at the greatest lengths (Table 3). Accordingly, we can assume that the increase in fungal biomass in these samples (substrates) is associated with a more intensive accumulation of spores, and not with the development of the mycelium. The predominance of small spores was earlier noted for such extreme habitats as the Arctic [29] and Antarctic ecosystems [7].

Thus, the investigated bottom sediments of relatively deep-water areas of the Barents and the Kara seas that are remote from the shore are extremely harsh habitats for fungi. This determines both the composition and abundance of mycobiota and the structure of fungal biomass. The fungi are extremely sparse: the number of CFUs is 2–4 orders of magnitude lower than in most of the previously studied marine sediments, and 2–6 orders of magnitude lower than in various terrestrial soils. A relatively high occurrence of yeast forms is unusual, which may be due to certain conditions that form in these ecotopes (constantly low temperature and oxygen content, increased salinity, etc.). At the same time, the species composition of the mycobiota is relatively diverse, but most species occurred as single colonies. The most frequent occurrence was recorded for representatives of the genus *Cladosporium* (which was first shown for marine sediments, although species of this genus are common in various cold and saline habitats) and for sterile forms (which is typical of a variety of extreme habitats). The biomass of fungi is very low: 3 to 6 orders of magnitude lower than in the substrates of the intertidal zone and in terrestrial soils. Similar values are known only for such extreme habitats as the Arctic deserts and Antarctica substrates. Spores predominate in the structure of the fungal biomass, which can also be considered as a marker of an extreme habitat.

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