

Study of contact of algal viruses and their hosts using the instrument Tox Y-PAM (Pulse amplitude modulation) (Walz, Germany) and measuring the dynamics of variable fluorescence

Stepanova Olga A.

Institute of Natural and Technical Systems, RF, Sevastopol, Lenin St., 28

solar-ua@ya.ru

Abstract

In these experiments were used the instrument Tox Y-PAM (Pulse amplitude modulation) (Walz, Germany) for study of contact between the algal virus (virus of *Tetraselmis viridis* - TvV), as viral suspension with infective titer 10⁸ IE/ml, and *Tetraselmis viridis* algae cultures, isolated from marine environment of Black Sea and White Sea. Algae cultures were in logarithmic phase of growth and development. Contact of algae cultures with TvV showed a reduction in the efficiency of the primary processes of photosynthesis, Fv / Fm. The higher sensitivity of the photosynthetic device to viral oppressing action was marked in infected *Tetraselmis viridis* algae of White Sea in comparison with algae of Black Sea.

Using of this device (instrument Tox Y-PAM) will allow to receive new knowledge about the ecology of algae and phytoplankton, about the role of a virus infection in such phenomena, as processes of photosynthesis and fluorescence, and also in interaction between the virus and host cells in an example of contact of algal virus and sensitive algae culture.

Key words: algal virus, microalgae *Tetraselmis viridis*, Black Sea, White Sea, instrument Tox Y-PAM (Pulse amplitude modulation) (Walz, Germany), photosynthesis and fluorescence.

Introduction

The young new science – marine (aquatic) virology – poses many problems for scientists, among which can be noted the task of study the contact of virus and its unicellular host-hydrobiont [Aquatic virus..., 2008; Ecology of..., 2003; JMBA, 2006].

Because methods of study of interaction between viruses and its unicellular hosts are always actual problem and will be claimed, the **purpose** of these

researches were devoted to receiving, discovering, fixing and interpretation the results of study of interaction of *Tetraselmis viridis* algae culture and its virus - TvV (strain TvV-S1), with using the instrument Tox Y-PAM (Pulse amplitude modulation) (Walz, Germany).

Measuring the relations of chlorophyll fluorescence intensity at saturating of photosynthesis exciting light (F_m) and conditions that do not cause changes in the state of the photosynthetic apparatus (F_o), allows to determine the efficiency of the primary processes of photosynthesis, which is equal to $(F_m - F_o) / F_m = F_v / F_m$ [Маторин и др., 2006].

Materials and Methods

The study of contacts algal viruses (viral suspension) with their unicellular hosts (cultures of microalgae) and measuring the variable fluorescence in dynamics of these contacts were carried out with using the device Tox Y-PAM (Pulse amplitude modulation) (Walz, Germany).

In these studies were used the strain of algae virus of microalgae *Tetraselmis viridis* –TvV-S1 (viral suspension with viral infective titer 10^8 IE/ml), which was isolated from samples of Black Sea [Stepanova, 2016; Степанова и др., 2005], and *Tetraselmis viridis* algae culture of the Black Sea (Institute of biology of southern seas, Sevastopol) and of the White Sea (Russia, Moscow State University), which are saved in collections of strains of the museums of those institutions. Algae cultures were used in these experiments in the logarithmic phase of their growth and development.

The contact of cultures of algae *Tetraselmis viridis* from Black Sea (BS) and White Sea (WS) with algal virus TvV-S1 was studied by the appropriate method as described in [Маторин и др., 2006]. The first information about this study was presented in 2007 [Stepanova et al., 2007].

Results and Discussion

The efficiency of the primary processes of photosynthesis (F_v / F_m) is a dimensionless energy characteristic of photosynthesis, which analogous (similar) to coefficient of performance and is independent on the species specificity of the organism.

The intensity of F_o with a high correlation coefficient corresponds to the total content of the pigments of the photosynthetic apparatus of phytoplankton (or

microalgae culture) that collects energy of light and therefore also is correlated with the abundance of phytoplankton (number of algal cells).

The measurement of variable fluorescence ($F_v = F_m - F_o$) reflects activity of the reactionary centers of photosystem 2, that is responsible for decomposition of H_2O and allocation of O_2 [Маторин и др., 2006].

It is logical to assume that change of parameter of fluorescence will be observed in infected algae culture that was caused by decrease of activity of cells as a result of viral lysis in comparison with control of noninfected culture.

This assumption was checked up in our experiment. Contact of algae cultures from Black Sea (BS) and from White Sea (WS) with algal virus TvV-S1 showed a reduction in the efficiency of the primary processes of photosynthesis, F_v / F_m (Fig.1).

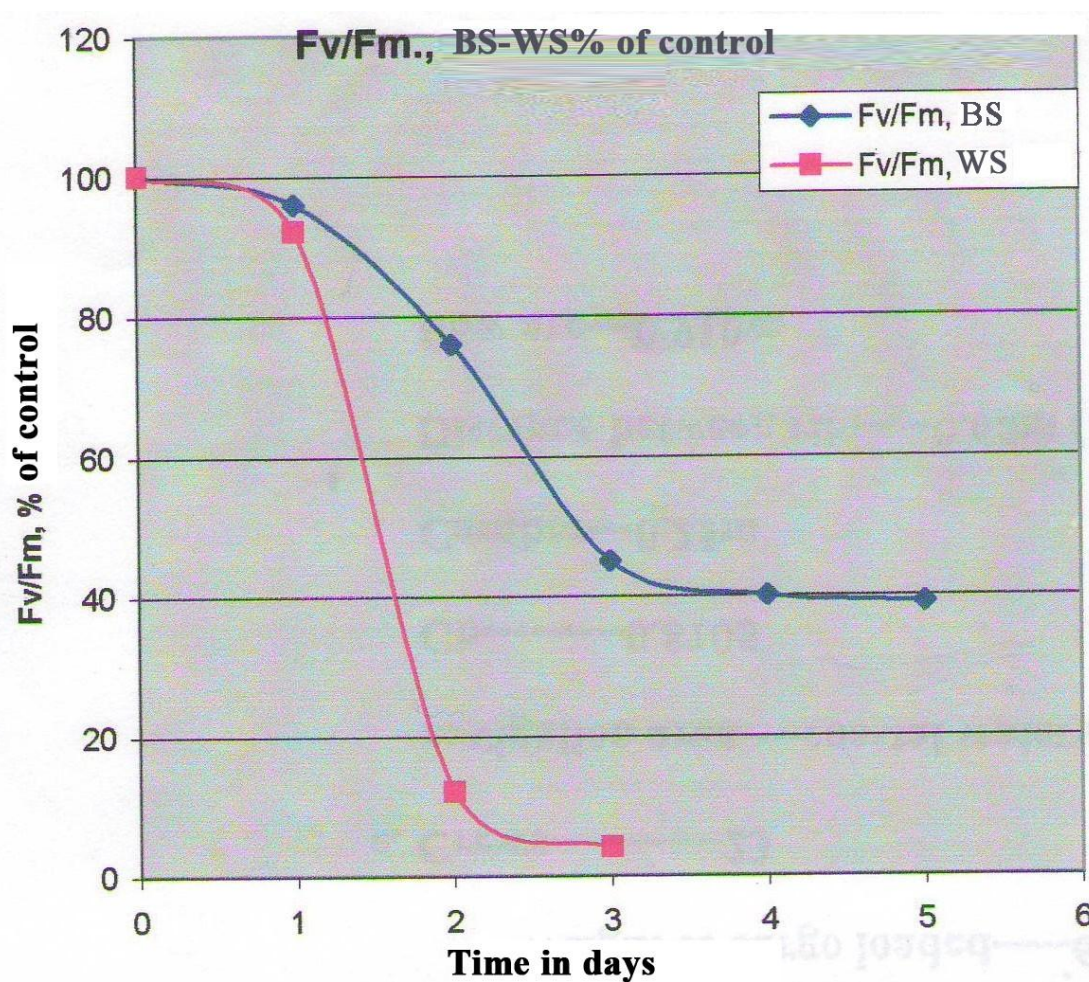


Fig.1. Efficiency of the primary processes of photosynthesis (F_v / F_m , % of control) of microalgae *Tetraselmis viridis* from Black Sea (BS) and White Sea (WS), infected by algal virus TvV-S1

It was also determined that the variable fluorescence (Fv) of infected by virus the algae *Tetraselmis viridis* from Black Sea to 4 days remains active, albeit slowly loses it. But infected by virus algae culture from White Sea begins to rapidly lose activity in the first day. On the second day there is a sharp drop in the curve of the variable fluorescence of infected culture from White Sea than in infected culture from Black Sea (Fig. 2).

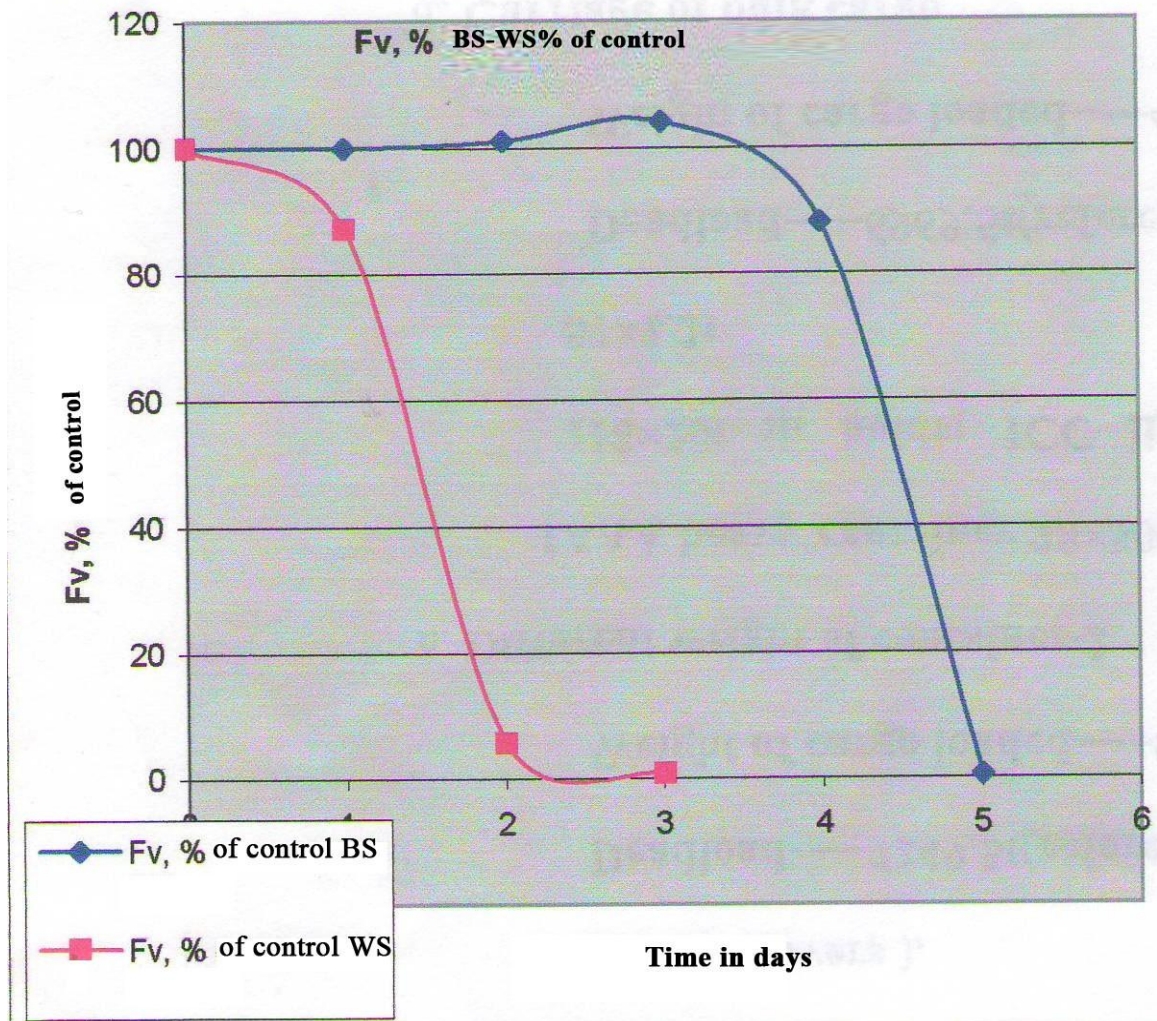


Fig.2. Variation in time of variable fluorescence (Fv, % of control) of infected by algal virus TvV-S1 *Tetraselmis viridis* algae from Black Sea (BS) and White Sea (WS)

Thus, the reduction in efficiency of the primary processes of photosynthesis (Fv / Fm), and the decreased levels of variable fluorescence in infected by algal virus algae cultures were registered. The higher sensitivity of the photosynthetic

device to viral oppressing action was marked in infected *Tetraselmis viridis* algae of White Sea in comparison with algae of Black Sea. Fact of contact of the Black Sea algal virus TvV-S1 with *Tetraselmis viridis* algae culture of White Sea was described for the first time in 2007 [Stepanova et al., 2007] and it represents the certain theoretical novelty.

Conclusion

In our experiments were used the instrument Tox Y-PAM (Pulse amplitude modulation) (Walz, Germany) for study of contact between the algal virus (virus of *Tetraselmis viridis* - TvV), as viral suspension with infective titer 10⁸ IE/ml, and *Tetraselmis viridis* algae cultures, isolated from marine environment of Black Sea and White Sea. Algae cultures were in logarithmic phase of growth and development. Contact of algae cultures with viral strain (TvV-S1) showed a reduction in the efficiency of the primary processes of photosynthesis, Fv / Fm. The higher sensitivity of the photosynthetic device to viral oppressing action was marked in infected *Tetraselmis viridis* algae of White Sea in comparison with algae of Black Sea.

In our opinion, using of this method and the instrument Tox Y-PAM for study of interaction of the virus and algae will allow to receive new knowledge about the ecology of algae and phytoplankton, about the role of a virus infection in such phenomena, as processes of photosynthesis and fluorescence, and also in interaction between the virus and host cells in an example of contact of algal virus and sensitive algae culture.

References

1. Aquatic virus workshop (July 6-11, 2008, The University of British Columbia Vancouver, B.C., Canada). Vancouver, 2008. 65 p.
2. Ecology of marine viruses (Banyuls-sur-mer, 19-22 March 2003). Monaco, 2003. 94 p. (CIESM Workshop Monographs N21).
3. JMBA (Journal of the Marine Biological Association of the United Kingdom). Aquatic Viruses. Cambridge University press. 2006. 86. P.449-451.
4. Stepanova O.A. Black Sea algal viruses // Russian Journal of Marine Biology, 2016, Vol. 42, No. 2, pp. 123–127.
5. Stepanova O.A., Osipov V.A., Matorin D.M., 2007. Use of a method of fluorescence at study of process of interaction between algae virus and sensitive

algae culture. Abstracts V Intern. conf. "Bioresources and Viruses" September 10-13, 2007 (Kyiv, Ukraine). Kyiv: Phitosociocenter, 59.

6. Маторин Д.Н., Погосян С.И., Осипов В.А., Хаптер Р., Рубин А.Б., 2006. Исследование состояния фотосинтетического аппарата фитопланктона Балтийского моря флуоресцентными методами. Вестник Московского Университета (Сер.16, Биология), 1, 61-66.

7. Степанова О.А., Бойко А.Л., Гордиенко А.И., Щербань С.А., Шевченко Т.П., Полищук В.П. Характеристика вируса *Tetraselmis viridis* Norris (Chlorophyta, Prasinophyceae) // Доп. НАН України. 2005. №1. С.158-162.