
EXPERIMENTAL
ARTICLES

Mycobiota of the Giant Oyster *Crassostrea gigas* (Thunberg, 1787) (Bivalvia) from the Peter the Great Bay of the Sea of Japan

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Abstract—Mycological investigation of the Pacific (giant) oyster *Crassostrea gigas* (Thunberg, 1793) (Bivalvia) from the Peter the Great Bay of the Sea of Japan was carried out. The taxonomic composition of filamentous fungi associated with *C. gigas* was studied. The taxonomic composition of the fungi associated with the giant oyster included 22 species of filamentous fungi of which 17 species were identified. The latter belonged to six genera: *Alternaria*, *Aspergillus*, *Botrytis*, *Fusarium*, *Penicillium*, and *Trichoderma*. The distribution of filamentous fungi in the internal organs of the bivalve mollusk was studied.

Keywords: marine filamentous fungi, bivalve mollusks, mycobiota, giant oyster, anamorphic fungi.

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The giant oyster is an important object of fishery and cultivation, both in the Far East and on the Black Sea coast [1].

Mycoses caused by pathogenic fungi pose a serious threat to oysters. The shell disease caused by the parasitic fungus *Ostracoblabe implexa* [1], both in natural populations and in artificially grown mollusks, is the most dangerous and widespread oyster disease of fungal etiology. In the Black Sea, oyster shell disease infecting up to 80% of the species was noted in all oyster shoals [2]. The mollusks may also be infected by toxicogenic species of filamentous fungi with the accumulation in their tissues of mycotoxins, which are hazardous for humans.

Many filamentous fungi, e.g., members of the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Trichoderma*, have pathogenic and toxicogenic properties: they cause mycoses and mycotoxicoses both in humans and terrestrial animals [3] and in marine invertebrates and fishes [4].

No mycological survey of the giant oyster *Crassostrea gigas* (Thunberg, 1793) (Bivalvia) has been previously carried out in the Russian Far Eastern seas. We obtained the first information on the taxonomic composition of filamentous fungi in the overgrowth (on the shell surface) of the giant oyster [5].

The goal of the present work was to study the taxonomic composition of the filamentous fungi associated with the giant oyster, and the character of their

distribution on the shell surface and in the internal organs of this mollusk.

MATERIALS AND METHODS

The giant oyster *C. gigas*, a bivalve mollusk, was the material for mycological investigations. The material was collected in the Peter the Great Bay near the Isle of Rikord at a depth of 3–4 m in April 2010.

The dissected internal organs of the mollusks—the gills, the mantle, the kidneys, the digestive gland (DG), the muscle, and the male gonads—were soaked in an antibiotic solution (500 000 U of penicillin and 0.5 g of streptomycin per 1 l of sterile seawater) for 2 h to inhibit the growth of bacteria. The material was then washed in sterile seawater and plated on the wort agar medium and Czapek medium with peptone.

The strains of filamentous fungi are stored in the Collection of Cultures of Marine Fungi of the Zhirmunskii Institute of Marine Biology, Far Eastern Branch, Russian Academy of Sciences.

RESULTS AND DISCUSSION

The mycological study of the giant oyster revealed 22 species of filamentous fungi; 17 species were identified [3, 7–11]. These species were anamorphic micromycetes (*Anamorphic fungi*). The species identified belonged to six genera: *Alternaria*, *Aspergillus*, *Botrytis*, *Fusarium*, *Penicillium*, and *Trichoderma* (table). The genus *Aspergillus* was the most common (five species). The fungi of the genera *Penicillium* and

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Distribution of filamentous fungi on the shell surface and in the internal organs of the giant oyster *Crassostrea gigas* (Thunberg, 1793) (Bivalvia) from the Peter the Great Bay of the Sea of Japan

Fungal taxon	Valve		Mantle		Muscle		DG		Gonads		Gills		Kidneys	
	1*	2*	1	2	1	2	1	2	1	2	1	2	1	2
<i>Alternaria alternata</i> (Fr.) Keissl.	+					+								
<i>A. litorea</i> (Pivkin et Zvereva) Ging.	+													
<i>A. tenuissima</i> (Fr.) Wiltshire		+												
<i>Aspergillus anthodesmus</i> Bartoll. et Maggi				+										
<i>A. candidus</i> Link			+											
<i>A. carbonarius</i> (Bain.) Thom	+	+					+	+						
<i>A. ochraceus</i> K. Wilh.			+											
<i>A. phoenicis</i> (Corda) Thom				+	+	+		+	+		+	+	+	
<i>Botrytis cinerea</i> Pers.									+				+	
<i>Fusarium oxysporum</i> var. <i>orthoceras</i> (App. et Wr.) Bilai	+	+												
<i>F. lateritium</i> Nees	+													
<i>Penicillium brevicompactum</i> Dierckx.			+											
<i>P. digitatum</i> Sacc. var. <i>californicum</i> Thom		+												
<i>P. lanosum</i> Westling	+	+					+	+						
<i>P. simplicissimum</i> (Oud.) Thom				+										
<i>Trichoderma aureoviride</i> Rifai	+				+									
<i>T. viride</i> Pers.			+	+						+			+	
Mycelia Sterilia 1		+						+				+		
Mycelia Sterilia 2												+		
Mycelia Sterilia 3											+			
Mycelia Sterilia 4		+												
Mycelia Sterilia 5									+					
Total: 22	7	7	4	4	2	2	2	4	2	2	2	3	3	0
	11		7		3		4		4		4		3	

Note: DG, digestive gland; 1*, wort–agar medium; 2*, Czapek medium with peptone.

Alternaria were also comparatively widely represented (four and three species, respectively). The genus *Botrytis* represented by one species, *Botrytis cinerea*, was the least numerous (table). The species designated by us as Mycelia Sterilia (five species) formed mycelium without sporification and differed from each other in colony morphology.

The character of the distribution of filamentous fungi in the mollusk internal organs was studied: the mantle contained seven species; the muscle, three species; the digestive gland, four species; the gonads, four species; the gills, four species; the kidneys, three species (table). Eleven species of filamentous fungi were revealed on the shell valves.

The species *Alternaria litorea*, *A. tenuissima*, *Fusarium oxysporum* var. *orthoceras*, *F. lateritium*, *Penicillium digitatum* var. *californicum*, and Mycelia Sterilia 4 were detected only on the valves of the giant oyster. The anamorphic fungus *Aspergillus phoenicis* was iso-

lated from all the internal organs studied, but not from the mollusk valves. The species *Aspergillus anthodesmus*, *A. candidus*, *A. ochraceus*, *Penicillium brevicompactum*, and *P. simplicissimum* were revealed only in the mantle. The Mycelia Sterilia 5 isolate was revealed in the gonads; Mycelia Sterilia 2 and Mycelia Sterilia 3 were isolated exclusively from the gills. The kidneys, the muscle, and the digestive gland were found to contain the species of filamentous fungi also detected in the other organs.

The fungi of the genus *Aspergillus* revealed in the internal organs of the oyster are assigned to the group of opportunistic and toxicogenic fungi.

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REFERENCES

1. McGladdery, S.E., Deinnan, R.E., and Stephenson, M.F., Oyster Parasites, Pest and Diseases. Shell Disease (*Ostracoblabe implexa*), *A Manual of Parasites, Pest and Diseases of Canadian Atlantic Bivalves*, 1993, pp. 59–61.
2. Pirkova, A.V. and Demenko, D.P., Cases of Shell Disease in the Giant Oyster *Crassostrea gigas* (Bivalvia) Cultivated in the Black Sea, *Russ. J. Marine Biol.*, 2008, vol. 34, no. 5, pp. 309–315.
3. Bilai, V.I. and Koval', E.Z., *Aspergilly* (Aspergilles), Kiev: Nauk. Dumka, 1988.
4. Sindermann, C.J. and Lightner, D.V., *Disease Diagnosis and Control in North American Marine Aquaculture*, Elsevier Sci, 1988.
5. Zvereva, L.V. and Borzykh, O.G., Filamentous Fungi in the Epigrowth of the Pacific Oyster *Crassostrea gigas* (Bivalvia) in Peter The Great Bay, Sea of Japan, *Proc. China–Russia Bilateral Symp. on “Comparison on Marine Biodiversity in the Northwest Pacific Ocean”*, Qingdao, 2010, pp. 10 – 11.
6. Egorova, L.N., *Pochvennye griby Dal'nego Vostoka. Gifomicety* (Far Eastern Soil Fungi. Hyphomycetes), Leningrad: Nauka, 1986.
7. Ainsworth, G.C., Sparrow, F.K., and Sussman, A.S., *The Fungi. Vol. 4A: A Taxonomic Review with Keys: Ascomycetes and Fungi Imperfecta*, New York: Academic, 1973.
8. Ellis, M.B., *Dematiaceous Hyphomycetes*, Kew, Surrey: Commonw. Mycol. Inst., 1971.
9. *Integration of Modern Taxonomic Methods for Penicillium and Aspergillus Classification*, Samson, R.A. and Pitt, J.I., Eds., Harwood Academic, 2000.
10. Rifai, M.A., A Revision of the Genus *Trichoderma*, *Mycol. Papers, Bogor*, 1969, vol. 116.
11. Thom, C and Raper, K.B., *A Manual of the Penicillia*, Baltimore, 1949.