The study of contact of Black Sea algal viruses and their hosts (algae) by microcalorimetry method

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Abstract

The investigations of study the contact of algal viruses (strains of TvV and PtV) and their hosts (*Tetraselmis viridis* and *Phaeodactylum tricornutum*) by microcalorimetry method were carry more 15 years ago. Heat production of contact of algal viruses and their hosts (algae) measured by Biological Activity Monitor (BAM) is presented in graphics-thermogrammes in μW. It was shown that heat production of algae cultures growth (in control) will depend on such factors as the species of algae, their concentration and the growth stage (phase) of culture, nutrient content and lighting, and also of other factors of biotical and abiotical origin. Viral infection, in our opinion, is one of the most significant factors affecting the viability of algae and therefore on the amount of heat production of algae cultures infected with the virus.

Interaction between algal viruses and sensitive algae culture leads to the production of virus and destruction (lysis) of host cells that manifests itself in reducing heat production in the experiment as compared with the control.

The contact between the algal viruses with their hosts was accompanied by a decrease of heat production in the first hours of their interaction, but the best difference was observed after 10–24 h.

We hope that this study can be used to study the contact of other viruses with host cells in order to assess the influence of various factors (UV, EMF, various substances and drugs) on this contact.

Key words: heat production, microcalorimetry method, algal viruses, *Tetraselmis viridis* and *Phaeodactylum tricornutum*

Introduction

The microcalorimetry method is used both for scientific and practical purposes in the study of ecology, biology and other characteristic features of various microbiological objects [Cabadaj et al., 2021; Harris et al., 2012; Menert et al., 2006]. The application of microcalorimetry in medicine is of particular interest [Bonkat et al., 2012; Monti, 2006].

Our research by microcalorimetry was devoted to the study of the contact of viruses, incl. and algal viruses, with their unicellular hosts, as well as detecting changes in heat production upon contact of viruses with DNA [Stepanova, 2005; 2006; Stepanova et al., 2003]. Many results, generalizations and conclusions related to the study of the contact of viruses with their unicellular hosts or with DNA and with its decay products were reflected in publications in Russian, incl. in [Степанова, 2004; Степанова, Шайда, 1998; 1999; 2002].

The purpose of this communication is to present in a generalized form the results of studying the contact of Black Sea algal viruses with their hosts (cultures of Black Sea microalgae) obtained over a long period of time using the microcalorimetry method.

Material and Methods

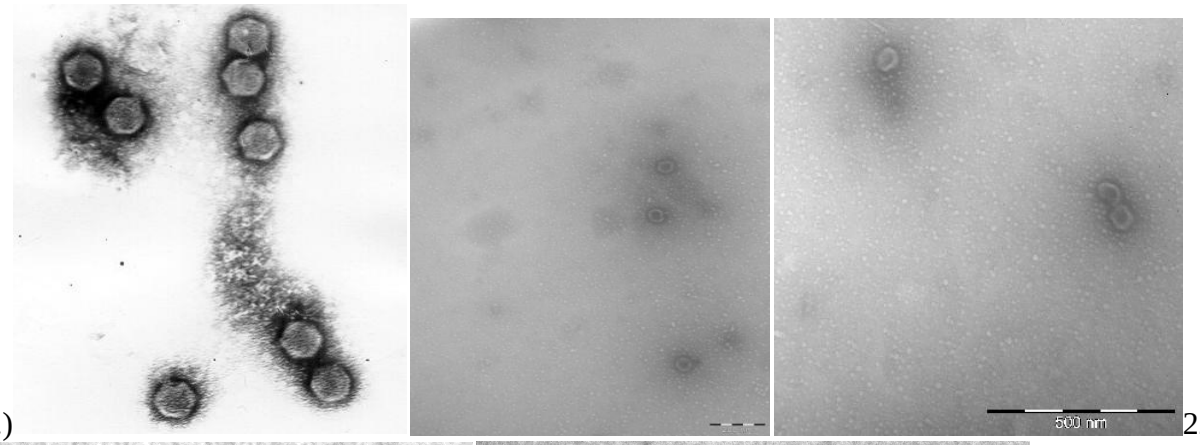
Contacts of algal viruses and algae were studied by microcalorimetry method using a Biological Activity Monitor (BAM) 2277 system (LKB, Sweden) - a device for continuous monitoring of the processes and reactions.

Heat production of contact of virus with algae culture (cell-hosts) was studied using method of closed ampoules under dark conditions. Also the heat production of contact of viruses and algae was tested in conditions with full lighting [Stepanova, 2006]. Heat production measured by Biological Activity Monitor (BAM) is presented in graphics-thermogrammes in μW.

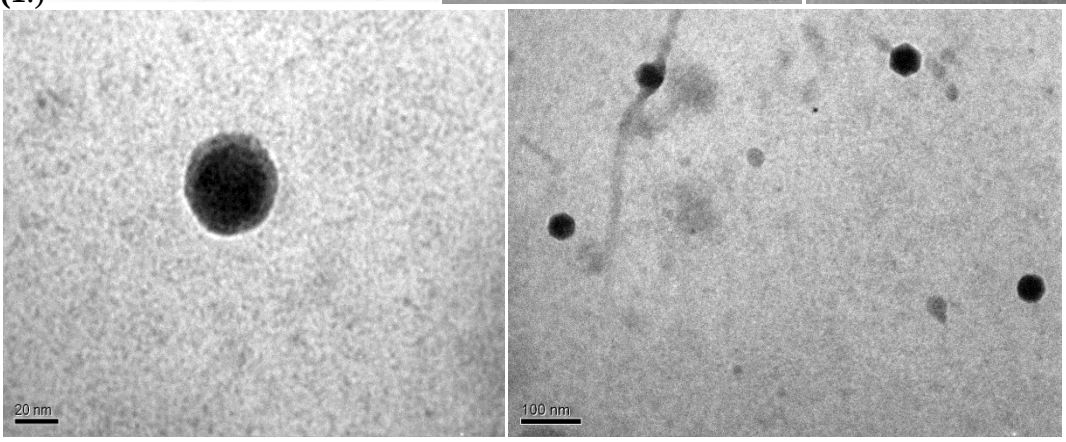
Volume of samples examined was of 2.0 ml, in which there was 1,0 ml of liquid cultures of microalgae and 1.0 ml – the volume of virus suspension (in experimental ampoules, experiment) or 1,0 ml of sterile marine water (in control ampoules, control).

The microalgae used in our research were *Tetraselmis viridis* (Rouchijajnen) R.E. Norris, Hori &Chihara, 1980 and *Phaeodactylum tricornutum* Bohlin, 1897. Liquid cultures of the microalgae were obtained from the Department of Ecological Physiology of Algae (Institute of Biology of the Southern Seas, Sevastopol).

Among algal viruses were used strains of *Tetraselmis viridis* Virus (TvV) and *Phaeodactylum tricornutum* Virus (PtV) from authors collection of algal viruses [Stepanova, 2016]. Electron microscopic photos of the strains of these viruses are shown in Fig. 1.



(1.)



(2.)

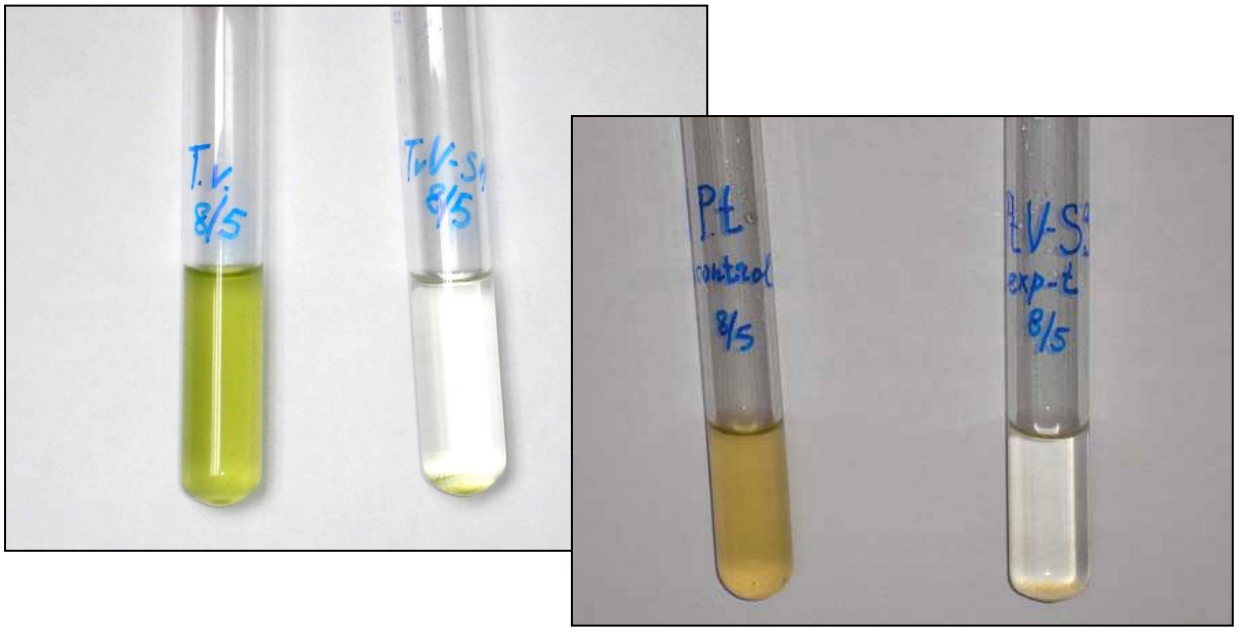
Figure 1. Electron microscopic photos (images) of virions of the Black Sea algal viruses in the upper row (1) and in the lower row (2):

1. Strains of the algal virus of *Tetraselmis viridis* (TvV), in the first picture on the left there is an instrumental magnification x120,000, in the other upper figures (pictures) the scale is 200 and 500 nm, respectively.

2. Strains of the algal virus of *Phaeodactylum tricornutum* (PtV), scale in the first figure on the left – 20 nm, in the figure on the right – 100 nm.

Results and Discussion

Upon the contact of virus and sensitive culture of microalgae with using our author's method, which was described in [Stepanova et al., 2021], the visual differences in the experiment (infected algae culture) compared to control (culture growth) usually appear within 24 h or more (**Fig. 2**).



A Control Experiment

B Control Experiment

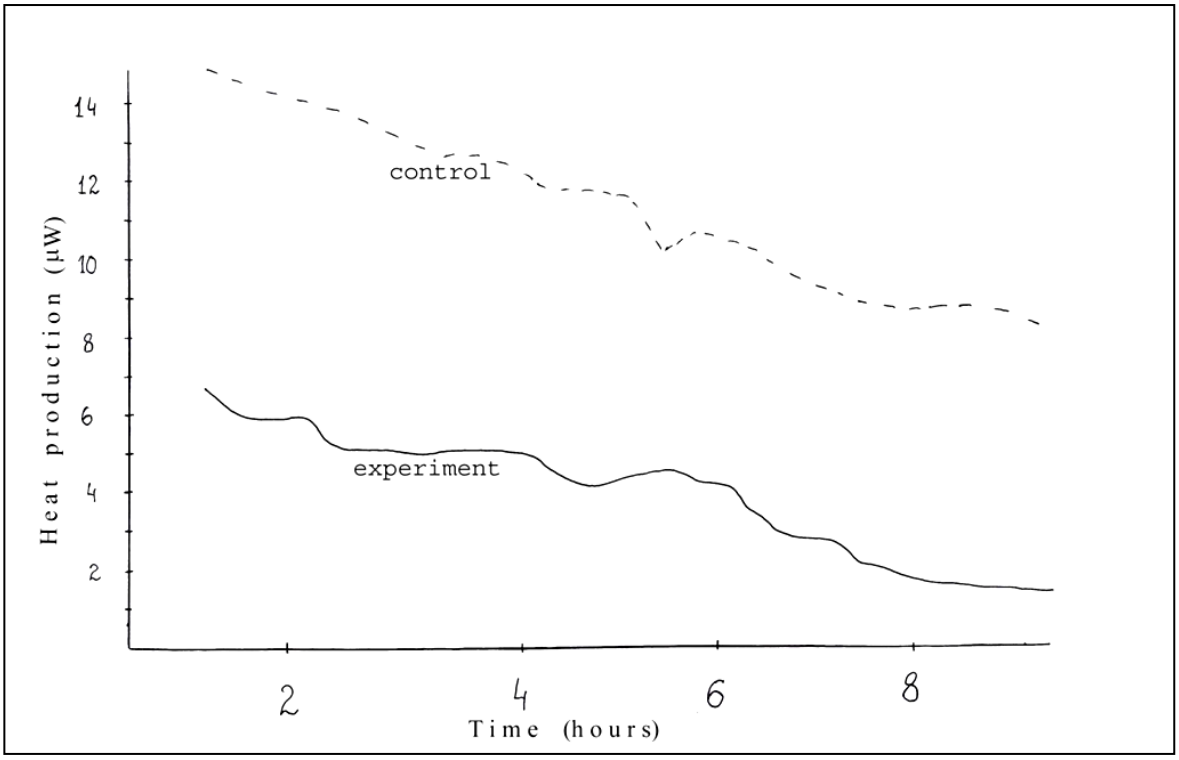
Figure 2. Visual differences between virus-inoculated and non-inoculated (control) microalgae cultures. A – control and TvV-inoculated *Tetraselmis viridis* culture. B – PtV-inoculated and control *Phaeodactylum tricornutum* culture

At the same period of time there is a difference in heat production in method of microcalorimetry. In **Fig. 3** a difference in heat production can be seen, that reflected the growth and development of algae culture in the control compared with infected culture in experiment.

Lighting is one of the most important factors in the study of the interaction between algal viruses and their hosts - microalgae, as well as in the study of heat production of their contact. Algae cultures need light for their growth that of course is reflected in their production of viruses. However, there is no lighting in using method with BAM. It is not possible to observe a natural change in the dynamics of heat production in contact between virus and the host throughout the experiment, and especially during the first hours. This problem was solved by using several ampoules for each of the samples (control and experiment) which were located exposed to light. Then they were changed every 4-5, 8, 24 h and over longer periods (up to 14-15 days) at BAM.

These conditions created by us gave the opportunity for the control and for the experience to get the same (identical) sufficient amount of illumination. Using this mode we got more information about heat production in contact dynamics of the virus and microalgae, which is reflected in **Table 1**.

A.



B.

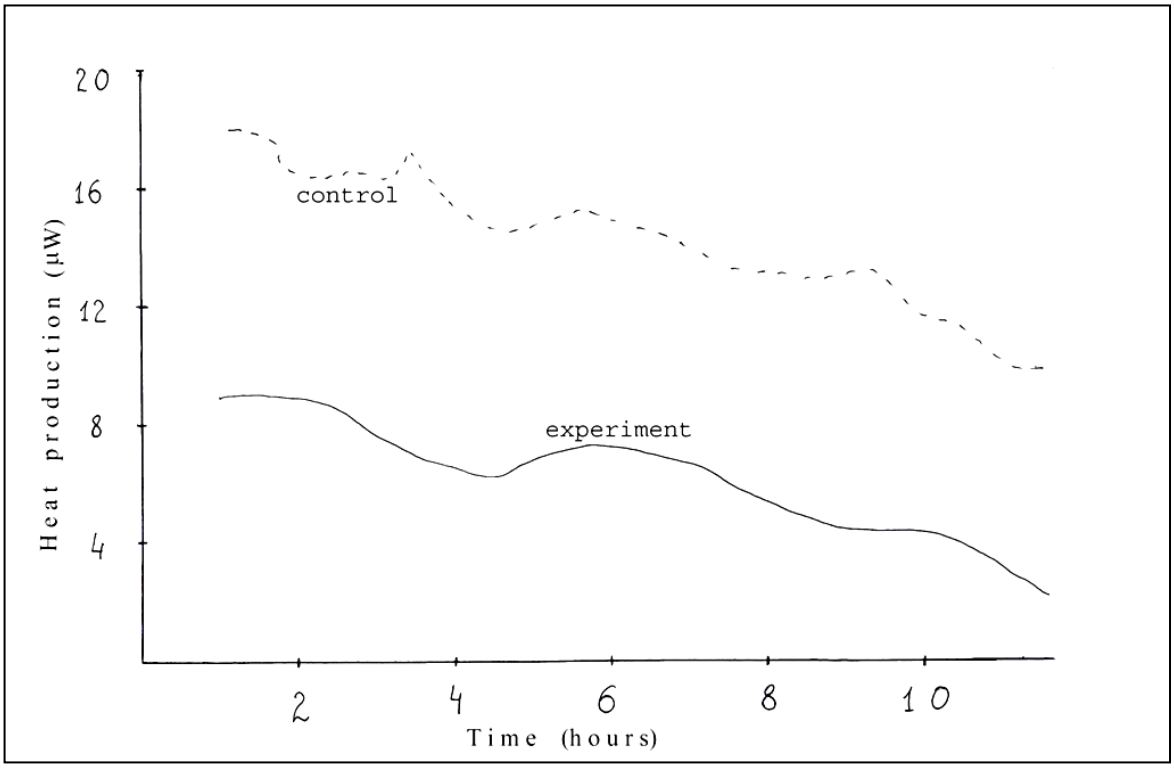


Figure 3. Heat production in virus-inoculated (experiment) and non-inoculated (control) microalgae cultures in the period of visual differences between experimental and control samples. A - TvV-inoculated of *T.viridis* culture and control *T.viridis* culture. B – control of *P.tricornutum* culture and PtV-inoculated *P.tricornutum* culture

Table 1

Heat production of *T.viridis* algae in contact with virus (experiment) and in control samples

Samples	Production of heat in different time periods (in h) after virus-algae contact, μW				
	1 - 4 h	4 – 6 h	8 -10 h	24 h	14 - 15 days
experimental	4 - 4,2	3 - 4	2,5 - 3	1 - 2	1 - 0,5
control	4,5 - 5	5 - 6	7 - 7,5	7 - 7,5	11 - 6,5

It has been revealed that the decrease of heat production is observed in the first hours of contact between viruses and algae in selected by us conditions (in light), and after 10-24 h the differences in heat production were the greatest.

The results of the study by microcalorimetry of the contact between algal viruses and their hosts (algae) were made by us for the first time and are likely to be interesting and useful for future researches in various fields - Algology, Hydrobiology, Microbiology, Virology, and in others, and in particular to study the effect of various biotic and abiotic origin factors on the contact between viruses with their hosts.

We think and hope that this information will be especially interesting for medicine - when studying the contact of pathogenic viruses with mammalian cell cultures. And this will be especially interesting and may be usefully in pandemic period of Covid-19.

Conclusion

This study, and consideration of the previously described results on the study of contact of viruses and single-celled viral hosts [Степанова, 2004] led to the following **conclusions** –

1. Heat production of algae cultures growth will depend on such factors as the species of algae, their concentration and the growth stage (phase) of culture, nutrient content and lighting, and other factors of biotic and abiotic origin. Viral infection, in our opinion, is one of the most significant factors affecting the viability of algae and therefore on the amount of heat production of algae cultures infected with the virus.
2. Interaction between algal viruses and sensitive algae culture leads to the production of virus and destruction (lysis) of host cells that occurs in reducing heat production in the experiment as compared with the control.
3. The contact between the algal viruses with their hosts was accompanied by a decrease of heat production in the first hours of their interaction, but the best difference was observed after 10-24 h. During the later date (some days) the start of bacterial infection of unicellular algae can be manifested in elevation of heat production.

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