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BACTERIOPHAGES FROM SAMPLES OF *PULSATILLA PRATENSIS* PLANTS OF NATURAL FLORA OF UKRAINE

We segregated the bacteriophages with long tails, which have different litic activity, size and consist of proteins from Pulsatilla pratensis plants. These plants were selected in the Kaniv Nature Reserve.

Key words: bacteriophages, natural flora, Pulsatilla pratensis.

Introdaction. Bacteriophages are the most widespread in the environments with high density of active metabolically bacteria [1]. In phytocenosis the reprodaction of a set of bacteria which significantly influence a physiological condition of plants is supported and can show phytopathogenic properties. In this regard studying of the interconnected processes of ecology of bacteria and their phages is an actual task. Despite a large number of the works dedicated to segregation and identification of phages, the processes associated with their ecology and evolution in the nature are studied deficiency [2]. Evolutionary approach allows to study them under the influence of environment where the titre of phages in an ecosystem, density of bacterial population and a physiological condition of microorganisms are important factors. Research of interaction of system of populations of phages and bacteria in the conditions of natural environment gives the chance to research the dynamics of interaction and development of populations under the influence of environment factors. It's allows a better understanding about bacteriophages. The relation of quantity of virus particles to quantity of bacteria in averages 10:1. Moreover, the phages are the most numerical organisms on Earth according to some data. Population of phages exceeds 10^{30} virus particles [3]. Transduktion is an improbable event [4]. But, with such number of phages, it happens quite often. The number of virus particles consists not only in their huge number, but also in a specific variety [5].

Material and methods. Sampling for analyses of features of segregation made from the rhizomes of plants of *P. pratensis* and soil around the roots during the period from June, 2011 to June, 2013. In researches used isolates of bacteriophages which are susceptible to cultures of bacteria of *Pseudomonas fluorescens* and *Seratia marcienses L-2*. After series of passages, pure phage clones with different phage plaque morphology were obtained for every isolate. Viruses accumulated on the cultures in nutrient broth with aeration at 25°C. C.In researches used lysates with concentration10^{9}-10^{10} plaque forming units per ml (PFU/ml). Titers were determined by agar-layer technique by Gratia [6].

For research of a range of lytic activity used pathogenic for plants cultures of bacteria: *Erwinia carotovora 216, Pseudomonas syringae pv. atrofaciens 1025, Pseudomonas viridiflava 8868, Pseudomonas fluorescens 8573* and *Seratia marcienses L-2*, the cultures were provided by the museum of phytopathogenic bacteria of the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

The protein composition of the isolated viruses was researched by electrophoresis by Laemmli [7]. Morphology of virus particles researched by means of electronic microscope (JEOL JEM – 1400).

Results and discussion. Phages from samples formed small clear negative colonies on *Seratia marcienses L-2* and colonies with an aureole on *Pseudomonas fluorescens* with diameter about 0,5-1 mm (fig.1).

Research of features of segregation of phages from rhizomes of plants and soil around the roots showed existence of variety of isolates of phages on the basis of lytic ability to used indicator bacteria. The received isolates were characterized by high titers of lytic activity. In total, 4 phage isolates were isolated and described. For determine of a range of hosts was conducted the research of lytic activity of phages on 5 strains of phytopathogenic bacteria. It is revealed that from four checked samples two (the sample №1 and №6) showed lytic activity to strains of different genus of phytopathogenic bacteria (*Pseudomonas* and *Seratia marcienses L-2*) while other two isolates was monovalent (samples №2, №3) – showed lytic activity only to one bacterial strain (tabl.1).

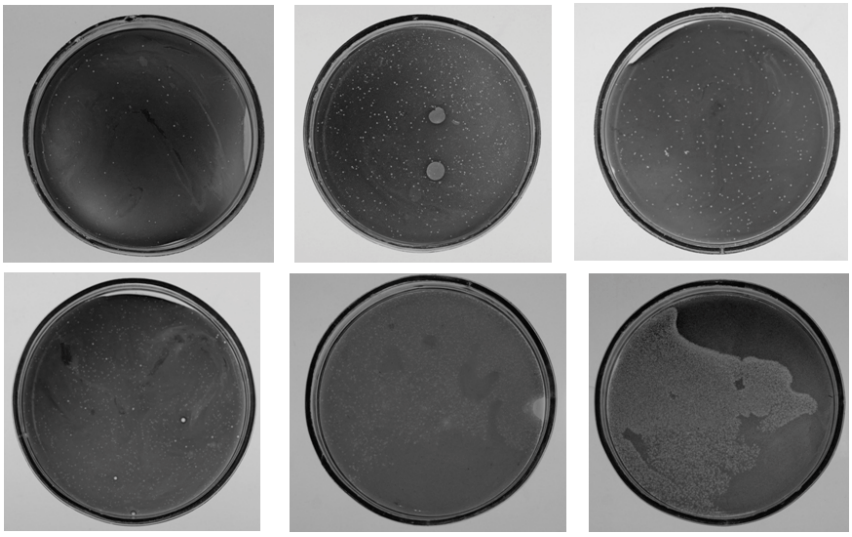

Fig.1. Negative colonies of phages on the culture:
Seratia marcienses L-2 – sample №1 (A), sample №2 (B), sample №3 (C), sample №6 (D)
and *Pseudomonas fluorescens* – sample №1 (E) and sample №6 (F)

Table 1. **Phage lytic activity to indicator bacteria**

Number of sample / Bacterial culture	№1	№2	№3	№6
Erwinia carotovora	-	-	-	-
Pseudomonas syringae pv. atrofaciens	-	-	-	-
Pseudomonas fluorescens	+	-	-	+
Pseudomonas viridiflava	-	-	-	-
Seratia marcienses L-2	+	+	+	+

Notes: "+" – negative colonias
"-" – colonias were absent

Virulent phages of samples №2 and №3 are narrowly specific. Phages of samples №1 and №6 show polyvalency reovercoming generic border, infecting 100 percent of representatives of *Pseudomonas fluorescens* and *Seratia marcienses L-2* respectively.

The nature of phage segregation allows to assume existence of the mechanisms providing preservation of populations of phages in biocenoses. The received results create a basis for studying of distribution of certain representatives of viruses of microorganisms in the nature, definition of their interrelations in biocenoses.

Analysis of the electron micrographs showed that the phages were different in structure and size of virions.

Among them the group of phages, with an icosahedral head and the long non-contractile tail, relates to family *Siphoviridae* and order *Caudovirales*. Phages have the sizes: diameter of a head – 97 ± 2 nm, length of a tail – 157 ± 3 nm, diameter of a head – 89 ± 2 nm, length of a tail – 154 ± 4 nm. Other isolate of a phage with an i icosahedral head without long tail relates to family *Podoviridae* and order *Caudovirales*. It had the size: diameter of a head – 37 ± 1 nm, length of a tail shoot – 137 ± 3 nm (fig.2).

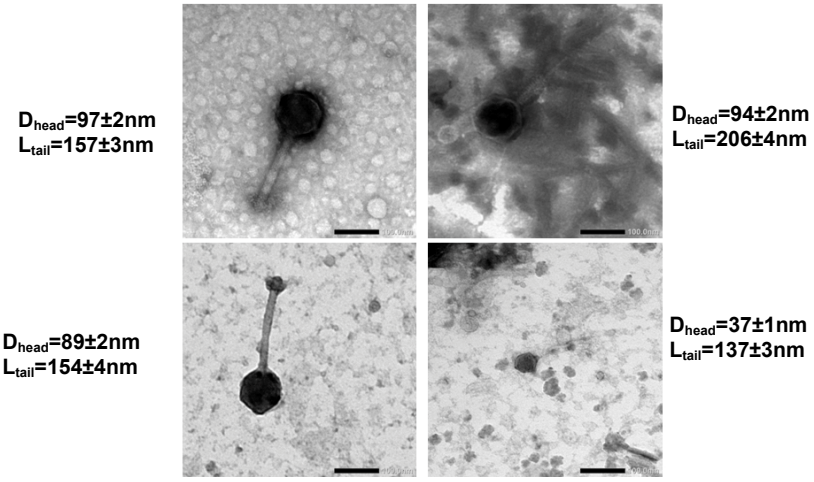

Fig.2. Electron micrographs of segregated phages. A – from sample №2, B – from sample №3, C-D – from sample №6

The protein composition of the isolated viruses was researched by electrophoresis by Laemmli. Research polypeptide structure of phages conducted for the purpose of detection of distinctive characteristics polypeptide structure. In work used preparations of the concentrated and cleared suspensions of phages. Analyzed allocation of samples on molecular masses. Compared on number and electrophoresis mobility the 4 isolates of phages: sample №1, №2, №3 and №6 – a susceptible bacteria of *Seratia marcienses L-2*, №21 and №26 – a susceptible bactera of *Pseudomonas fluorescens*. Them electron micrographes differed and had characteristic individual distinctions (tabl.2).

Table 2. **Molecular weight of phages polypeptides**

Ps. fluorescenns	Sample №6, kDa					66						31	27				16
	Sample №1, kDa						63			40	34						
S. marcienses L-2	Sample №6, kDa							60	47			31	26	24	19	18	
	Sample №3, kDa		167		104		63			39	33	31	28		19	18	
	Sample №2, kDa		167		104	66				40		31	27				
	Sample №1, kDa	190		123						40		31	28		19		

Thus, phages from samples №2 and №3 have high degree of similarity that allows to assume their general origin. Other samples of phages considerably differ on some polypeptides. Results of polypeptide structure can reflect a certain evolutionary process in population of phages. They had the general ancestor. Comparison of proteins of phages of different hosts shows a variety of molecular mass of their polypeptides. According to literary data a variety of structural proteins is characteristic for viruses of microorganisms into which structure can enter to several tens proteins.

Conclusions. Nature of allocation of phages allows to assume existence of the mechanisms providing preservation of populations of phages in biocenoses. The received results create a basis for studying of distribution of certain representatives of viruses of microorganisms in the nature, definition of their interrelations in biocenoses. Comparison of the allocated phages will allow to understand deeper an evolutionary way of viruses in natural ecosystems.

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БАКТЕРИОФАГИ ЗІ ЗРАЗКІВ РОСЛИН *PULSATILLA PRATENSIS* ПРИРОДНОЇ ФЛОРИ УКРАЇНИ

При дослідженні зразків з рослин Pulsatilla pratensis, відібраних на території Канівського природного заповідника, було виділено 6 бактеріофагів з довгими хвостовими відростками, що відрізнялися за розмірами, біологічною (літичною) активністю та білковим складом.
Ключові слова:бактеріофаги, флора України, Pulsatilla pratensis.

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БАКТЕРИОФАГИ ИЗ ОБРАЗЦОВ РАСТЕНИЙ *PULSATILLA PRATENSIS* ПРИРОДНОЙ ФЛОРЫ УКРАИНЫ

При исследовании образцов из растений Pulsatilla pratensis, отобранных на территории Каневского природного заповедника, было выделено 6 бактериофагов с длинными хвостовыми отростками, которые отличались по размерам, биологической (литической) активностью и белковому составу.
Ключевые слова: бактериофаги, флора Украины, Pulsatilla pratensis.

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DIAGNOSTICS OF SEED-BORNE CEREAL VIRUSES IN AGRIECOSYSTEMS OF UKRAINE

Using various complementing diagnostics techniques, we have analyzed spread of cereal viruses capable of seed transmission for Ukraine. Testing different cultivars and lines of cereal plants massively cultivated in Ukraine showed that seed(s) of 10 cultivars (11,8% of their total quantity) contains Barley stripe mosaic virus (BSMV).

Key words: cereal viruses, seed transmission, Barley Stripe Mosaic Virus.

Introduction. Conservation of a virus in a seed for subsequent virus transmission is an ingenious strategy for virus survival because the seed virtually links sowing seasons. This approach is of special importance for viruses having narrow host range and for viruses which are not readily transmitted by vectors. For cereals, *Barley stripe mosaic virus* (BSMV) is a showcase of probably most specialized virus as the seed transmission is vital for its survival [1; 2; 3]. In addition to BSMV it is known that *Wheat streak mosaic virus* (WSMV) may also be transmitted with seeds with the rate of 0,1-0,2% for maize [4] and 0,5-1,5 % for different wheat genotypes [5]. In the early 2000ies, seed transmission has brought WSMV from the USA to Australia where this virus (having probably adopted to a new vector species, *Aceria tosichella*) induced heavy epidemics [6]. Different authors pointed that even such small seed transmission rate of the virus as 1% (i.e., when 1% of seeds contain virus) may lead to multiple virus infection of plant generation (105-107 infected plants per hectare). This is why annual yield losses of cereals attributed to WSMV in the North America make approximately 5%. However, local outbreaks favored by intensive virus spread by *Aceria tritici* at the early stage of plant growth may lead to total loss of the crop yield [1].

As regarding BSMV infection in the USA, natural virus infection typically leads to barley yield losses of 30-31%. Virus induced losses are normally due to flower sterility (BSMV is transmitted by both seeds and pollen) [2]. Efficiency of seed transmission of this virus depends on virus strain, stage of plant growth at which it became infected, and also on species and cultivar of the crop. Available data suggest that BSMV retains its infectivity even in the seeds stored for more than 19 years [2]. This fact is of importance when choosing material for plant selection aiming at breeding new virus-resistant cereal crops. In addition it's worth to say that BSMV-infected plants produce 20-50% less seed, mainly due to the decrease of the number productive stems and number of seeds in a spike. BSMV is spread worldwide where cereals are grown. The virus cannot be inactivated by chemical or temperature seed treatment (in spite of the fact that temperature point for virus inactivation is 70°C) [2; 3].

Starting from the 1960ies, many authors described diseases of cereal crops induced by WSMV and BSMV in Ukraine. Main foci of research were biological properties of these viruses, their spread, visual appearance of the diseases on various cultivars, harmfulness, etc. [7; 8; 9]. Today, however, these pathogens (and especially seed transmission) is totally neglected. BSMV is a good example of the virus which spread remains unknown.

This work was aimed at analyzing spread of WSMV and BSMV in Ukrainian agriecosystems using different diagnostic techniques, and also at testing plant selection material of major cereal crops (available at the Bank of plant genetic resources of Ukraine) for BSMV infection.

Materials and methods. For obtaining reliable data on detection and spread of seed-borne cereal viruses we have conducted 10-year monitoring of wheat and barley commercial sowings showing symptoms typical for these pathogens. The monitored areas were Vinnytsya, Dnipropetrivsk, Kyiv, Lviv, Mykolayiv, Odessa, Poltava, Kherson, Kmelnytskiy and Cherkassy regions. During the visual assessment of the fields attention was paid to the percentage and relative spread of diseased plants, to occurrence of insect vectors, and to the abundance of concurrent bacterial and fungi infections. WSMV and BSMV were detected using DAS-ELISA with commercial polyclonal test systems (Loewe Biochemica, Germany) following the manufacturer's recommendations. Samples with optical density of 0,2 and higher were considered positive in ELISA [10].