On the basis or test plants' reaction data, ELISA, particle morphology and literature data [4, 8] the virus was identified as TAV.

Conclusion. The plants of different cultivars of gladiolus from Kyiv region were analyzed for presence of viruses. The pathogen detected in gladiolus plant was identified as *Tomato aspermy virus*. Methods of controlling viral diseases assume growing and propagating healthy planting material tested as virus-free, inspection of plants during vegetation for occurrence of symptoms and elimination of affected plants.

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Г. Коротєєва, канд. біол. наук
КНУ імені Тараса Шевченка, Київ

ІДЕНТИФІКАЦІЯ ВІРУСІВ, ЯКІ УРАЖУЮТЬ ГЛАДІОЛУС У КИЇВСЬКІЙ ОБЛАСТІ

Проведено обстеження рослин різних сортів роду Gladiolus, відібраних на території Київської області, на наявність вірусних патогенів. Віруси детектували за допомогою методів біотестування, імоунофрментого аналізу та електронної мікроскопії. У зразках рослин було ідентифіковано вірус аспермії томатів.

Ключові слова: імуноферментний аналіз, віруси, гладіолус

А. Коротеева, канд. біол. наук
КНУ имени Тараса Шевченко, Киев

ИДЕНТИФИКАЦИЯ ВИРУСОВ, КОТОРЫЕ ПОРАЖАЮТ ГЛАДИОЛУС В КИЕВСКОЙ ОБЛАСТИ

Проведено обследование растений разных сортов рода Gladiolus, отобранных на территории Киевской области, на наличие вирусных патогенов. Вирусы детектировали с помощью методов биотестирования, имоунофрментого анализа и электронной микроскопии. В образцах растений был идентифицирован вирус аспермии томатов.

Ключевые слова: иммуноферментный анализ, вирусы, гладиолус

UDK 575.22 + 578.53

L. Leibenko, PhD, S. Babii, PhD stud., L. Radchenko, PhD stud., O. Smutko, stud.
Taras Shevchenko National University of Kyiv, Kyiv

PHYLOGENETIC ANALYSIS OF B/VICTORIA-LIKE INFLUENZA VIRUSES ISOLATED IN 2008-2012 IN UKRAINE

The article presents results of phylogenetic analysis of B/Victoria-like influenza viruses isolated in 2008-2012 epidemic seasons in Ukraine. Key mutations in amino acid sequences in hemagglutinin and neuraminidase proteins of Ukrainian isolates were analyzed. All Ukrainian B/Victoria-like influenza viruses type B located in Brisbane/60 cluster as the most isolates from all over the world according our data.

Key words: B/Victoria-like, phylogenetic analysis, Ukraine, Brisbane/60.

Introductoin. Family *Ortomyxoviridae* contains five different genes: *Influenza virus* A, *Influenza virus* B, *Influenza virus* C, *Isavirus* and *Togotovirus* [1]. Influenza B viruses belonged to the two genetic lineages: B/Victoria/2/87 and B/Yamagata/16/88 since the end of the XX century. Influenza viruses type B are less discovered than A type. Type B haven't animal host reservoir and pandemic potential. However, influenza B viruses could cause severe diseases and become the main infectious agent of influenza epidemic every three years [2,3].

The phylogenetic analysis applied to new influenza isolates allows monitor the rate and intensity of virus variations practically in real time. Moreover, the comparative analysis of their protein sequences allows reveal point amino acid replacements providing the mechanism of virus adaptation to human immune system [4]. Sequences of surface antigens – hemagglutinin (HA) and neuraminidase (NA) – are usually used for genetic analyses [5].

The **aim** of our work was to analyze the genetic diversity of B/Victoria-like influenza viruses isolated in Ukraine in 2008-2012.

Materials and methods. Nasal-throat swabs taken from patients with influenza-like illnesses (ILI) or severe acute respiratory infection (SARI) from different regions of Ukraine during 2008-2012 epidemic seasons in the study. They were analyzed using real-time polymerase chain reaction (RT-PCR). Influenza viruses type B were isolated in MDCK cell culture from samples positive in PCR. Hemagglutinin (HA) and neuraminidase (NA) gene sequences of Ukrainian isolates were selected to perform phylogenetic comparisons. Phylogenetic analysis was performed using MEGA 5 software [6].

Sequences of influenza viruses from other countries were received from GISAID resource (http://platform.gisaid.org/), using BLAST (Basic Local Alignment Search Tool) analysis (http://www.ncbi.nlm.nih.gov/ Blast.cgi). Sequences were aligned using ClustalW algorithm. Phylogenetic trees were built by the neighbor joining method [7] applying Kimura 2-parameter model [8]. Evolutional distances were calculated in terms of the number of base substitutions per site. A bootstrap technique with 1000 replications was used to test statistical validity of received data [9]. Nucleotide sequences were translated into amino acid sequences using MEGA 5 software.

Results and discussion. The B/Victoria-like influenza viruses circulated in 2008-2009, 2010-2011 and 2011-2012 epidemic seasons.

Season 2008-2009

*Comparison of hemagglutinin (HA) genes***.** Genetic comparison of the influenza virus type B HA genes shown that all investigated isolates were genetically related to vaccines strain B/Brisbane/60/2008 (Fig. 1). The Brisbane/60 cluster consists mainly of viruses among the different countries, but a small virus population from Middle East genetically related to the strain B/Malaysia/2506/2004.

Amino acid analyses of explored influenza HA genes identified four substitutions (K48E, K80R, K129N, A199T) in viruses of 2008-2009 influenza season compared with B/Brisbane/32/2002. That indicates the lower mutation rate of influenza viruses type B than type A, approved by the theoretical date [158]. The Brisbane/60 cluster viruses selected additional substitutions: N75K, N165K and S172P, also majority of viruses contain also mutation V146I, including explored isolates B/Kiev/69/2009 and B/Kiev/222/2009. The substitutions L58P (together with isolate from Madagascar), K136Q and S140T were occurred in the B/Kiev/58/2009 virus (Fig. 1).

*Comparison of neuraminidase (NA) genes***.** Genetic comparison of the 2008-2009 influenza season virus type B NA genes shown that all investigated isolates were closed to vaccines strain B/Brisbane/60/2008, estimated rate 98% (Fig. 2). As in the comparison of HA genes (Fig. 1), most of viruses from that season were presented in the Brisbane/60 cluster, but a short virus population from Middle East preserved genetical similarity to the strain B/Malaysia/2506/2004.

For all isolates from 2008-2009 influenza season were distinguished substitutions I248V and L396F, compared with the B/Brisbane/32/2002 strain. For B/Malaysia-like viruses group were noted by presence of mutations S41P and D463N (Fig. 2).

The Brisbane/60 cluster was indicated by existence of substitutions I204V and A358E, significant part of viruses also contain – D329N, including investigated isolates B/Kiev/69/2009 and B/Kiev/222/2009. Either Kiev isolates № 69 and 222, together with viruses from Norway, Russia, USA, Germany etc. received additional amino acid substitutions G378E and D463N (Fig. 2). The B/Kiev/58/2009 isolate, as in case with HA gene, became certain differences in neiraminidase that distanced it from other Kiev isolates on the phylogenetic tree. Along with Uganda virus Kiev isolate B/Kiev/58/2009 gain the I249V substitution.

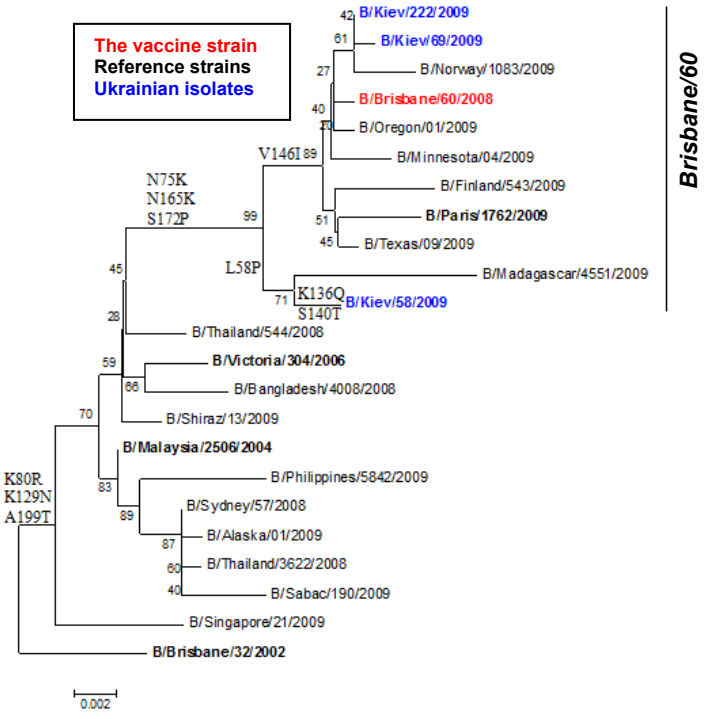

Figure 1. Molecular phylogenetic analysis of HA nucleotide sequences B/Victoria-like influenza viruses isolated in 2008-2009

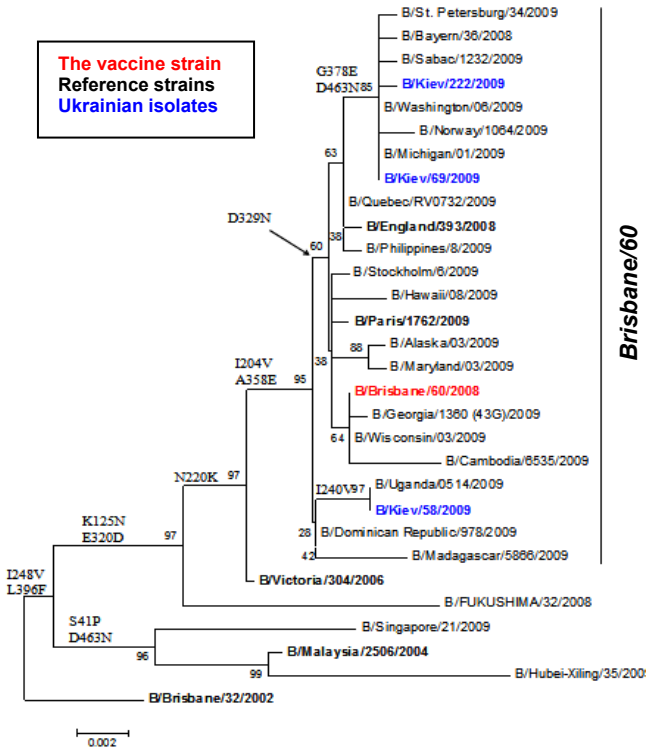

Figure 2. Molecular phylogenetic analysis of NA nucleotide sequences B/Victoria-like influenza viruses isolated in 2008-2009

Season 2010-2011. B/Victoria lineage viruses were prevalence in the world during that epidemic season (approximately 85%).

Comparison of hemagglutinin (HA) genes. Results of phylogenetic analyses are shown in the Figure 3. As in the previous 2008-2009 influenza season (Fig.1), that season viruses were similar to the vaccine strain B/Brisbane/60/2008. Low mutation rate with single amino substitution was observed in the investigated samples.

The B/Brisbane cluster viruses proceed selection of the mutations N75K, N165K, S172P i I199T. Isolates with L58P substitution developed new subcluster 2, which contains majority of the Ukrainian isolates from current season (Fig. 3). Urkainian isolate B/Odessa/3886/2010 was elected as reference strain by the Center for disease control and prevention (CDC) in London (Fig. 3).

In the investigated isolates were revealed single amino acid substitutions (Fig. 3): H122N – in the B/Zaporizzya/210/10; N197S – in the B/Kharkov/4260/10; T222A – in the B/Odessa/145/10 i B/Odessa/3886/10.

Comparison of neuraminidase (NA) genes. By genetic analyses results of neiraminidase genes (Fig. 4.), mostly explored isolates, including all Ukrainian viruses, were related to the vaccine strain Brisbane/60/2008 and belonged to the equal cluster. The isolates from Brisbane/60 cluster received the mutations I204V, A358E and most viruses had additional substitution P51S, L73F, N199D, but part of virus population also contained S27L.

Our isolated influenza viruses type B of the discovered influenza season selected such additional substitution: I45T and I455T – in the B/Ukraine/7/11 and B/Zaporizzya/87/11 together with isolates from Myrmansk and Minsk; L75F – in the B/Ukraine/7/11; E44K and D329N – in the B/Zaporizzya/87/11; V248I – in the B/Zaporizzya/210/11 and isolate from England; S51L – in the B/Ukraine/142/11.

Partly isolates from 2010-2011 influenza season (viruses from Bolivia, Indianan, Irkytsk and England) by NA sequence were related to the reference strain B/Malaysia/2506/2004. In the all idem isolates revealed substitution K220N, however viruses from Irkytsk and England also contained mutations P42S, N127K and D463N (Fig. 4).

The phylogenetic analyses of the surface influenza B/Victoria virus antigens revealed that all investigated Ukrainian isolates were related to vaccine strain B/Brisbane/60/2008 in the 2010-2011 influenza season with estimated rate 97%.

Season 2011-2012. Influenza B viruses has been isolated in the world much less compared with A type during the 2011-2012 epidemic season. They formed 9.5% of the total number of isolated influenza viruses, but 31.5% in the previous 2010-2011 season. We isolated only 2 B/Victori-like influenza viruses in Ukraine.

Comparison of hemagglutinin (HA) genes. The results of hemagglutinin genes phylogenetic comparison are presented in theFigure 5. All influenza viruses have taken for the analysis continued to carry the amino acid substitution N75K, N165K and S172P, compared with strain B/Malaysia/2506/2004. They located within the dominant in Victorian branch Brisbane/60 cluster (Fig. 5). There three groups of viruses are isolated during the 2011-2012 season have been formed in this cluster. The amino acid sequences of viruses from different groups were allmost identical with the exception of single substitutions.

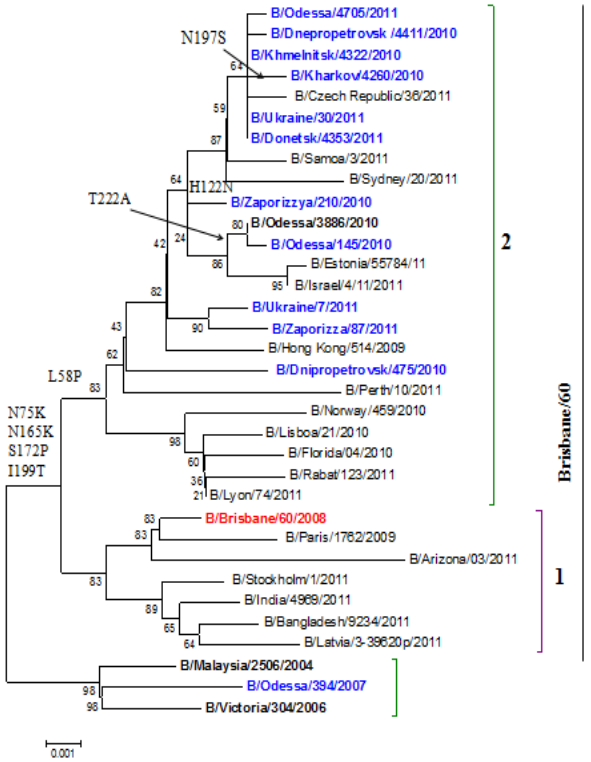

Figure 3. Molecular phylogenetic analysis of HA nucleotide sequences B/Victoria-like influenza viruses isolated in 2010-2011

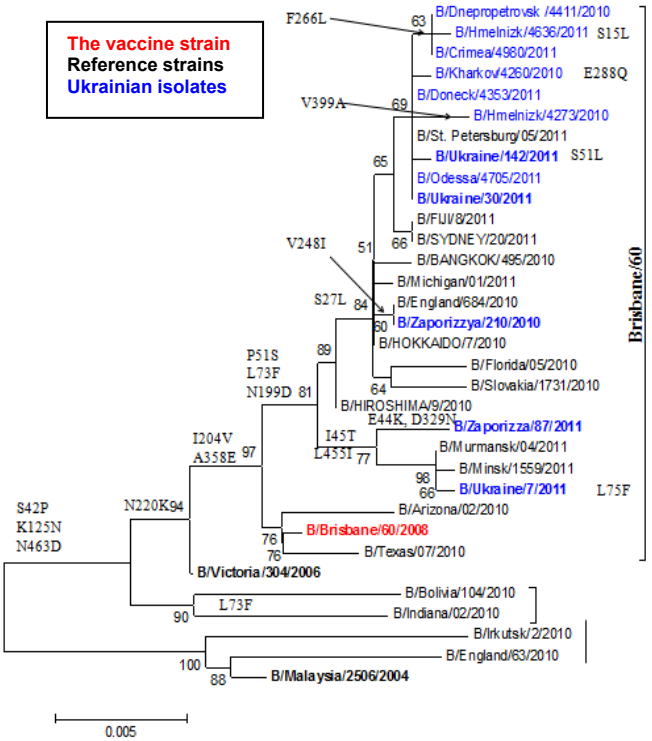

Figure 4. Molecular phylogenetic analysis of NA nucleotide sequences B/Victoria-like influenza viruses isolated in 2010-2011

B/Ukraine/104/2012, the only one sequenced in Ukraine influenza B isolate, was similar to viruses from group 2. Its sequence of hemagglutinin gene was the 100% similar to the virus from Italy – B/Milano/03/2012. PCR positive results for influenza B in Italy showed from 2012 week 6, while the first positive in PCR in Ukraine was in 2012 week 10 according EuroFlu data. Therefore, the higher likelihood was the entry of influenza B from Italy to Ukraine than vice versa. Reference strain for the group 2 was B/Malta/Mv636714/2011 virus.

Comparison of neuraminidase (NA) genes. The isolate B/Ukraine/104/2012 located within the dominant

B/Brisbane cluster according to the analysis. Comparison the neuraminidase genes of all viruses isolated in 2011-2012 in the world showed that they arranged within three clusters. Viruses of clusters 1 and 2 gained P41S, S42P, K125N and E320D substitutions, compared with B/Malaysia/2506/2004 strain (Fig. 6).

The most circulating influenza viruses type B as the B/Ukraine/104/2012 strain has located in cluster B/Brisbane in this epidemic season. This cluster reflects the main direction of evolutionary changes among influenza B viruses circulating since 2006. Strain B/Ukraine/104/2012 has located in the "top" of cluster – from B/Florida/07/2012 to B/Ukraine/5374/2012 (Fig. 6) that gained S295R and E358K substitution.

The second cluster included isolates located in the United States, Japan and Iceland in 2011. They gained a number of additional amino acid substitutions in the neuraminidase (T8M, Q61H, L73F, M375K, A389T and S397R).

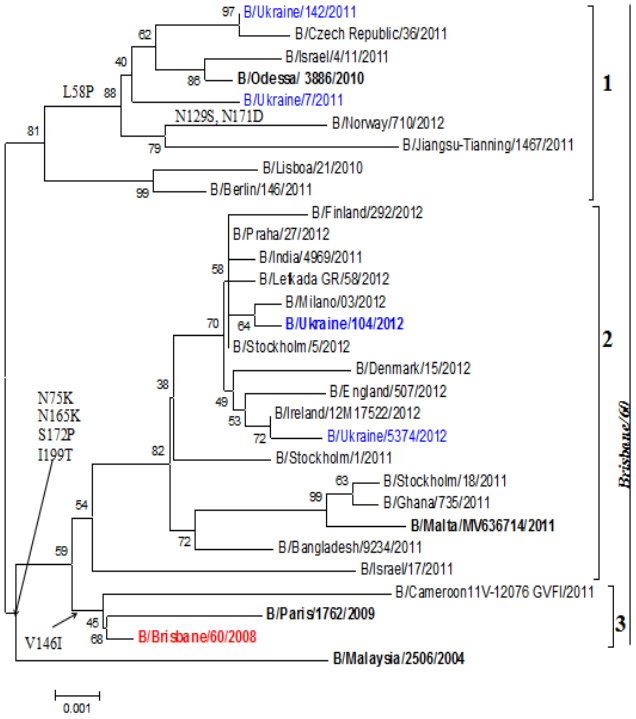

Figure 5. Molecular phylogenetic analysis of HA nucleotide sequences B/Victoria-like influenza viruses isolated in 2011-2012

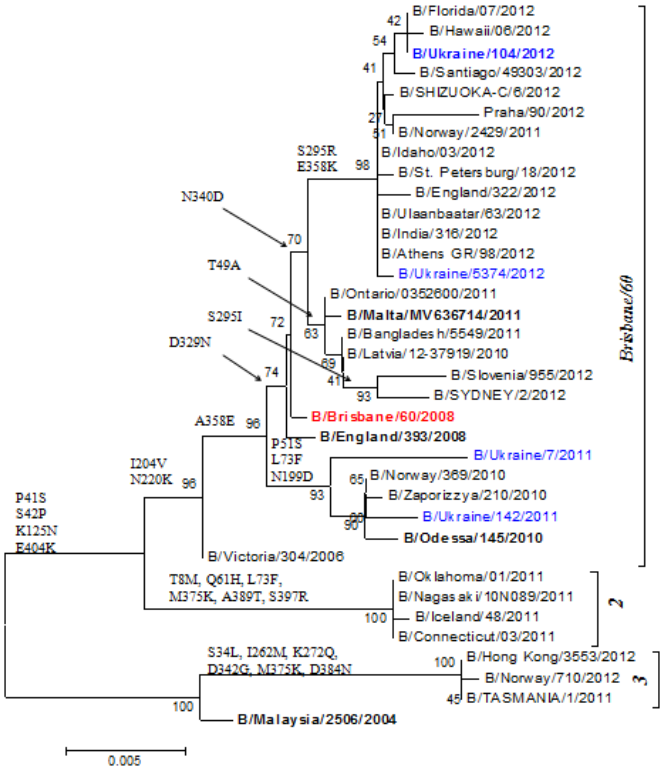

Figure 6. Molecular phylogenetic analysis of NA nucleotide sequences B/Victoria-like influenza viruses isolated in 2011-2012

A small group of viruses has been separated in third cluster – the branch genetically closer to B/Malaysia/2506/2004 strain than to viruses isolated from 2008. These viruses gained a number of amino acid substitutions: S34L, I267M, K272Q, D342G, M375K and D384N. The hemagglutinin genes of these isolates were similar to viruses from B/Brisbane cluster (Fig. 5), but their neuraminidases were separated into third cluster (Fig. 6).

Conclusions.

Influenza viruses type B isolated in Ukraine during 2008-2012 has located in common cluster Brisbane/60 and their hemagglutinin and neuraminidase genes almost hasn't changed. Most of B/Victoria-like influenza viruses allocated in the world also located in Brisbane/60 cluster. The evolutionary "towing" of Ukrainian isolates of the three seasons was shown from our analysis. The level of influenza B virus evolution is significantly lower than influenza A according to research of foreign authors [10]. Our studies confirmed this regularity.

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Л. Лейбенко, канд. біол. наук, С. Бабій, асп., Л. Радченко, асп., О. Смутко, студ.
КНУ имені Тараса Шевченко, Київ

ФІЛОГЕНЕТИЧНИЙ АНАЛІЗ ВІРУСІВ ГРИПУ ТИПУ B/VICTORIA, ВИДІЛЕНИХ В УКРАЇНІ В 2008-2012 РОКАХ

В статті представлені результати філогенетичного аналіу вірусів шрипу типу В генетичної лінії B/Victoria, виділених в Україні в 2008-2012 епідемічних сезонах. В роботі проаналізовано основні заміни в амінокислотних послідовностях гемаглютиніту та нейрамінідази українських ізолятів. За нашими даними всі укріїнські B/Victoria-подібні віруси гриппу В розмістились в кластері Brisbane/60, як і більшість виділених вірусів в світі.

Ключові слова: B/Victoria, філогенетичний аналіз, Україна, Brisbane/60.

Л. Лейбенко, канд. биол. наук, С. Бабий, асп., Л. Радченко, асп., О. Смутко, студ.
КНУ имени Тараса Шевченка, Киев

ФИЛОГЕНЕТИЧЕСКИЙ АНАЛИЗ ВИРУСОВ ГРИППА ТИПА B/VICTORIA, ВЫДЕЛЕННЫХ В УКРАИНЕ В 2008-2012 ГОДАХ

В статье представлены результаты филогенетического анализа вирусов гриппа В генетичаской линии B/Victoria, выделенных в Украине в 2008-2012 эпидемических сезонах. В работе были проанализированы основные замещения в аминокислотных последовательностях гемагглютинина и нейраминидазы украинских изолятов. По нашим данным все украинские B/Victoria-подобные вирусы гриппа В расположились в кластере Brisbane/60 как и большинство выделенных вирусов в мире.

Ключевые слова: B/Victoria, филогенетический анализ, Украина, Brisbane/60.

UDK 578.81

O. Mas, PhD student, O. Andriychuk, PhD., V. Polischuk, Prof
Taras Shevchenko National University of Kyiv, Kyiv

SYSTEMIC APPROACH TO STUDYING VIRUSES OF BACTERIA FROM ANTARCTIC SOIL BIOTOPES

Sub-Antarctic climate and specific geological/biological characteristics created unique environment for development of bacterial and viral populations in soil. This paper reviews environmental factors which influence the maintenance of virus infectivity and must be taken into account when treating the samples in the lab.

Key words: Sub-Antarctic climate, bacteriophages, soil biotopes.

Introduction. Exogenic viral pathogens traditionally remained the main aspect in research focused on soil ecology of viruses. As regarding bacterial viruses and their populational dynamics, most of the work was done using indicator species of bacteria. Classical approach for evaluation of virus numbers in soil samples typically came to counting negative colonies (plaques) on lawn of indicator bacterium. Such type of research allows obtaining rather limited data on a specific group of viruses and not the general knowledge on viral community. This is not enough, however, for comprehensive understanding of the ecological role the bacterial viruses play in soil biotopes. Moreover, complex insight in phage-bacteria-soil environment interactions requires paying attention to a number of factors affecting each element of this system, both separately and in combinative effect. In addition, influence of such factors must be considered in laboratory research.

It was demonstrated that relative bacteria number and diversity in soil may be fairly considerable and depend on sampling location and soil properties [1]. Research on density and species diversity of bacterial populations in biotopes of Dry Valleys confirmed that such locations might be characterized with 10^6-10^8 prokaryotic cells per gram of substrate [2]. For cold climates it was shown that gram-negative bacteria, α, β and γ-proteobacteria (*Pseudomonas spp*. and *Vibrio spp*.), as well as phylum *Cytophaga–Flavobacterium–Bacteroides* were detected most often. In case of gram-positive microorganisms, coryneforms (*Ar-*