Short Communications

Cultivation, purification and crystallization of virus of green algae *Tetraselmis viridis*

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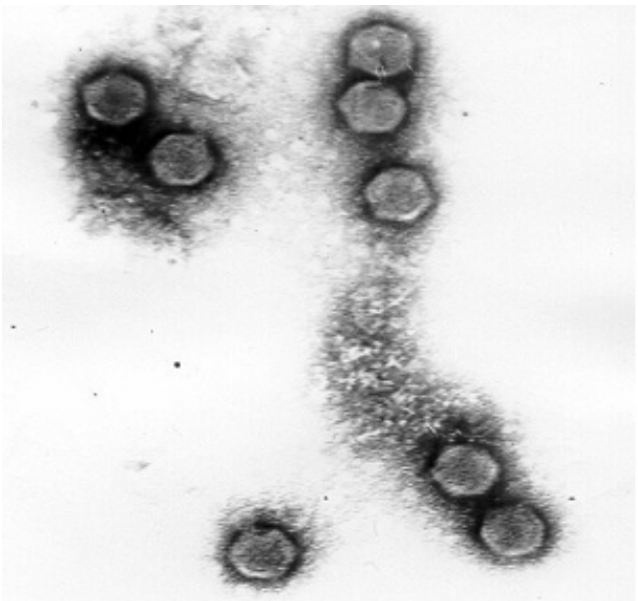
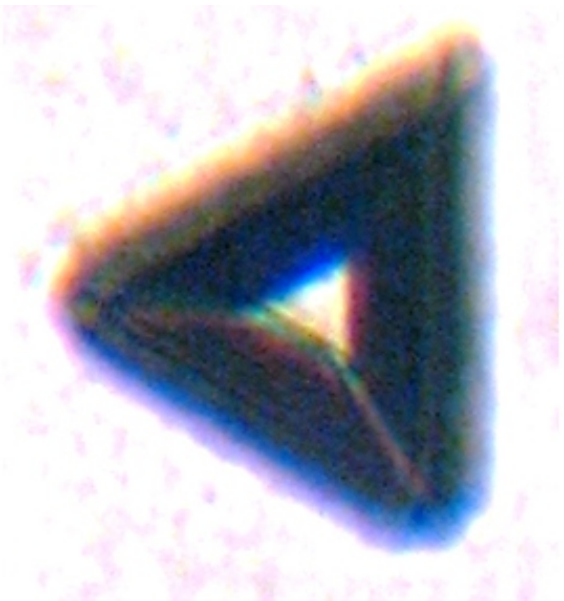
The method of obtaining the virus of algae Tetraselmis viridis in high concentration is presented and the scheme of its crystallization is developed which will henceforth enable the further determination of virion structure if crystals correspond to the requirements of the X-ray structural analysis. The obtained data are also useful for the elaboration of a new model system for cultivating viruses, in particular, phycodnaviruses.

***Key words**: virus Tetraselmis viridis, virion structure, cultivation of viruses, phycodnaviruses*

In the recent 10 years, the active study on viruses, infecting water organisms, resulted in the extension of knowledge in the filed of microalgae viruses. These achievements allowed uniting the representatives of algoviruses into the new family – phycodnaviruses, which are characterized by the icosahedral form of a virion with the diameter of up to 220 nm and a genome, consisting of double-strand DNA. At present at least one representative of phycodnaviruses for 10 (44 taxons) out of 14 described classes is known [1, 2].

Microalgae are at the basis of food pyramid in the World Ocean and are the main primary producers. Their photosynthetic activity provides the enrichment of hydro- and atmosphere with oxygen. It is evident that the decrease in microalgae biomass will result in the destruction of ocean food chains as well as in the change of oxygen and carbonic acid concentration in the atmosphere and hydrosphere. The latter may become the reason for development of green house effect. At present algae are also considered to be the potential rough resource for humanity. At the same time algoviruses are a factor which may influence the total quantity of phytoplankton significantly and decrease its





total biomass considerably [1]. Hence, thorough study on algoviruses is of vital practical and scientific interest, with the consideration of almost complete absence of scientific information concerning their biological peculiarities.

The virus isolation. Algologically pure culture of one-celled seaweed *T.viridis Norris (Chlorophyta, Prasinophyceae)* as well as pre-culture of virus TvV-S1 was used to obtain a large quantity of virus.

The culture of algae was grown in Goldberg medium, prepared on sterile sea water. After 4-5 days of incubation at 27°C and 24-hour-illumination the algae were infected by the virus pre-culture (1:100) and incubated at the room temperature with the artificial 12-hour illumination for 3-4 days, after which clear cultural liquid was separated [3, 4]. The virus was obtained from the cultural liquid by centrifugation for 30 min at 5000 rpm. The supernatant was centrifuged for 2.5 hours at 28000 rpm, after which the obtained glass-like sediment was re-suspended in the least possible volume of physiological solution. Electronic microscopy was used to control the clearness of the virus preparation.

Obtaining the virus crystals. The virus was crystallized using standard screening solutions Crystal Screen 2 (“Hampton Research”, the USA) according to the method of “sitting drop” mixing virus-containing material with the screening solution 50:50 in crystallization dishes of “greiner” type (Falcon) [5]. The reservoir volume was to 80 мl, the drop – 1 мl, virus concentration (by the protein, determined by Loury method) ~4 mg/ml. Crystallization conditions were the following: 0.05M CsCl, 0.1M MES, pH 6.5, 30% Jeffamine M – 600 Reagent.

Icosahedral virions TvV-S1 with the diameter of 56-58 nm were detected using electronic microscopy of samples. Their image is presented in Fig 1.

It was determined that at long-term keeping at the temperature of 4°C the quantity of empty capsids, which is insignificant in a fresh preparation, increases. Evidently, it is the reason of considerate loss of infection, observed in the previous research [7].

After the introduction of virus preparation into the crystallization dish the crystals of pyramidal form appeared on the 2nd day of incubation at the room temperature. The crystal size amounted to about 0.3mm (Fig 2), they turned out to be stable and stayed in the solution for several months. It is worth mentioning that the crystals may be obtained only from the fresh virus preparations. Keeping the virus at the room temperature for several days causes significant decrease in concentration and destruction of virion structure, which makes crystal formation impossible.

The scheme of cultivation and obtaining some representatives of phycodnaviruses out of green algae *T.viridis Norris* as a specific highly sensitive host was elaborated. The regime of cultivation and obtaining the virus in high concentrations was worked out. The cultivation of the algae is rather simple and does not require special conditions and reagents. Thus, the green sea microalgae *T. viridis* may be used successfully as a model organism to study other

algoviruses. The obtaining of crystals of phycodnavirus TvV-S1 is the first step for further structural research. Henceforth, in case of corresponding quality of crystals it will be possible to obtain a three-dimensional model of capsid proteins which will allow analyzing their structure and potential functional meaning.

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Культивирование, выделение и получение кристаллов вируса зеленых водорослей *Tetraselmis viridis*

Резюме

Представлена методика получения вируса водорослей T. viridis в больших количествах и разработана схема его кристаллизации, что в дальнейшем (при условии соответствия кристаллов требованиям рентгеноструктурного анализа) даст возможность определить структуру вирионов. Полученные данные могут также быть полезными для разработки новой модельной системы культивирования вирусов, в частности, фикоднавирусов.

Ключевые слова: вирус Tetraselmis viridis, структура вирионов, культивирование вирусов, фикоднавирусы.

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