ISOLATION AND PRELIMINARY STUDIES OF NEW ALGAL VIRUS OF MICROALGAE *PROROCENTRUM CORDATUM*

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Abstract

For the first time, from the Black Sea water samples taken from the waters of three bays of the Sevastopol city, strains of the new to science algal virus of the microalgae *Prorocentrum cordatum* (PcV), were isolated by the author's patented methods. Using the methods and means available in the laboratory of the Institute of Natural and Technical Systems (Sevastopol, RF), some characteristics of this algal virus were studied. A short incubation period (24 h) and a relatively high infectious titer in the viral suspension (10^7 IE / ml) were established; the sensitivity of the algal virus to freezing (-18^0 C) and chloroform was revealed, which indicates a complex morphology of the virion (the presence of a supercapsid). The long-term survival of the virus under indoor conditions (up to 6 months) was also recorded, the spring-autumn seasonality and narrow species specificity in relation to the closely related host *Prorocentrum pusilla* were determined. Our results of monitoring during 6 months this new for science algal virus of the microalgae *Prorocentrum cordatum* in different in ecological status bays testified, that it can be used as an ecological indicator.

Key words: strains of a new for science algal virus of the microalgae *Prorocentrum cordatum* - PcV, characteristics and properties of PcV based on the results of its preliminary study, Black Sea, bays of Sevastopol.

Introduction and short Review of Literature

Currently, a young science – aquatic (marine) virology – is at the stage of globalization of research on the viral component and its individual representatives in the World Ocean [1, 2]. So already at the beginning of the 21st century, marine (aquatic) viruses are defined by scientists as key players in global ecology, which indicates the relevance of knowledge in the field of marine virology [3, 4].

According to The Scientific Committee on Ocean Research Viral Ecology Working Group, understanding the role of viruses in the circulation of organic carbon in the Ocean, in food chain processes and in biodiversity is the basis for assessing the stability of marine systems and their biogeochemical significance, increasing the

predictability of the impact of global changes on biogeochemical processes in the World Ocean, which will undoubtedly affect the biosphere of our entire Planet [5].

Of particular interest have always been and continue to cause algal viruses, the key position of which in the regulation of phytoplankton abundance, which is of great importance for our Planet, without a doubt. The search, isolation and study of algal viruses from the hydrosphere remain at the forefront of scientific interests, which is associated with the theoretical and practical significance of these studies. According to the literature, 65 eukaryotic algae viruses preserved in collections have already been described [2].

Since 2002, over the 300 strains of new to science and the Black Sea ecosystem algal viruses of microalgae *Tetraselmis viridis, Dunaliella viridis, Phaeodactylum tricornutum, Prorocentrum pusilla*, *Isochrysis galbana*, *Tisochrysis lutea, Emiliania huxleyi* and also strains of cyanophage of cyanobacterium *Synechococcus*, have been isolated from various samples, taken from the ecosystem of the Black Sea of the Crimean region [6, 7]. It was found by electron microscopy that the virions have the form of icosahedrons with sizes of 56-60, 45-48, 50-53, 128-132 and 88-92 nm, respectively, in algal viruses of next algae *Tetraselmis viridis*, *Phaeodactylum tricornutum*; *Dunaliella viridis*; *Prorocentrum pusilla*; *Isochrysis galbana* [6, 7].

Two strains of algal virus of microalgae *Tetraselmis viridis* (TvSI1, TvS20) and one strain of the algal virus of microalgae *Dunaliella viridis* (DvVSI2) were studied at the level of genetic research in the international project " Gordon & Betty Moore Foundation Marine Phage, Virus, & Virome Sequencing Project (Broad Institute, MTI, USA, www.broadinstitute.org/annotation/viral/Phage) [8]. The results obtained during on the genomes of Black Sea algal viruses sequenced, assembled, and annotated by the Broad Institute researchers are presented in CAMERA (Community Cyberinfrastructure for Advanced Marine Microbial Research and Analysis).

The strains of algal viruses of 7 species of microalgae and strains of cyanophage of the cyanobacterium *Synechococcus* which preserving in the author's collection, can be used and have already been repeatedly used in various model experiments and for approbation of laboratory stands and installations, created on the basis of the Institute of Natural and Technical Systems and the FRC Marine Hydrophysical Institute [6].

The **purpose** of the study, taking into account the indicated relevance of the scientific problem, were the search, isolation and preliminary study of a new for science algal virus of the microalgae *Prorocentrum cordatum*.

Materials and Methods

Isolation of algal virus strains of the microalgae *Prorocentrum cordatum* (PcV) was carried out from samples of both fresh and stored 1–2 months of seawater according to the author's patented methods [9, 10]. At the same time, the same samples were used to search and isolate algal viruses of two other microalgae

Prorocentrum pusillum and *Prorocentrum micans* related to the microalgae *Prorocentrum cordatum*. Moreover, the isolation of the algal virus of the microalgae *Prorocentrum pusillum* was already performed for the first time in 2010 [6, 7], and the microalga *Prorocentrum micans* was used for the first time to isolate the algal virus.

Seawater samples were taken from January to May, as well as in September 2021, from three differing in ecological status (well-being) bays of Sevastopol. In the submitted communication, the bays of Sevastopol were designated as 1 (open, relatively ecologically safe bay), 2 (closed, with a functioning mussel farm, relatively ecologically safe bay) and 3 (closed, exposed to anthropogenic impact and pressing, unfavorable bay).

A liquid, algologically pure culture of the microalgae *Prorocentrum cordatum*, as well as the cultures of two other microalgae, *Prorocentrum pusillum* and *Prorocentrum micans*, were obtained from the collection of live microalgae cultures of the Department of Ecological Physiology of Algae of the Federal Research Center, A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS (Sevastopol, RF)".

Results and Discussion

In the first half of 2021 (January – May), 15 samples of seawater were tested during the research (5 samples from each bay). At the same time, 8 PcV strains were isolated: in January and February 2 strains were isolated in each month (saved seawater samples were used), in March – 1, and in April – 3 PcV strains. The results obtained indicate the spring seasonality of PcV, as a reflection of the seasonality and its host, the microalga *Prorocentrum cordatum*. In May, PcV strains were not isolated from seawater samples.

The results obtained in the course of the research are reflected in **table 1**, which also presents for comparison the data on the search and isolation of algal viruses of the microalgae *Prorocentrum pusillum* (PpV) and *Prorocentrum micans* (PmV) from seawater samples, as well as the results of the PcV search in September 2021.

The results of isolation algal virus of the microalgae *Prorocentrum cordatum* (PcV) from seawater samples from three bays of Sevastopol in September 2021 also reflect the autumn seasonality, which is typical of the Black Sea microalgae [11], and, consequently, of their viruses [6, 7].

From the standpoint of the distribution of the algal virus of the microalgae *Prorocentrum cordatum* (PcV) in the three studied bays, depending on their ecological well-being, it can be noted that PcV strains were most often isolated from relatively ecologically safe bays 1 and 2. There were 4 strains each from water samples from these bays during 6 months. But in total, 2 strains of the algal virus of microalgae *Prorocentrum cordatum*, a new for science algal virus, were isolated from the unfavorable closed bay 3 exposed to anthropogenic impact and pressure.

Table 1

Results of search and isolation of algal viruses of microalgae *Prorocentrum pusillum* (PpV), *Prorocentrum micans* (PmV), and *Prorocentrum cordatum* (PcV) from seawater samples from bays 1, 2, and 3 in Sevastopol in 2021

Date of selection (collection) of studied samples	Studied material (number of samples) – seawater	Localization (bay) of gathering the samples	Algal viruses isolated during the study		
			Prorocentrum pusillum Virus	*Prorocentrum micans* Virus	*Prorocentrum cordatum* Virus
23.01.21* (*22.03.21*)	1	1	PpV-S-21-1	-	PcV-S-21-1
	2	2	-	-	PcV-S-21-2
	3	3	PpV-S-21-2	-	-
23.02.21* (*22.03.21*)	4	1	-	-	PcV-S-21-3
	5	2	-	-	PcV-S-21-4
	6	3	-	-	-
19.03.21	7	1	-	-	-
	8	2	-	-	PcV-S-21-5
	9	3	-	-	-
20.04.21	10	1	-	-	PcV-S-21-6
	11	2	-	-	PcV-S-21-7
	12	3	PpV-S-21-3	-	PcV-S-21-8
19.05.21	13	1	-	-	-
	14	2	-	-	-
	15	3	-	-	-
29.09.21	16	1	nr	nr	PcV-S-21-9
	17	2	nr	nr	-
	18	3	nr	nr	PcV-S-21-10

Designations: 23.01.21* (or 01/23/21*) and 23.02.21* (or 02/23/21*) - samples were stored at room temperature and illumination until 22.03.21 (or 03/22/21), i.e. the search and isolation of algal viruses was carried out according to the author's patented technique described in US Pat. 97293 C2 UA, IPC C12N 1/12 [9];

(nr) - no research was carried out; (-) - negative result

This fact, recorded in our studies, testifies to the peculiarities of the distribution of the viral host, the microalgae *Prorocentrum cordatum*, which apparently prefers ecologically safe waters. Probably, the algal virus of the microalgae *Prorocentrum*

cordatum, new to science, can be used as an ecological indicator, as well as the previously described indicator algal viruses of the microalgae *Tetraselmis viridis* and *Phaeodactylum tricornutum* [6, 7, 12, 13]

Some characteristics of this new to science algal virus were also studied, using available methods based on Institute of Natural and Technical Systems. It was fixed a short incubation period (24 h), a relatively high infectious titer in the viral suspension (10^7 IE / ml) was determined. The revealed sensitivity of the algal virus to freezing and to chloroform indicates a complex morphology of the virus (the presence of a supercapsid).

The test for possible cross links of the algal virus of the microalga *Prorocentrum cordatum* (PcV) with the closely related host of the microalga *Prorocentrum pusilla*, as well as the algal virus of the microalgae *Prorocentrum pusillum* (PpV) with the probable host of the microalgae *Prorocentrum cordatum*, is negative, which is reflected in the **table 2**.

Table 2

Study of cross links of closely related algal viruses of microalgae *Prorocentrum cordatum* and *Prorocentrum pusillum*

Cultures of microalgae	Strains of algal viruses	
	PpV	PcV
Prorocentrum pusilla	Viral lysis	growth of culture
Prorocentrum cordatum	growth of culture	Viral lysis

Presented in **table 2** data indicate a narrow species specificity of the new algal virus of the microalgae *Prorocentrum cordatum* in relation to closely related microalgae - viral unicellular hosts.

Checking the persistence (vitality, saving) of the infectious activity of the isolated new for science algal virus of the microalgae *Prorocentrum cordatum*, has established that under the room lighting and room temperature the virus does not degrade (not decayed) for more than 6 months, although its infectious titer decreases by several orders of magnitude, which indicates the possibility of its long-term storage.

Conclusion

For the first time, 10 strains of a new for science algal virus of microalgae *Prorocentrum cordatum* (PcV) were isolated from samples of the Black Sea water from the water area of the bays of the Sevastopol city with using the author's patented methods.

Several characteristics of this algal virus were studied by using available methods. So a short incubation period (24 h) and a relatively high infectious titer in the viral suspension (10^7 IE / ml) were established, the sensitivity of the algal virus to freezing and chloroform was revealed, which indicates a complex morphology of virions (the presence of supercapsid). Also, its spring-autumn seasonality, long-term survival (preservation) of up to 6 months under room lighting and temperature conditions, and its narrow species specificity in relation to a closely related host were established. Our results of monitoring during 6 month this new for science algal virus of the microalgae *Prorocentrum cordatum* in different in ecological status bays testified, that it can be used as an ecological indicator

Further studies of this new algal virus will be devoted to the study of its morphology (size, EM image, etc.).

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