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EXPERIMENTAL ARTICLES

Comparison of Fungal Complexes of Japanese Scallop *Mizuhopecten yessoensis* (Jay, 1856) from Different Areas in the Peter the Great Bay of the Sea of Japan

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Abstract—Mycological investigation of the Japanese scallop *Mizuhopecten yessoensis* (Jay) (*Bivalvia*) from different areas of the Peter the Great Bay (Sea of Japan) was conducted. Isolates from internal organs of *M. yessoensis* scallop comprise 72 species of filamentous fungi from 30 genera of ascomycetes, anamorphic fungi, and zygomycetes. Species richness of filamentous fungi—fungi of the genera *Aspergillus, Penicillium, Cladosporium*, and *Chaetomium*—in the internal organs of bivalve mollusks increases in polluted coastal waters.

Keywords: marine filamentous fungi, Japanese scallop *Mizuhopecten yessoensis*, Peter the Great Bay, Sea of Japan

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Japanese scallop *Mizuhopecten yessoensis* (Jay, 1856) (*Bivalvia*) is a valuable trading and mariculture asset [1]. Its natural realm spreads over the coasts of the southern Kuril Islands, Sakhalin, Primor'e, Korea, and Japan. Within the continental coast of the Sea of Japan, the scallop is most abundant in the Peter the Great Bay [2].

Studies of pathogenic and toxicogenic fungi inhabiting the internal organs of Japanese scallop are necessary for the safe use of the mollusks for food, for prevention of toxic infections of men upon consumption of fungi-contaminated seafood, as well as for the successful development of sea farming. With the Russia's accession to the World Trade Organization (WTO), the requirements for seafood regimentation in terms of the content of pathogenic microorganism and their toxins will toughen.

The aim of the work was to study the taxonomic composition of the mycobiota of Japanese scallop *Mizuhopecten yessoensis* (Jay, 1856) from various regions of the Peter the Great Bay of the Sea of Japan.

MATERIALS AND METHODS

Subjects of mycological studies were the specimens of Japanese scallop *Mizuhopecten yessoensis* collected in various regions of the Peter the Great Bay from 2008 to 2012. The following sites were selected for mollusk collection: Amur Bay aquatic area exposed to industrial and household wastewaters of the city of Vladivostok [3]; aquatic area around the Rikorda Island, where the bivalve mollusk breeding farm is located; and the relatively pure waters of the Vostok Bay [4].

Methods of mycological investigation of the mollusks. Mollusks were collected by divers at the depth of 4–10 m into sterile bags. Prior to chamber treatment, the samples were stored at −18°C in a freezer. Then, the samples were unfrozen for preparation. Internal organs (mantle, gills, digestive gland, muscle, kidneys, and gonads) were washed three times with sterile seawater. Then, they were soaked in an antibiotic solution (500000 U penicillin and 0.5 g streptomycin per 1 L sterile seawater [5]) for 2 h to suppress the accompanying bacterial flora, and washed in sterile seawater to remove the antibiotics. Fragments of the organs were laid over the surface of agarized nutrient medium. Prior to soaking, the scallop shells were cleared from encrustations, washed in sterile seawater, broken into pieces 2 × 2 cm, and subjected to the same procedure as the organs.

Fungi grown in mixed cultures were inoculated onto fresh nutritive medium to prepare pure cultures.

Nutritive media. To isolate and cultivate the fungi, the following nutritive media were used: universal Sabouraud's medium and the medium based on the Japanese scallop tissue broth supplemented with glucose (30 g/L), yeast extract (0.5 g/L), and bacto-agar (15 g/L) [6, 7]. The media were prepared on filtered and diluted (75%) seawater, pH 7.5–7.8 [5].

Prior to pouring the media in Petri dishes, penicillin (500000 U/L) and streptomycin (0.5 g/L) were

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added and the solution was mixed to complete dissolution of the components.

To identify the isolated fungi, common keys and guides were used [8–13].

RESULTS AND DISCUSSION

In total, 72 species of filamentous fungi from 30 genera were isolated from internal organs of Japanese scallop, with 60 of the species belonging to the 23 genera: *Acremonium* (4 species), *Alternaria* (3), *Aphanocladium* (1), *Aspergillus* (13), *Aureobasidium* (1), *Beauveria* (1), *Botrytis* (1) *Chrysosporium* (2), *Cladosporium* (3), *Cochliobolus* (1) *Fusarium* (2), *Geomyces* (1), *Gliomastix* (1), *Hyalocylindrophora* (1), *Isaria* (1), *Monilia* (1), *Oidiodendron* (1), *Paecilomyces* (1), *Penicillium* (17), *Phialophorophoma* (1), *Sarocladium* (1), *Scopulariopsis* (1), and *Trichoderma* (1); 5 species belonged to ascomycetes from four genera: *Chaetomium* (1), *Eurotium* (1), *Purpureocillium* (1), and *Talaromyces* (2); and 6 species were from the 3 genera of zygomecetes: *Mucor* (4), *Pilaira* (1), and *Rhizopus* (1) (table).

The complex of filamentous fungi associated with the Japanese scallop from the Amur Bay includes 35 species of fungi from 16 genera; 33 of the species belong to anamorphic micromycetes from 14 genera: *Acremonium* (3), *Aspergillus* (10), *Beauveria* (1), *Chrysosporium* (2), *Cochliobolus* (1), *Geomyces* (1), *Gliomastix* (1), *Phialophorophoma* (1), *Scopulariopsis* (1), and *Trichoderma* (1); also, there is a single species of ascomycetes (*Chaetomium globosum*) and a single zygomycete (*Pilaira anoma*la) (Fig. 1).

The complex of filamentous fungi associated with the Japanese scallop from the aquatic waters of the Rikorda Island comprises 39 species of filamentous fungi from 19 genera; among them, 30 species belong to anamorphic fungi from 15 genera: *Acremonium* (3), *Alternaria* (2), *Aphanocladium* (1) *Aspergillus* (5), *Botrytis* (1), *Cladosporium* (1), *Fusarium* (2), *Geomyces* (1), *Gliomastix* (1), *Hyalocylindrophora* (1), *Isaria* (1), *Penicillium* (8), *Phialophorophoma* (1), *Saroclasium* (1), and *Scopulariopsis* (1); 4 species belong to ascomycetes from 3 genera: *Eurotium* (1), *Purpureocillium* (1), and *Talomyces* (2); 4 species belong to zygomycetes from the *Mucor* genus; and one species belongs to Mycelia Sterilia.

The Vostok Bay was chosen as a control aquatoria that is subjected to anthropogenic influence the least [4]. The complex of fungi associated with Japanese scallop from the region comprised 15 species from 8 genera; among them, 14 species belonged to anamorphic fungi from 7 genera: *Alternaria* (3), *Aureobasidium* (1), *Aspergillus* (1), *Cladosporium* (3), *Geomyces* (1), *Penicillium* (4), *Trichoderma* (1); and a single zygomycete *Rhizopus stolonifer* (Fig. 1).

Apparently, fungi were the most numerous in the scallop collected in the aquatic waters of the Rikorda Island. The genera *Penicillium* and *Aspergillus* were represented the most (Fig. 1). In the Vostok Bay, the *Aspergillus* genus was represented by a single species, *Aspergillus nidulans*. The genera *Acremonium* and *Alternaria* were also represented rather well, although not in all of the investigated aquatic waters. Zygomycetes from the *Mucor* genus (4 species) were detected only in mollusks from the aquatic waters of the Rikorda Island. Mucoral species require easily consumed organic compounds [14]. Why, then, were mucoral fungi not detected among the mollusks from the Amur Bay? And why is the Amur Bay inferior to the aquatic waters of the Rikorda Island by the species richness of fungi associated with the mollusks? Apparently, this is due to the fact that the biogenic substances in Amur Bay are of anthropogenic origin, while in the aquatoria of the Rikorda Island they are mainly formed due to living activities of a lot of cultured bivalve mollusks. Besides, in the Amur Bay heavy metals (Cd, Ni, and Mn) are present at higher concentrations [15].

Among the total number of fungi species only two were common for all studied regions (Fig. 2): *Geomyces pannorum* and *Penicillium simplicissimum*. *Geomyces pannorum* is a species widely spread in both soil and marine grounds [16, 17]. It is characterized by a wide range of thermal stability and is capable of growth in the Antarctic region at negative temperatures [18]. The *Penicillium simplicissimum* is also common in grounds of surface and marine habitats; it has been isolated from sea sponges [19]. It produces a secondary metabolite sherinin exhibiting anticancer activity [20].

Complexes of fungi associated with the Japanese scallop from the Amur Bay and aqatoria of the Rikorda Island were the most similar with each other with a total of 11 common species. The species belong to the group of anamorphic fungi: *Acremonium charticola, A. roseogriseum, Aspergillus fumigans, A. ochraceus, A. wentii, Gliomastix murorum, Penicicclium chrysogenum, Phialophorophoma litoralis, Scopulariopsis brumptii, Geomyces pannorum*, and *Penicillium simplicissimum* (Fig. 2).

Fungi complexes of the mollusks from the aquatoria of Rikorda Island and in the Vostok bay include five common species: *Alternaria alternate, A. litorea, Cladosporium cladosporioides, Geomyces pannorum*, and *Pemicillium simplicissimum* (Fig. 2).

The least number of common fungi species associated with the mollusks in the Amur Bay and the Vostok Bay was noted, with three common species: *Trichoderma aureoviride, Geomyces pannorum*, and *Penicillium simplicissimum* (Fig. 2).

Therefore, qualitative composition of the complex of filamentous fungi associated with the Japanese scallop is defined by the quality of its environment.

The observed filamentous fungi of the genera *Aspergillus, Penicillium, Cladosporium, Chaetomium*, etc. belong to the group of conditionally pathogenic

Species composition of the filamentous fungi complexes associated with the Japanese scallop from various regions of the Peter the Great Bay of the Sea of Japan

Number	Fungi species	Regions of the mollusk collection		
		Amur Bay	Rikorda Island	Vostok Bay
1	*Acremonium charticola* (Lindau) W. Gams	+	+	
2	*Acremonium breve* (Sukapure et Thirum.) W. Gams		+	
3	*Acremonium fusidioides* (Nicot) W. Gams	+		
4	*Acremonium roseogriseum* (S.B. Saksena) W. Gams	+	+	
5	*Alternaria alternata* (Fr.) Keissl.		+	+
6	*Alternaria litorea* (Pivkin et Zvereva) E.G. Simmons		+	+
7	*Alternaria tenuissima* (Fr.) Wiltshire			+
8	*Aphanocladium album* (Preuss) W. Gams		+	
9	*Aureobasidium pullulans* (de Bary) G. Arnaud			+
10	*Aspergillus awamori* Nakaz.	+		
11	*Aspergillus candidus* Link	+		
12	*Aspergillus flavus* Link	+		
13	*Aspergillus foetidus* Thom et Raper	+		
14	*Aspergillus fumigatus* Fresen.	+	+	
15	*Aspergillus lanosus* Kamal et Bhargava	+		
16	*Aspergillus nidulans* (Eidam) G. Winter			+
17	*Aspergillus niger* v. Tiegh.	+		
18	*Aspergillus ochraceus* G. Wilh.	+	+	
19	*Aspergillus repens* (Corda) Sacc.		+	
20	*Aspergillus terricola* Marchal et É.J. Marchal		+	
21	*Aspergillus ustus* (Bainier) Thom et Church	+		
22	*Aspergillus wentii* Wehmer	+	+	
23	*Beauveria bassiana* (Bals.-Criv.) Vuill.	+		
24	*Botrytis cinerea* Pers.		+	
25	*Chaetomium globosum* Kunze	+		
26	*Chrysosporium merdarium* (Ehrenb.) J.W. Carmich.	+		
27	*Chrysosporium tropicum* J.W. Carmich.	+		
28	*Cladosporium cladosporioides* (Fresen.) G.A. de Vries		+	+
29	*Cladosporium oxysporum* Berk. et M.A. Curtis			+
30	*Cladosporium sphaerospermum* Penz.			+
31	*Cochliobolus sativus* (S. Ito et Kurib.) Drechsler ex Dastur	+		
32	*Eurotium cristatum* (Raper et Fenell) Malloch et Cain		+	
33	*Fusarium oxysporum* Schltdl.		+	
34	*Fusarium trichothecioides* Wollenw.		+	
35	*Geomyces pannorum* (Link) Sigler et J.W. Carmich.	+	+	+
36	*Gliomastix murorum* (Corda) S. Hughes	+	+	
37	*Hyalocylindrophora rosea* (Petch) Réblová & W. Gams		+	
38	*Isaria farinosa* (Holmsk.) Fr.		+	
39	*Mucor abundans* Povah		+	
40	*Mucor circinelloides* f. *janssenii* (Lendn.) Schipper		+	
41	*Mucor hiemalis* Wehmer		+	
42	*Mucor racemosus* f. *racemosus* Fresen.		+	
43	*Monilia brunnea* J.C. Gilman et E.V. Abbott	+		

Table. (Contd.)

Number	Fungi species	Regions of the mollusk collection		
		Amur Bay	Rikorda Island	Vostok Bay
44	*Oidiodendron cereale* (Thüm.) G.L. Barron	+		
45	*Paecilomyces variotii* Bainier	+		
46	*Penicillium atramentosum* Thom	+		
47	*Penicillium aurantiogriseum* Dierckx		+	
48	*Penicillium canescens* Sopp	+		
49	*Penicillium chrysogenum* Thom	+	+	
50	*Penicillium citrinum* Thom			+
51	*Penicillium citreonigrum* Dierckx		+	
52	*Penicillium dierckxii* Biourge		+	
53	*Penicillium griseofulvum* Dierckx	+		
54	*Penicillium humuli* J.F.H. Beyma	+		
55	*Penicillium lanosum* Westling		+	
56	*Penicillium multicolor* Grig.-Man. et Porad.	+		
57	*Penicillium rubrum* Stoll		+	
58	*Penicillium simplicissimum* (Oudem.) Thom	+	+	+
59	*Penicillium vulpinum* (Cooke et Massee) Seifert et Samson	+		
60	*Penicillium* sp. 1		+	
61	*Penicillium* sp. 2			+
62	*Penicillium* sp. 3			+
63	*Phialophorophoma litoralis* Linder	+	+	
64	*Purpureocillium lilacinum* (Thom) Luangsa-ard, Hywel-Jones et Samson		+	
65	*Pilaira anomala* (Ces.) J. Schröt.	+		
66	*Rhizopus stolonifer* (Ehrenb.) Vuill.			+
67	*Sarocladium strictum* (W. Gams) Summerb.		+	
68	*Scopulariopsis brumptii* Salv.-Duval.	+	+	
69	*Talaromyces purpureogenus* Samson, Yilmaz, Houbraken, Spierenburg, Seifert, Peterson, Varga & Frisvad		+	
70	*Talaromyces verruculosus* (Peyronel) Samson, Yilmaz, Frisvad et Seifert		+	
71	*Trichoderma aureoviride* Rifai	+		+
72	Mycelia Sterilia		+	
	Total	36	39	15

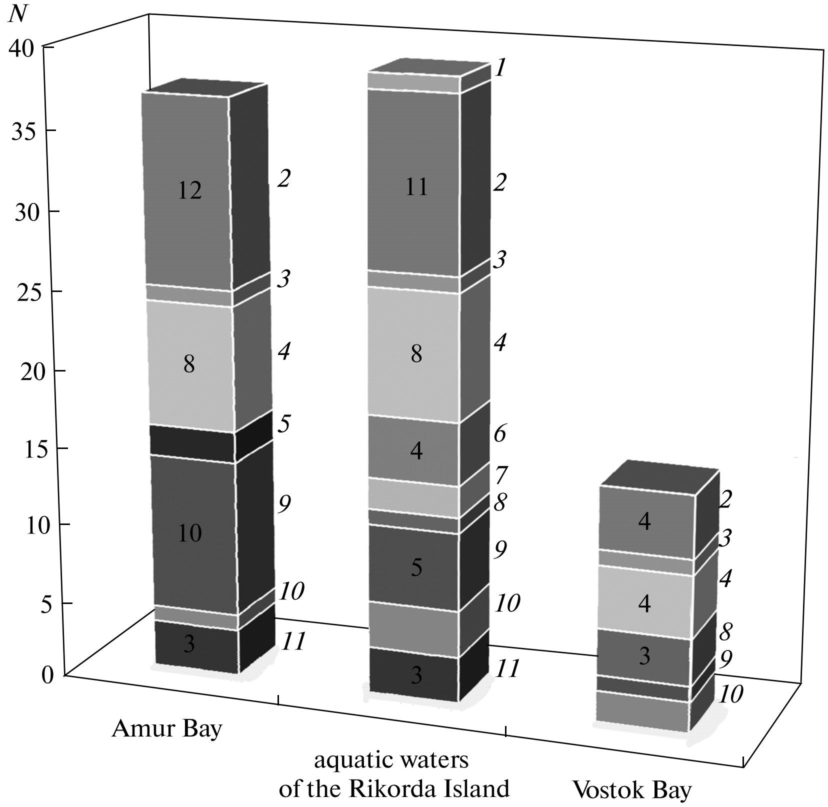

Fig. 1. Taxonomic composition of the complexes of filamentous fungi associated with the Japanese scallop from various regions of the Peter the Great Bay: *1*, Mycelia Sterilia; *2*, minor genera; *3*, Trichoderma; *4,* Penicillium; *5,* Chrysosporium; *6,* Mucot; *7,* Fusarium; *8,* Cladosporium; *9,* Aspergillus; *10,* Alternata; and *11*, Acremonium. *N*, the number of fungi species.

and toxigenic microorganisms [5, 12] that can cause mycoses and mycotoxicoses of hydrobionts. Previous mycological and toxicological studies of the Japanese scallop *Mizuhopecten yessoensis* using the enzyme-linked immunosorbent assay (ELISA) demonstrated that internal organs of the mollusks accumulated aflatoxins produced by a filamentous fungus *Aspergillus flavus* [21].

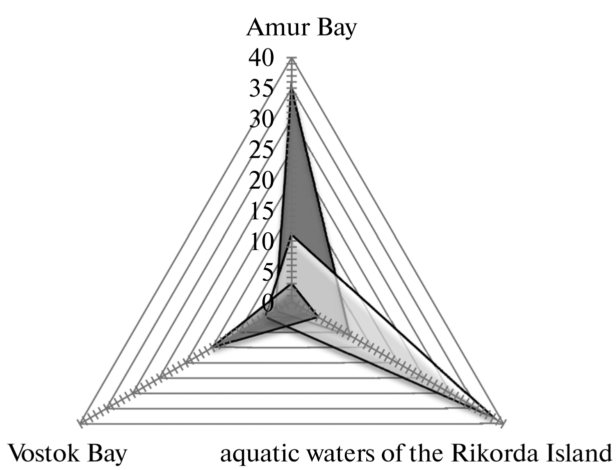

Fig. 2. Comparative characteristics of the species richness of filamentous fungi associated with the Japanese scallop from various regions of the Peter the Great Bay.

Microbiotic monitoring of the mollusks from various regions of the Peter the Great Bay of the Sea of Japan demonstrated that the species richness of the conditionally pathogenic and toxigenic filamentous fungi, primarily the fungi of the genera *Aspergillus, Penicillium, Cladosporium*, and *Chaetomium*, in internal organs of the bivalve mollusks increases in contaminated coastal waters [22–24].

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