This is the fist our attempt to isolate phytopathogenic bacteria from diseased plants together with their specific bacteriophages. Newly isolated bacteria are probably members of family Xanthomonadaceae that include common pathogenes of tomatoes and peppers according to data of other scientists [10]. Our results are speculative, but we can found a lot of viruses to potentially phytopathogenic microorganisms, isolated from rotten tomatoes and sweet papers. In future researches we intend to identify microorganisms to species and pathovars and confirm their influence on plants *in vitro* and *in vivo*.

Readable results were observed during second trials, phages from 2 samples formed plagues on 10 phytopathogenic microorganisms from different regions, it means that isolated viruses are probably polyvalent or their hosts are relatives and have the same receptors. We plan to investigate all isolated phages in details after identification of target microorganisms.

Noteworthy is also the fact of insensitivity of laboratory bacterial strains to newly isolated bacteriophages. These results may be explained in two ways. The first explanation is the absence of bacterial strain related to laboratory strains in samples that were collected. According to second suggestion laboratory strains have lost susceptibility to mostly bacteriophages due to the numerous passages.

Conclusions. In this survey 22 isolates of bacteria were plated from infected tomato and sweet pepper. Identification of these bacteria is in progress. Bacteriophages, specific to the pathogenic microorganisms, were isolated, accumulated and examined by the method of electron microscopy. Three distinct groups of bacteriophages, based on their virion morphology, were identified.

References

1. Атабеков И.Г. Практикум по общей вирусологии. – М.: Издательство Московского университета. – 1981. – 192 с.

2. Cappuccino J. G., Sherman N. Microbiology: a laboratory manual. 4-th edition. – Menlo-park, CA: The Benjamin-Cumming Publishing Company Inc. – 1990. – 186 p.

3. Carlson K., Kutter E., Sulakvelidze A. Bacteriophages: biology and applications. Appendix: Working with bacteriophages: Common techniques and methodological appproaches. – Boca Raton: CRC Press. – 2005. – PP. 437-494.

4. Clockie M.R.J., Millard A.D., Letarov A.V., at all. Phages in nature. // Bacteriophage. – 2011. – Vol.1. – № 1. – P. 31-45.

5. Гвоздяк Р.І., Пасічник Л.А., Яковлева Л.М. та ін. Фітопатогенні бактерії. Т 1. Бактеріальні хвороби рослин. – К.: ТОВ "НВП Інтерсервіс". – 2011. – 444 с.

6. Ho K. Bacteriophage therapy for bacterial infections. Rekindling a memory from the pre-antibiotics era. // Perspect. Biol. Med. – 2001. – Vol. 44. – P. 1–16.

7. Jarvis W.R. Preventing the emergence of multidrug-resistant microorganisms thought antimicrobial use controls: the complexity of the problem. // Inf. Con. And Hosp. Epidem. – 1996. – Vol. 17. – № 8. – P. 10-15.

8. Jones J.B., Iriarte F.B., Obradovic A., at all. Management of bacterial spot on tomatoes with bacteriophages. // Proc. Int. Symp. Biol. Control Bact. Plant Dis., Land-Forstwirtsch: Mitt. Biol. Bundesanst. – 2006. – № 408. – P. 154.

9. Jones J.B., Jackson L.E., Balogh B., at all. Bacteriophages for plant disease control. // Annu. Rev. Phytopathol. – 2007. – Vol. 45. – P. 245-262.

10. Jones J.B., Lacy G.H., Bouzar. H., at all. Reclassification of the Xanthomonads associated with bacterial spot disease of tomato and paper // Systematic and Applied Microbiology. – 2004. – Vol. 27. – P. 755-762.

11. Khachatourians G.G. Agricultural use antibiotics and the evolution and transfer of antibiotic-resistant bacteria. // CMAJ – 1998. – vol. 159. – № 9. – P. 1119-1120.

12. Loper J.E., Henkels M.D., Roberts R.G., at all. Evaluation of streptomycin, oxytetracycline and copper resistance of Erwinia amylovora isolated from pear orchards in Washington State. // Plant Dis. – 1991. – Vol. 75. – P. 287-290.

13. Mallmann W.L., Hemstreet C.J. Isolation of an inhibitory substance from plants. // Agric. Res. – 1924. – P. 599–602.

14. Миронов А. А., Комиссарчик Я. Ю., Миронов В.А. Методы электронной микроскопии в биологии и медицине: Методологическое руководство. – СПб.: Наука, 1994. – 400 с.

15. Остерман Л.А. Методы исследования белков и нуклеиновых кислот: электрофорез и ультрацентрифугирование. – М.: Наука, 1981. – 288 с.

16. Tanaka H., Negishi H., Maeda H. Control of tobacco bacterial wilt by an avirulent strain of Pseudomonas solanacearum M4S and its bacteriophage. // Ann. Phytopathol. Soc. Jpn. – 1990. – Vol. 56. – P. 243–246.

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У ПОШУКАХ ВБИВЦЬ: БАКТЕРІОФАГИ ПРОТИ ФІТОПАТОГЕННИХ МІКРООРГАНІЗМІВ

Зразки перців і томатів із симптомами бактеріальної гнилі були відібрані у різних регіонах України. Фітопатогенні мікроорганізми – збудники хвороб рослин були виділені із овочів і введені в культуру для подальшого визначення до штамів. Присутність вірусів до виділених мікроорганізмів в отриманих зразках було підтверджено за допомогою методу агарових шарів та методу електронної мікроскопії.

Ключові слова:фітопатогенні мікроорганізми, бактеріофаги.

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В ПОИСКАХ УБИЙЦ: БАКТЕРИОФАГИ ПРОТИВ ФИТОПАТОГЕННЫХ МИКРООРГАНИЗМОВ

Образцы перцев и томатов с симптомами бактериальной гнили были отобраны в разных регионах Украины. Фитопатогенные микроорганизмы – возбудители болезней растений были выделены из овощей и введены в культуру для дальнейшего определения штаммов. Присутствие вирусов к выделенным микроорганизмам в полученных образцах были подтверждены с помощью метода агаровых слоев и метода электронной микроскопии.

Ключевые слова: фитопатогенные микроорганизмы, бактериофаги.

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ADAPTATION OF BIOTESTING METHOD FOR DETECTION OF *PBCV-1* IN WATER SAMPLES

Here we report on the adaptation of biotesting method for detection of algae viruses in samples of water and bottom sediments. We have shown that the modification involving the use of two media layers of different density with application of samples previously enriched in aeration box proved to be most efficient. Here we also describe that the water samples collected from technical water reservoirs of the National Exhibition Center of Ukraine demonstrate lytic activity towards test culture of symbiotic algae Chlorella sp. (ACKU 95-02). The stage of initial accumulation of a virus has been carried out.

Key words: algae viruses, Chlorella sp (ACKU 95-02), PBCV-1.

Introduction. Viruses are typical for any water system. These organisms are vastly abundant; their content may reach over 10 millions of particles per milliliter of water [1, 2]. The viruses are thought to influence great part of genetic and species biodiversity in seas and oceans [3]. Despite wide range of virus species found in water reservoirs,

only a small part of these (118 virus species) may directly infect algae [4]. This phenomenon is uncommon, especially in the light of strict virus-host specificity and wide diversity of algae – potent hosts for viruses. Only *Chlorophyta* division contains approximately 20-25 thousand species of algae typical for fresh-water and hyperhalic reservoirs, seas, oceans and terrestrial biotopes [5].

Research on ecology of algae viruses has scientific, fundamental and applied value. Lack of food resources and energy sources is a major social issue of modern living, and microalgae represent one of the few practical solutions for this challenge. They are considered as a potent source of nutrients and biofuel. In turn, algae viruses cause raw material waste and consequential significant financial losses. It is also known that lysis of about 25% of water (phytoplankton) bloom is attributed to virus-induced lysis [6,7]. Such viruses are thought of as candidates for biological control of water blooms. On the other hand, algae viruses (whether directly or indirectly) are connected with global warming due to the generation of dimethyl sulfoxide (DMSO) when the algae are lysed. This gas is a major biogenic source of sulfur. Generation of dimethyl sulfoxide leads to progressive accumulation of greenhouse gases [8, 9].

We also know that some algae viruses are involved in horizontal gene transfer, contributing to genetic diversity of microalgae associations [10, 11].

Nearly 60 years have passed since the first report of virus-like particles (VLPs) in the cells of microalgae [12]. However, research of algae viruses still remains at the beginning descriptive stage. This may be explained by the difficulties in virus isolation from the environment using traditional methods of virology. Many algae viruses may be detected by modern molecular biological methods but still lack information on susceptible algae cultures, preventing virus isolation and accumulation [13].

Ambiguous results of interaction of a virus with susceptible cell pose another issue of algae virus research. High concentration of algae culture and small amount of virus typically lead to nearly complete lysis of susceptible test culture. In turn, small numbers of algae cultures in the media coupled with specific conditions may lead to cytoproliferative effect. In addition, routine cultivation of a test culture of microalgae needs certain skills, time-consuming and costly.

In spite of said above we turned to adaptation of biotesting method for detection of PBCV-1 in samples from fresh water reservoirs.

Materials and methods. The samples were collected following general recommendations pursuing virus preservation in a sample and preventing sample contamination with secondary microflora. We have used different methods when sampling water for detection of algae viruses: washing out mantle cavity of bivalved mollusks, sampling bottom sediments, and direct sampling of water from reservoir. Further, collected and prepared samples were kept in a fridge at +4^{o}C. For initial virus detection we have collected 74 water samples plus 32 samples of soil and washouts/swabs from the foulings of water-immersed objects.

Samples of unicellular fresh-water green algae *Chlorella sp.*(ACKU 218-03), *Chlorella sp.* (ACKU 531-02), *Chlorella sp.* (ACKU 532-03) and symbiotic culture of *Chlorella sp.* (ACKU 95-02) were used as test objects. These cultures were kindly provided by the Department of Botany, Taras Shevchenko' National University of Kyiv

Different modifications of biotesting were used for virus detection in the collected samples. PBCV-1 was used as a positive control.

Results and Discussion. Biotesting is a classical approach in virology. The method comes to virus detection using test objects. Alterations of vital functions of a test object (or its death) serve as a signal of virus presence. Biotesting approach is widely used due to its simplicity, quickness and availability. Biotesting is a historically proven technique in virology and forms a part of Koch's triad – necessary element for confirmation of the infectious nature of any pathogen.

However, biotesting approach has several limitations when used for algae viruses. Firstly, many detected algae viruses still have no established susceptible microalgae cultures. Secondly, virus interaction with sensitive cells does not always follow cytopathic pathway. Cytoproliferative effect is also possible. Thirdly, the algae demonstrate varying degree of susceptibility to virus infection at different stages of their life cycle. We should also point that microalgae are rather problematic to cultivate, they often require specific media and regimes of light and temperature. However it is impossible to refuse using this method totally, and therefore there's a need for its modification and selecting optimal conditions for each test culture and the virus.

In the course of this work we have tried several biotesting techniques: 1) biotesting in liquid media; 2) biotesting on solid media; 3) biotesting on solid media using two media layers of different density. Every technique was tried with both native and synchronized test cultures (Fig.1). We have also used water samples filtered via bacterial filter (Millex GV, 0,22) and enriched filtered samples previously enriched in a special aeration box.

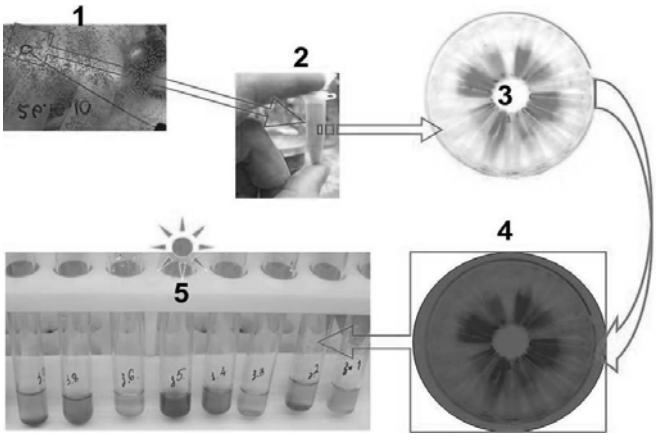

Fig.1. Scheme for synchronization of microalgae test culture:
1-10-14-day lawn of test cultures; 2,3 – cultures resuspended in 1,5 ml of liquid media; 4 – cultures kept in the dark, 12-hour incubation; 5 – 2,5 ml of liquid media is added to the culture which is further cultivated at normal regime of light

The modification of biotesting method involving the use of two media layers of different density with application of water samples previously enriched in aeration box proved to be most efficient in our work.

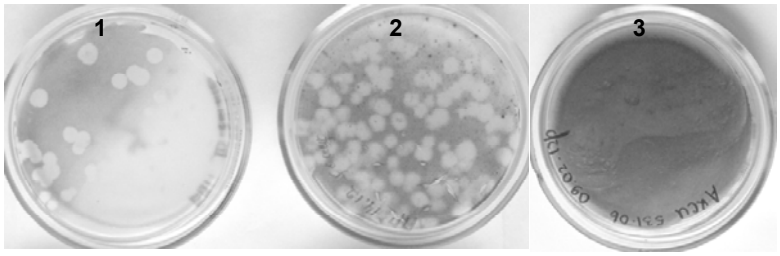

Fig.2. Results of biotesting in the modification involving the use of two media layers of different density with application of water samples previously enriched in aeration box:
1 – positive control контроль (PBCV-1); 2 – experimental samples; 3 – control of test culture

Conclusion. We have demonstrated that water samples collected from technical water reservoirs of the National Exhibition Center of Ukraine (50.370651; 30.474002) showed lytic activity towards symbiotic algae *Chlorella sp.* (ACKU 95-02) and induced lysis zones of approximately 1 cm in diameter (Fig.2). We have also carried out the stage of initial accumulation of the virus. The modification of biotesting method involving the use of two media layers and water samples previously enriched proved to be most efficient. Other modifications were less efficient or provided no results at all. However we need to mention that chosen modification is more time-consuming and requires specialized equipment.

References

1. Weinbauer, M., Rassoulzadegan, F., 2004. Are viruses driving microbial diversifica-tion and diversity? Environ. Microbiol. 6, 1–11.

2. Sobecky, P.A., Hazen, T.H., 2009. Horizontal gene transfer and mobile genetic ele-ments in marine systems. Methods Mol. Biol. 532, 435–453.

3. Suttle, C.A., 2005. Viruses in the sea. Nature 437, 356–361.

4. Virus Taxonomy. Classification and Nomenclature of Viruses Ninth Report of the International Committee on Taxonomy of Viruses Editors, Andrew M.Q. King, Michael J. Adams, Eric B. Carstens, and Elliot J. Lefkowitz.

5. Костіков І.Ю., Джаган В.В. та ін. Ботаніка. Водорості та гриби.

6. Keizo Nagasaki, Yuji Tomaru, Noriaki Katanozaka, Yoko Shirai, Kensho Nishida, Shigeru Itakura, and Mineo Yamaguchi. Isolation and Characterization of a Novel Single-Stranded RNA Virus Infecting the Bloom-Forming Diatom Rhizosolenia setigera // Applied and Environmental Microbiology, February 2004, p. 704-711, Vol. 70, No. 2.

7. Castdery T. Algal viruses: Characteristics and ecological effects. – PY: Dep.of Micr.,University of Bergen, 2001. – 204 p.

8. Hill, R.W.,White, B.A., Cottrell,M.T.,Dacey, J.W.H., 1998.Virus-mediatedtotal release of dimethylsulfoniopropionate frommarine phytoplankton: a potential climate process. Aquat. Microb. Ecol. 14, 1–6.

9 Wilson, W.H., Turner, S., Mann, N.H., 1998. Population dynamics of phytoplankton and viruses in a phosphate-limited mesocosm and their effect on DMSP and DMS production. Estuar. Coast Shelf Sci. 46, 49–59.

10. Sobecky, P.A., Hazen, T.H., 2009. Horizontal gene transfer and mobile genetic ele-ments in marine systems. Methods Mol. Biol. 532, 435–453.

11. Weinbauer, M., Rassoulzadegan, F., 2004. Are viruses driving microbial diversifica-tion and diversity? Environ. Microbiol. 6, 1–11.

12. Spencer, R., 1955. A marine bacteriophage. Nature 175, 690–691;

13. Suttle, C.A., Fuhrman, J.A., 2010. Enumeration of virus particles in aquatic or sed-iment samples by epifluorescence microscopy. In: Wilhelm, S.W., Weinbauer, M.G., Suttle, C.A. (Eds.), Manual of Aquatic Viral Ecology. American Society of Limnology and Oceanography, Waco, TX, USA, pp. 145–153.

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АДАПТАЦІЯ МЕТОДИКИ БІОТЕСТУВАННЯ ДЛЯ ВИЯВЛЕННЯ *PBCV-1* У ПРОБАХ ВОДИ

Проведено адаптацію метода біотестування для детекції альговірусів у пробах води та придонних осадах. Встановлено, що найефективнішою є модифікація методу біотестування з використанням двох шарів середовища різної щільності та додаванням попередньо збагачених в аераційній камері проб. Показано здатність зразків води відібраних з технічних озер Національного виставкового центру України лізувати тест культуру симбіотичної водрості Chlorella sp (ACKU 95-02). Проведено первинне накопичення вірусу.

Ключові слова: альговіруси, симбіотична водорость Chlorella sp (ACKU 95-02), PBCV-1.

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Проведено адаптацию метода биотестирования для детекции альговирусов в пробах воды и придонных осадках. Установлено, что наиболее эффективной является модификация метода биотестирования с использованием двух слоев среды различной плотности и добавлением предварительно обогащенных в аэрационной камере проб. Показана способность образцов воды отобранных из технических озер Национального выставочного центра Украины лизировать тест культуру симбиотической водрости Chlorella sp (ACKU 95-02). Проведено первичное накопление вируса.

Ключевые слова: альговирусы, симбиотическая водоросль Chlorella sp (ACKU 95-02), PBCV-1.