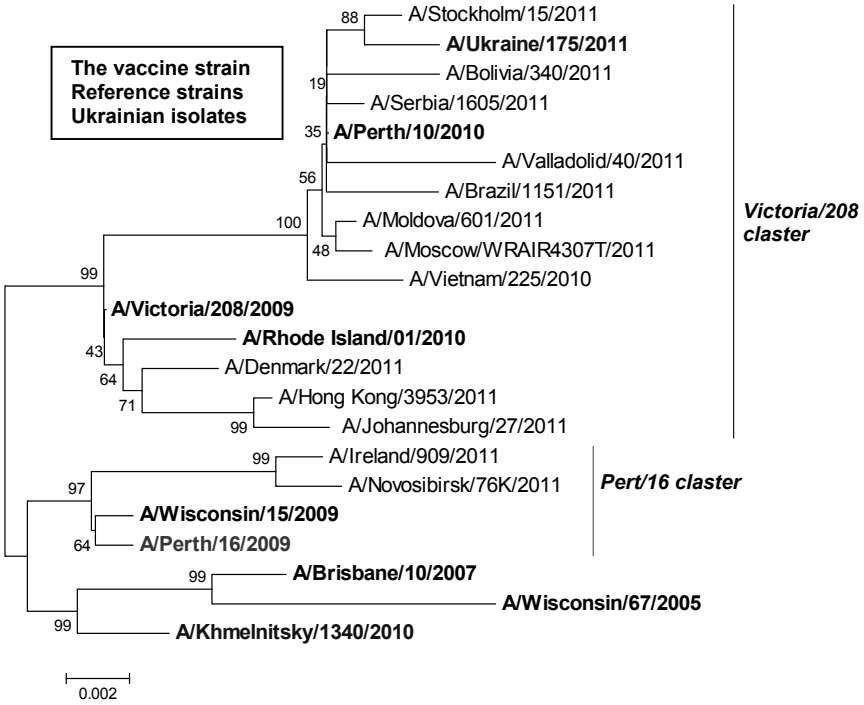

Fig. 4. Phylogenetic tree of hemagglutinin(HA) gene influenza A(H3N2)viruses constructed by NJ method with Kimura 2-parameter model (Bootstrap values are indicated)

Influenza A(H3N2)viruses, isolated during the 2010-2011 epidemic season in the world belonged to both Victoria and Pert clusters due to their genetic differences in the hemagglutinin. MutationsR261Q, E50K, P162S, N133D, R142G A212T and V213A were observed within A/Perth/16/2009 cluster, that differ viruses from A/Brisbane/10/2007. In cluster A/Victoria/208/2009 were observed next substitution in the hemagglutinin gene: N145S, V223I, N312S, D53N, Y94H, I230V, E280A and S312N (Fig.4.).

Hemagglutination inhibition assay showed that all Ukrainian isolates reacted in high enough titres with strainspecific serum to reference strains. It demonstrates their antigenic affinity to the vaccine strain A/Perth/16/2009.

Conclusions. The analysis of circulating influenza viruses in 2010-2011 epidemic season in Ukraine showed that epidemic was caused mainly by influenza viruses type B (up 55% of all selected viruses). Almost 45% of influenza isolates were similar to pandemic A(H1N1)sw strain. And only 2 isolates belonged to subtype A(H3N2).

Pandemic viruses A (H1N1)sw isolated in Ukraine were placed within two clusters, according their genetic structure. The amino acid substitutions N75K, N165K and S175P identified Ukrainian isolates type B as B/Brisbane/60/2008-like viruses. Phylogenetic analysis of influenza A(H3N2) viruses showed that isolate A/Khmelnitsky/1340/2010 located near A/Brisbane/10/2007 viruses – earlier representatives of this genetic branch. And virus A/Ukraine/175/2011 located within A/Victoria/208/2009 cluster, and showed the greatest genetic affinity to the A/Pert/10/2010-like viruses.All isolated in 2010-2011 influenza season viruses were antigenically related to vaccine strains.

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O. Mas, PhD student, O. Puhach, student, O. Andriychyk, PhD, V. Polischuk, Prof

FEATURES OF POLYPEPTIDE COMPOSITION OF PHAGES ISOLATED FROM ANTARCTIC SAMPLES OF SOIL AND MOSS ON DIFFERENT BACTERIA STRAINS – *XANTHOMONAS AXONOPODIS PV. BETICOLA 7325* AND *ERWINIA CAROTOVORA 216*

Исследование морфологии и полипептидного состав фагов, выделенных из антарктических образцов почвы и мха на бактериях Xanthomonas axonopodis pv. beticola 7325 и Erwinia carotovora 216 IMV. Для исследуемых фагов, подобных морфологически, показана подобность и в полипептидном составе. Группа подобных фагов диференциировалась по составу минорных белков.

The morphology and polypeptide composition of phages isolated from Antarctic samples of soil and moss on bacteria X. axonopodis pv. beticola 7325 and E. carotovora 216 IMV were studied. For phages similar morphologically, similarities and polypeptide composition were shown. Group of similar phages differ in composition of minor proteins.

Introduction. Even though bacteriophages play a major role in maintaining ecological balance of microbial communities, their potential influence on biogeographical patterns of bacterial communities remain greatly under-investigated. Features of their evolutionary relationships, adaptation to

bacteria hosts in extreme climatic conditions and functioning of such communities still require a detailed investigation [1].

One of the most important tasks is characterization of phages isolated directly from environment. Generalization of patterns will allow understanding the circulation of phages in nature, evolutionary changes in their populations within local regions.

However, Antarctic viral ecology is not sufficiently studied, there's virtually no dataon functioning of "phage-bacteria" system in ecological niches characterized with extreme conditions. Therefore, description of biodiversity phages and bacteria in Antarctic ecosystems and determining their ecological role is necessary [2].

The aim of this work was a comparative characterization of protein composition of phages which were isolated from Antarctic samples of soil and moss on bacterial strains *X. axonopodis pv. beticola* 7325 and *E. carotovora 216* IMV. An investigation of these phages characteristics is important to establish the range of phage propagation, to study evolutionary processes in phage populations and to analyze dependence of changes in the structure of phage reproduction on different hosts.

Materials and methods. Samples of soil and moss, which were selected during seasonal work on the Argentine Islands archipelago in locations Ukrainian Antarctic station "Akademik Vernadsky", were analyzed. Samples of soil and moss were selected from different areas of the islands. It were taken and kept in sterile plastic bags in the freezer. All further studies were conducted after delivery to Ukraine.

5 g of sterile material were taken and brought to 50 ml of 0.1 M Tris-HCl buffer. The filtrate was collected and bacteriophage particles were collected by ultracentrifugation at 100,000 x *g* for 2 h. The pellet was resuspended in 0.5 ml 0.1 M Tris-HCl buffer (pH 7.0).

Phages detected by direct inoculation. Titers were determined in plaque forming unit per ml (PFU/ml) by agar-layer technique by Gratia [3].

Preparations of concentrated phage were analyzed by TEM [4]. Electrophoretic characteristic of polypeptide composition of phages analyzed by Laemmlimethod using 14% resolving gel and 5% stacking gel, followed byCoomassie Brilliant Bluestaining [5]. Determination of molecular weight of proteins was performed using a set of marker proteins LMW (Fermentas): 116 kd – β-galactosidase from *E.coli*; 66,2 kd – Serum Albumin Bovine; 45 kd – Ovalbumin; 35 kd – Lactate dehydrogenase; 25 kd – REase Bsp98I from *E.coli*; 18,4 kd – β-Lactoglobulin from bovine milk, 14,4 kd – Lyzozyme from chicken egg white.

Results and discussion. Electron microscopy studies revealed that isolated phages fall into two morphological groups characterized with: i) isometric heads and long non-contractive tail (B1-morphotype, relates to the *Siphoviridae* family, isolated from moss and soil and accumulated on *X. axonopodis pv. beticola 7325;* isolates №3, 4, 8, 9); and ii) isometric heads and short non-contractive tail (C1-morphotype, relates to the *Podoviridae* family, order *Caudovirales,* accumulated on *E. carotovora 216*; isolate №11).

Polypeptide composition of phages is represented in Figure 1. Account of electrophoregram performed using the software "TotalLab TL120". The results of calculations are presented in Table 1.

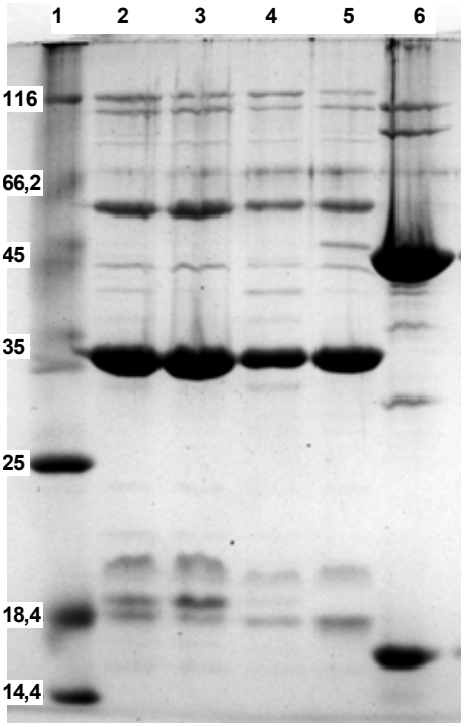

Fig. 1. Electrophoregram of polypeptide separation of phages in the system Laemmli.
Lanes: 1 – molecular weight marker LMW (Fermentas); 2, 3 – phages isolated from moss on sensitive bacterium *X. axonopodis pv. beticola 7325*, 4, 5 – phages isolated from soil on sensitive bacterium *X. axonopodis pv. beticola 7325*,
6 – phage isolated from soil on sensitive bacterium *E. carotovora 216*

Table 1. **Molecular weight of phages polypeptides**

	Molecular weight, kd					
Lane № / Band №	1	2	3	4	5	6
	Marker (kd)	Isolate 3	Isolate 4	Isolate 8	Isolate 9	Isolate 11
1	116	116,851	118,147	118,554	119,376	108,931
2	66,2	107,935	108,805	109,82	109,878	94,187
3	45	72,461	71,389	72,079	72,039	71,304
4	35	56,273	55,573	57,559	57,337	44,247
5	25	43,275	43,196	43,412	46,986	39,842
6	18,4	34,356	34,292	39,877	43,182	37,099
7	14,4	20,427	20,371	34,477	34,54	30,808
8		19,139	19,081	32,128	20,208	16,873
9		18,579	18,519	20,048	18,43	
10				18,4		

Research of protein composition of virions by electrophoresis system Laemmli allowed to set specific features among phages. Similarity of profiles identified polypeptides were characterized for all phages. Maybe, first passages on some bacteria-hosts influenced on phages. As seen in Fig. 1, phages formed two conventional groups according to the virions morphology and to the bacteria strains at which they were isolated (tracks 2, 3, 4, 5 – the first group; track 6 – the second group). Differences were observed mainly on the composition of minor polypeptides within the group of phages depending on thesource ofvirus isolation.Isolates №3 and №4 have a similar polypeptide composition by number of proteins and in molecular weight (may belong to the samebasicgroup) which differs from the polypeptide of isolates №8 and №9 thatwere isolatedfrom another source (moss).

A difference in composition of minorproteins within the group of phages suggests the impact of restriction – modification system or recombination processes in the host cells. In our opinion, the presence of minor differences in each studied phages may be result of mosaic theory of DNA phages evolution. Evolution of phages is associated with horizontal transfer of certain genetic modules [6]. Active phages, which take part in recombination exchanges, are source of genetic modules [7].

Conclusions. Phages, which were studied, have a high rate of similarity. Phages could get individual features by mutation or by exchanging DNA modules with other phages within their areal. In consideration of phages were isolated from nature, these results can reflect some evolutionary process in phage population.

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N. Matvienko, N. Kharkavliuk

FISH RNA VIRUSES

Было проведено обследование рыб из разных ферм и водоемов Украины на наличие вирусной инфекции. Получены изоляты вирусов весенней виремии карпа (SVCV) и инфекционного панкреотического некроза (IPNV), а также изучены их биологическте свойства на культуре клеток. Для молекулярной диагностики SVCV, методом ПЦР, подобрана пара праймеров.

The fish from different farms and water bodies of Ukraine were observed for the presence of viral infection. Spring viraemia of carp virus (SVCV) and Infectious pancreatic necrosis* virus *(IPNV) were isolated and their biological properties were investigated. The pair of primers was designed for PCR identification of SVCV in fish clinical material.

The main objectives of ichtyopatological research at this stage of development of Ukrainian aquaculture are studies of epizootic situation in fish farms in current environmental conditions, prevention of fish diseases and their early diagnostics based on modern techniques. Especially dangerous viral pathogens of fish are a group of RNA-containing viruses such as rhabdoviruses, which include the spring viraemia of carp virus (SVCV), virus haemorrhagic septicemia (VHS) virus and infectious hematopoietic necrosis of tissue (INN). It is important to mention also another representative of this group – aquabirnavirus, which causes salmon pancreas disease (IPNV) [1,2].

SVCV causes carp spring viraemia. The disease occurs as an epizootics type, is characterized by the development of septic process and ends with high fish mortality reaching 30-40% and sometimes 70% [3]. Reproduction of SVCV occurs in the endothelial cells of blood capillaries, hematopoietic tissue and nephrons. Infectious pancreatic necrosis virus was isolated from young rainbow trout (*Oncorhynchus mykiss*) held under intensive rearing condition. As his infection develops in trout, following pathological sings are recorded: disorders of motor coordination, skin darkening, significant pathological changes in the pancreas, liver, spleen and other parenchymal organs, and mortality of fish juveniles reaches 50-70%.

An optimal composition of the buffer was selected for preserving virus during its transporting to the laboratory. Electron microscopic investigations demonstrated that IPNV is an icosahedral-shaped virus appearing as a six-sided structure having 65-85 nm in diameter (*Fig.1*).