BOOK OF ABSTRACTS

11-13 SEPT, 2023, KYIV, UKRAINE

X INTERNATIONAL
CONFERENCE
BIORESOURCES & VIRUSES

SPONSORS & PARTNERS

TABLE OF CONTENTS

Plenary Session

International Conference „Bioresources and Viruses“– A good tradition preserved in different times. Some personal memories.

Thomas Kuehne

Julius Kuehn Institute, Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Quedlinburg, Germany

As a student, I lived five years in Odessa. After returning home and starting my scientific career in GDR I was always interested to keep contacts to colleagues in Soviet Union. The political changes in Eastern Europe and German reunification in 1990 interrupted almost all my contacts. Therefore, I was very happy and thankful for an invitation to take part in a conference on “Bioresources and Viruses”, organized in May 1994 in Yalta. Here I met Valery Polischuk and other plant virologists from Kyiv State University. The meeting in Yalta marked the starting point of a series of conferences with the 10th taking place this year. I had the chance to participate in 2001, 2004 and 2019. For all years from 1994 until today, the conference remained a stable factor, while life around it was eventful and changed permanently. This is a successful story. Some of my personal memories will presented.

Virology in Ukraine: past, present, future

I.Budzanivska
ESC “Institute of Biology and Medicine”, Taras Shevchenko National University of Kyiv, Ukraine
(budzanivskai@knu.ua)

Historically, Ukraine has been distinguished by the contribution to virology of such famous scientists as Dmytro Ivanovsky. His discoveries about the nature of viruses had a significant impact on the development of this field. Modern virology in Ukraine is developing in different directions. Research groups in Ukraine are working on the study of viruses that infect humans, animals, and plants. In addition, the role of bacteriophages in the fight against bacterial infections is being actively studied.An essential aspect of virology development in Ukraine is the challenges faced by the country, including war and geopolitical instability. The ongoing conflict has an impact on public health and the agricultural sector, and Ukrainian virologists are actively involved in addressing these issues. There are many problems associated with the decrease in the number of scientists and students and the relocation of institutions from the occupied territories. The future of Ukrainian virology promises to be exciting. Innovations in biotechnology and molecular biology are opening up new opportunities for research and applications. However, challenges related to the war must be overcome. Here, we discuss these challenges and ways to overcome them.

Accelerating expansion of the RNA virosphere

Valerian V. Dolja

Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331, USA

RNA viruses were the first viruses to be discovered 130 years ago and became particularly notorious in recent decades, with endless human epidemics and pandemics of influenza, polio or COVID-19 as well as short-lived but scary outbreaks of Ebola or SARS-CoV-1. Despite their outsized impact on humans, however, our knowledge of the RNA virosphere remained quite narrow, with only about a thousand of RNA viruses known by the middle of the previous decade. As of now, however, this number is around quarter million and counting. Thanks primarily to advent of metavirome and metatranscriptome sequencing, this explosive growth revealed previously unimaginable genomic and ecological diversity of RNA viruses and provided deep insights into the origins and evolution of this virus realm. This expansion is particularly striking among RNA bacteriophages and viruses of unicellular eukaryotes that now represent a lion share of the RNA virospere diversity. Along with the discovery of novel virus families, orders and classes, a broad variety of novel RNA virus genes and genome architectures has been revealed. In this presentation, I will cover recent advances in understanding RNA virus origins and evolution from precellular replicator systems to reconstructed RNA viromes of the Last Universal Common Ancestor and the Last Eukaryotic Common Ancestor to the contemporary global RNA virome. I will also emphasize the evolutionary connectivity of RNA virome due to the rampant horizontal transfer of genes and gene modules among RNA and DNA viruses and their hosts, horizontal virus transfer among diverse virus hosts and, more generally, the lack of defined borders within the global RNA virome and between bona fide viruses and viroid-like circular RNAs. In other words, RNA viruses are all they can possibly be.

Age-dependent ADAR-deamination signatures are associated with lower viral loads in influenza-infected patients

Joshua Fingal, Johan Ringlander, Hanna Kann, Magnus Lindh, Maria Andersson, Martina Wahlöf, Johan Westin, Julia Widström, Michael Kann

The effect of adenosine deaminases acting on RNA (ADAR) on replication of RNA viruses is controversial. ADAR cause hypermutations on dsRNA by deaminating adenosine to inosine, which is interpreted as guanosine during translation and replication, resulting in an A→G substitution. Among isoforms described is ADAR1p150, which is an interferon-inducible, predominantly cytosolic enzyme. While deamination of a few viral genomes such as HIV and SARS-CoV-2 was found to be associated with decreased viral loads, most publications report a replication-promoting effect on other RNA viruses. This includes *in cellulo* replication studies performed on influenza viruses.

Influenza A virus (IAV) is a negative-sense, segmented RNA virus which replicates within the nucleus of infected cells. To determine the effect of ADAR on IAV replication, we investigated samples from 76 patients collected at Gothenburg hospitals during the 2016-2017 influenza epidemic. Viral RNA mutations (>0.2% frequency) were detected by deep sequencing where only nucleotides with a sequencing depth >1000 were analyzed (>88% of full genome of 13.5kb).

A→G substitution was the most frequent mutation detected in viral RNA and showed the best association with low viral load ($p<0.0001$). Remarkably, A→G substitutions were age-related showing a sharp drop in patients older than 70 – 75 years ($p=2.5 \times 10^{-6}$). As extrapolation of deamination frequencies below the technical limits implies c. 110 A→G changes per genome, we hypothesize that deamination may directly affect viral fitness and is not just an inflammation marker. Supported by the low frequency of T→C changes, which is the ADAR signature for negative-sense RNA, we theorize that the observed signatures are primarily on viral (pre)mRNA rather than genomic or antigenomic viral RNA. Both synonymous ($p=0.0002$) and non-synonymous ($p=0.0057$) A→G changes were associated with a reduction in viral load. A link between deamination signatures and viral load was found throughout multiple segments and genes, indicating a polygenic effect. In agreement with mRNA deamination, dominant A→G mutations did not differ between samples from early and late in the epidemic. Taken together, our results suggest an anti-IAV role for ADAR1p150 by altering viral mRNA, leading to destabilization of mRNA secondary structures, and affecting the functionality of viral proteins through synonymous and non-synonymous mutations, respectively

Endogenous “retrobiome” in mammalian evolution, cancer and aging

Andrei V. Gudkov
Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA
(andrei.gudkov@)roswellpark.org)

Nearly half of the human genome is occupied by highly repetitive interspersed virus-like genetic elements that replicate via reverse transcription – active or inactive, autonomous and non-autonomous retrotransposons, known as the “retrobiome”. Phylogenetic analysis indicates that retrobiome formation in mammalian evolution involved multiple explosive amplifications of its major families – SINEs and LINEs – that drove major evolutionary events and significantly contributed to the current archetypical diversity of mammals. While the retrobiome is epigenetically silenced in normal cells, it frequently becomes derepressed in cancer cells triggering numerous genetic, epigenetic, and physiological processes contributing to cancer development and progression. In addition to tumors, retrobiome expansion also occurs in aging somatic cells contributing to aging-associated DNA damage and inflammation. Our team is focused on studying mechanisms of retrobiome control, its impact on tumor progression and aging and developing diagnostic and therapeutic approaches targeting retrobiome activity.

Global organization and evolution of the virus world

Eugene V. Koonin

National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, USA

Viruses and virus-like mobile genetic elements are ubiquitous parasites (and sometime symbionts) of all cellular life forms and the most abundant biological entities on earth. The recent, unprecedented advances of comparative genomics and metagenomics have led to the discovery of diverse novel groups of viruses and a rapid expansion of the chartered region of the virosphere. These discoveries provide for a vastly improved understanding of the evolutionary relationships within the virosphere. Arguably, we are approaching the point when the global architecture of the virus world can be outlined in its entirety, and the key evolutionary events in each of its domains can be reconstructed. I will present such an outline of the global organization of the virosphere and the corresponding megataxonomy, including 6 distinct virus realms, that has been recently approved by the International Committee on Taxonomy of Viruses. The expansion of the prokaryotic virosphere that is being shown to include many groups of viruses, particularly, those with RNA genomes, previously thought to be eukaryote-specific, will be emphasized. I will further discuss the position of viruses within the wider space of replicators and the recent dramatic expansion of the “alternative virosphere” that includes viroids and diverse viroid-like viruses that seem to have evolved on multiple, independent occasions.

Principles of organization, formation and functioning of alphavirus replicase complex

Merits A., Lello L.S., Wang S., Utt A.
Institute of Technology, University of Tartu, Nooruse 1, Tartu, Estonia
(andres.merits@ut.ee)

The unique property of RNA viruses (realm *Ribovira*) is an ability to perform replication of their RNA genomes. As host cells do not have such an ability, these viruses must encode an RNA dependent RNA polymerase. In addition to this enzyme, they also encode additional enzymes needed for replication. Together with multiple of host cell encoded proteins, these viral enzymes form replicase complexes (RC). In eukaryotic cells, the RCs of positive strand RNA viruses are anchored to cellular membranes and represent molecular machines capable for synthesis and modification on viral RNAs.

Alphaviruses (family *Togaviridae*) are emerging arthropod-transmitted pathogens. Alphavirus Infection in humans is associated with arthritic or encephalitic symptoms and may be fatal. The most medically important alphavirus is chikungunya virus that has caused several massive outbreaks in this century and represents major medical burden due to its tendency to cause long lasting chronic symptoms. RC of alphaviruses consist from four viral subunits called nsP1-4. Using reverse genetics, synthetic and structural biology methods we have demonstrated that: i) binding of RC to the cellular membranes is mediated by nsP1 – an enzyme, responsible for capping of positive strand RNAs of alphaviruses. NsP1 forms the structural bases of entire RC. ii) binding of specific RNAs by RC of alphaviruses is a complex process. Template selectivity is determined by sequences located at 5’ end of viral genome that are recognized by viral RNA polymerase and/or by viral helicase/protease (nsP2). The interaction between viral helicase and virus genome involves hydrophobic stacking between RNA bases and aromatic amino acid residues; iii) virus encoded nsP3 is covalently modified by phosphorylation of serine/threonine residues; the impact of this modification is different for different alphaviruses. Presumably, this is caused by interactions of nsP3 with cellular proteins that are altered upon phosphorylation of nsP3. iv) viral RNA polymerase (nsP4) has typical right-hand conformation and contains regions that in an individual nsP4 are disordered. The nsP4 acquires stable conformation upon interaction with other replicase subunits, most importantly with nsP1. The interaction between template RNA, different nsPs and host factors is specific yet flexible. For example, functional RCs can be assembled from subunits originating form heterologous viruses and interactions with host proteins normally mediated by nsP3 can be performed by nsP2 as well. However, as the architecture of RC as certain clearly defined principles and viral replication depends on specific interactions between viral and host components these high-order structures and their functions represent promising targets for novel drug leads.

Dynamic of changes of genetic variants of SARS-CoV-2 during pandemic of COVID-19 in Ukraine

Mironenko A., Radchenko L., Kravchuk I., Teteriuk N., Holubka O., Zakharchuk I.
L.V.Gromashevsky Institute of Epidemiology and Infectious Diseases NAMS of Ukraine, Kyiv, Ukraine
(*miralla@ukr.net*)

The emergence of new pathogen – coronavirus SARS-CoV-2 in the world set global tasks for humanity and for virologists regarding study of changes in this virus and changes of its properties. The aim of our work was looking for changes in genetic variants during COVID-19 pandemic in Ukraine. We performed the molecular-genetic analysis of SARS-CoV-2 viruses' characteristics, which circulated in Ukraine from May 2020 till June 2023 according to data of sequences which were uploaded to database GISAID (www.gisaid.org). There were analyzed the mutations which occurred during the whole period, which caused the changes of genetic variants and subvariants of SARS-CoV-2 virus. We analyzed the genetic sequences of 605 SARS-CoV-2 viruses, which circulated in Ukraine. Since the appearance of the pathogen in the territory of Ukraine in March 2020 there was the accumulation of specific mutation in the virus because of natural epidemic process. The first wave of morbidity increase was in autumn 2020. The main pathogen was classic or Wuhan strain. In December 2020 was informed about the emergence of new variant Alpha, but it appeared in Ukraine in February 2021, that was confirmed by sequencing for the first time in the country by the scientists of «L.V.Gromashevsky Institute of Epidemiology and Infectious Diseases NAMS of Ukraine». This variant caused the second wave of morbidity, which was in spring 2021. The next pathogen – Delta variant caused the huge increase of morbidity, which was in autumn 2021. Omicron variant appeared in December 2021 and circulates for this day in Ukraine. There is the permanent and fast change of its subvariants. The subvariant of Omicron XBB.1.9.1 was dominant in second quarter of 2023 in Ukraine. All four waves of COVID-19 pandemic were caused by different variants of the pathogen: the first wave – by primary Wuhan, the second one - by Alpha variant, the third – by Delta variant and the last one – by Omicron variant. The quick change of subvariants of Omicron follows.

HBV Cure – how can we get there?

Ulrike Protzer

Institute of Virology, Technical University of Munich / Helmholtz Munich, Munich, Germany

Hepatitis B virus (HBV) is a major public health threat with more than 290 million humans chronically infected worldwide at risk to develop end-stage liver disease and hepatocellular carcinoma. Each year, more than 880,000 people die from the consequences of HBV infection. Available antivirals control HBV replication but do not cure HBV infection. Immune therapies including therapeutic vaccines, antibody and T-cell therapies are most promising to achieve a cure.

We designed a chimeric antigen receptor (CAR) and cloned T-cell receptors (TCR) recognizing all HBV envelop proteins. Grating our CAR and TCR on T cells, we proved T-cell therapies to be effective in relevant preclinical models. In a first clinical application for HBV-associated hepatocellular carcinoma we were able to confirm on-target activity and safety of TCR T cells. To avoid T-cell manipulation under GMP condition, we designed T-cell engager antibodies that proved to be of comparable efficacy to a CAR in controlling HBV infection.

Knowing that efficient activation of T-cell responses is crucial, we designed a therapeutic hepatitis B vaccine that allows activation of CD4 and CD8 T cells. Such a therapeutic vaccine represents a broadly applicable treatment approach. However, development of such a vaccine is challenging since simultaneous stimulation of vigorous antibody- as well as T-cell responses is necessary to overcome the HBV-specific immune tolerance and the therapeutic vaccine needs to cover different HBV strains. The heterologous *TherVacB* prime-boost vaccination scheme proved most suited to achieve this in preclinical models and will soon enter clinical trials.

The neurobiology of herpes simplex virus

Anders Vahlne, MD, PhD
Professor emeritus in Clinical Virology, Karolinska Institutet, Stockholm, Sweden

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are neurotropic viruses and both after primary infection establish latent infection in sensory neurons. The mechanisms for controlling the virus latent in the sensory neurons will be discussed. In the central nervous system HSV-1 infection (both primary and recurrent) may cause a mortal encephalitis. Except in the perinatal period, HSV-2 in the CNS will not cause encephalitis but a meningitis, that may recur. The reasons for this difference in CNS pathogenesis between HSV-1 and HSV-2 will be discussed. Furthermore, in experimental animals HSV-1 infection in the CNS can cause localized demyelinations with destruction of oligodendroglia. Possible involvement in multiple sclerosis and idiopathic facial (Bell’s) palsy will also be discussed.

Arboviruses: an urgent problem in Ukraine

N.O. Vynograd[1], and O.O. Yurchenko[2]
[1] *Danylo Halytsky Lviv National Medical University, Lviv, Ukraine;*
[2] *Branch «Mechnikov Anti-Plague Institute» of the SI "Public Health Centre of the Ministry of Health of Ukraine", Odessa, Ukraine*
(vynogradno@ukr.net)

Arboviruses are an ecological group of viruses that are maintained in nature among susceptible vertebrates through biological transmission by blood-sucking arthropods. More than 500 species of arboviruses are known in the world, and 150 of them can cause human diseases. At present, five arboviruses belonging to four families of RNA-containing viruses (*Flaviviridae*, *Nairoviridae*, *Phenuiviridae*, and *Togaviridae*) have the greatest clinical significance in Europe. West Nile (WNV), tick-borne encephalitis (TBEV) and Toscana (TOSV) viruses can cause febrile diseases with injuring the central nervous system, Crimean-Congo hemorrhgic fever virus (CCHFV) – hemorrhagic fever, and Sindbis virus (SINV) – fever with arthralgia. WNV and SINV are transmitted by mosquitoes, TBEV and CCHFV viruses – by ticks, and Toscana virus – by sandflies. The geographical distribution of arboviruses and the spring-autumn seasonality of diseases caused by them coincide with the range and period of activity of blood-sucking arthropod vectors. Dengue (DENV), Zika (ZIKV), and Chikungunya (CHIKV) viruses are exotic for Europe, but in recent years there has been an increase in cases of them being introduced during travel. Unlike arboviruses endemic to Europe, the main host of DENV, CHIKV and ZIKV is a human, and the vectors are tropical mosquitoes *Aedes aegypti* and *Ae. albopictus*. WNV, TBEV, and CCHFV are endemic for Ukraine that was confirmed by the detection of the pathogens in vertebrate reservoirs and blood-sucking arthropod vectors and registration of human diseases caused by the viruses. In recent years, sporadic imported cases of exotic dengue fever have been registered in Ukraine. Different factors influence the development of the epidemic process: biological, climate-geographic, socio-economic. Worsening the epidemic situation on arbovirus infections may be caused by emergence or introduction of pathogens with increased virulence, increasing arboviruses prevalence in vectors and reservoirs. Climatic changes, such as an increase in temperature and relative humidity, contribute to an increase in the number and expansion of the range of vectors of arboviruses. The risk of infection increases with an increase in the duration and frequency of people's stay in the territory of natural foci. In addition to the above listed factors, russian military aggression which led to destruction of civil infrastructure and ecological systems on the large areas should be taken into account in Ukraine. Thus, the presented data indicate the arboviruses are an urgent problem in Ukraine and the deterioration of the epidemic situation with TBE, WNF, CCHF, as well as other arboviral diseases can be expected.

Section 1
Medical Virology

Antiviral potential of bimetallic nanoparticles

L.O. Artiukh, O.Y. Povnitsa, S.D. Zahorodnia
Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine, Kyiv, Ukraine
(bilyavskal@ukr.net)

Bimetallic nanoparticles (BNPs) have increasingly attracted the attention of researchers around the world, because their uniqueness in terms of chemical, physical and biological properties significantly distinguishes them from their monometallic counterparts. Au and Ag have a long history of anti-infective properties however, bimetallic nanoparticles based on them still remain understudied. The aim of this study to investigate antiviral properties of NPs alloys composed of various ratios of noble metals such Au and Ag against different type of viruses. BNPs were obtained using method of chemical or photochemical synthesis. Antiviral efficacy of bimetallic nanoparticles was estimated by the reduction of virus infectivity. Cytopathic, virucidal and antiviral effects of new mono- and bimetallic silver and gold nanoparticles were analyzed. It should be noted that extent detection of toxic or antiviral effect of NPs depended on their nature, composition, and surface functional groups. Mono silver and gold nanoparticles and their alloys exhibit a weak cytopathic effect, but NPs synthesized by heating to boiling were more toxic to all tested cell cultures. It was found that synthesized by irradiation alloy where the ratio of silver and gold NPs was 3:1 exhibit a pronounced virucidal effect against herpes simplex virus. The decrease in the infectious titer of the herpes virus was ~2 $\log_{10}$. The striking finding of our study is that BNPs exert their antiviral effects against adeno- and herpes viruses. So, post-exposure of herpes simplex virus with BNPs (Ag:Au ration 1:3) obtained both chemical and photochemical synthesis leads to reduction of virus titer by 1.5 - 1.8 $\log_{10}$. Instead, for alloy of silver with gold nanoparticles in equal volumes synthesized using the photochemical method anti-adenoviral effect was found (decrease in adenovirus titer is 1.9 - 2.1 $\log_{10}$). Thus, obtained data indicates high antiviral potential of bimetallic nanoparticles of gold and silver with different composition and methods of synthesis.

Virological monitoring of Measles in Zhytomyr Oblast in 2017-2019

Boialska O.
SI Zhytomyr Oblast Center for Diseases Control and Prevention of the Ministry of Health of Ukraine
(*virus_29@ukr.net*)

Virological monitoring plays an important role in the epidemiological surveillance of measles. It has been established that observation based on clinical detection of cases is inaccurate, and laboratory confirmation of suspected cases with subsequent genotyping of measles viruses is extremely important for effective monitoring of measles.

The low vaccination coverage of both the Zhytomyr oblast and Ukraine as a whole has led to a significant increase in measles morbidity (less than 95%). Therefore, for the registration of measles cases and the successful implementation of the measles elimination program, it is necessary to perform measles virological monitoring in each administrative territory of our country. Most positive findings were found among people in the age groups of 20-29 and 30 and above that indicates the absence or weakening of immunity to measles. The rapid evolution of the virus with the aim of avoiding the virus from immune response leading to changes in circulating measles virus genotype in the region from D4 to D8 and B3.

Vaccines: a never ending evolution

Konstantin Chumakov
President and CEO of NextGen Bio Consulting
Adjunct Professor, George Washington University
Adjunct Professor, University of Maryland

Vaccines are among the most cost-effective public health interventions. While the first vaccine was created in the 18 century, most of them were created in the last 50 years. There are several reasons why the work on vaccines does not stop after successful licensure and rollout. The first is that vaccines alter the epidemiological situation, and the small rate of adverse reactions that was socially acceptable when the disease was rampant, becomes unacceptable when disease is under control or completely eliminated. This demands the improvement of safety profile. The second reason is the rapid progress of biotechnology that allows old vaccines to be improved. This is closely related to yet another reason – the need to decrease cost of vaccines, some of which are still out of reach in resource-limited countries. The presentation will provide several examples of this evolution, including smallpox vaccine, rabies vaccine, vaccine against pertussis, and others. The main focus will be on the recent development of novel genetically stable Oral Poliovirus Vaccine.

A novel precision-serology assay for SARS-CoV-2 infection based on linear B-cell epitopes of Spike protein

Samuel B Lundin[1,2,3], Hanna Kann[1], Alma Fulurija[2,3,4], Björn Andersson[1], Sravya S Nakka[1], Lars-Magnus Andersson[5,6], Magnus Gisslén[5,6], Ali M Harandi[1,7]

[1] Department of Microbiology and Immunology, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden; [2] Biotome Pty Ltd, Perth, WA, Australia; [3] Biotome AB, Kullavik, Sweden; [4] School of Biomedical Sciences, Marshall Centre, University of Western Australia, Perth, WA, Australia; [5] Department of Infectious Diseases, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden; [6] Region Västra Götaland, Sahlgrenska University Hospital, Department of Infectious Diseases, Gothenburg, Sweden;
[7] Vaccine Evaluation Center, BC Children's Hospital Research Institute, University of British Columbia, Vancouver, BC, Canada.

Introduction: The COVID-19 pandemic illustrates the need for serology diagnostics with improved accuracy. While conventional serology based on recognition of entire proteins or subunits thereof has made significant contribution to the antibody assessment space, it often suffers from sub-optimal specificity. Epitope-based, high-precision, serology assays hold potential to capture the high specificity and diversity of the immune system, hence circumventing the cross-reactivity with closely related microbial antigens. **Methods:** We herein report mapping of linear IgG and IgA antibody epitopes of the SARS-CoV-2 Spike (S) protein in samples from SARS-CoV-2 exposed individuals along with certified SARS-CoV-2 verification plasma samples using peptide arrays. **Results:** We identified 21 distinct linear epitopes. Importantly, we showed that pre-pandemic serum samples contain IgG antibodies reacting to the majority of protein S epitopes, most likely as a result of prior infection with seasonal coronaviruses. Only 4 of the identified SARS-CoV-2 protein S linear epitopes were specific for SARS-CoV-2 infection. These epitopes are located at positions 278-298 and 550-586, just proximal and distal to the RBD, as well as at position 1134-1156 in the HR2 subdomain and at 1248-1271 in the C-terminal subdomain of protein S. To substantiate the applicability of our findings, we tested three of the high-accuracy protein S epitopes in a Luminex assay, using a certified validation plasma sample set from SARS-CoV-2 infected individuals. The Luminex results were well aligned with the peptide array results, and correlated very well with in-house and commercial immune assays for RBD, S1 and S1/S2 domains of protein S. **Conclusion:** We present a comprehensive mapping of linear B-cell epitopes of SARS-CoV-2 protein S, that identifies peptides suitable for a precision serology assay devoid of cross-reactivity. These results have implications for development of highly specific serology test for exposure to SARS-CoV-2 and other members of the *coronaviridae* family, as well as for rapid development of serology tests for future emerging pandemic threats.

Keywords: B-cell epitope; SARS-CoV-2; Spike protein; cross-reactivity; precision serology.

Effects of light sources and distance on the photocatalytic activity of TiO_2 with the possibility of human adenovirus remediation

Povnitsa O.Y[1]., Artiukh L.O.[1], Zahorodnia S.D.[1], Zahornyi M.M.[2], Ievtushenko A.I.[2]

[1]Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, 154 Acad. Zabolotny Str., Kyiv, 03143, Ukraine

[2]I.M. Frantsevich Institute for Problems of Materials Science, National Academy of Sciences of Ukraine, 3 Krzhizhanovsky str., Kyiv, 03142, Ukraine

(povnitsa@ukr.net)

The search for safe ways to inactivate pathogens has become especially relevant in connection with the coronavirus pandemic. Existing methods using chlorides and ultraviolet irradiation (UVA and UVB spectrum) have disadvantages related to toxicity and low efficiency. Relative to other pathogens, adenovirus demonstrates strong resistance to UV light. It was shown that damage to the adenovirus genome by LP lamp radiation (254 nm) does not lead to a decrease in virus infection. This indicates the possibility of DNA repair and resistance of adenovirus to UV disinfection. Photodynamic inactivation by nanoparticles is used to disinfect water and air from microorganisms and enveloped viruses, such as human herpes simplex virus, vesicular stomatitis virus, human immunodeficiency virus, and hepatitis B and C viruses etc. Thus, the development of advanced photocatalyst for the effective remediation of viruses without the substantial formation of toxic byproducts is necessary. The **aim** of this work was to evaluate the possibility of inactivation of human adenovirus type 5 in an organic medium using titanium dioxide nanoparticles irradiated with sunlight and lamps with different distance. **Methods.** The nano-TiO_2 material was obtained by thermal decomposition of a suspension of hydrated titanium dioxide $TiO(OH)_2$ (metatitanic acid). The concentration of nanoparticles was 1 mg/ml. Scanning electron microscopy (SEM) results showed that TiO_2 nanopowder contained soft aggregates, nanoparticles mostly 20-30 nm in size. Determination of cytotoxicity, virulicidal and antiviral action of titanium dioxide by standard methods using (3-(4,5-dimathylthiazol-2-yl)-2,5-dipheniltetrazolium bromide (MTT). Two experimental schemes were used: an adenovirus suspension with a titer of 6.0 $\log_{10}$ $TCID_{50}$/ml was added to the nanoparticles before irradiation or immediately after irradiation. The titer of a virus, synthesized in the presence of titanium dioxide was determined by the end of dilution of the virus, which causes 50% developed of the cytopathic effect of the virus on cells. **Results.** Regardless of the solvent, NPs had low toxicity at a concentration of 0.5 mg/ml. The TiO_2 NPs dissolved in glycerin-water had no virucidal effect; bet dissolved in propanediol-ethanol reduced the infectious titer of the virus by 6.0 $\log_{10}$, which indicates their high virucidal effect. Irradiation of a mixture of virus - nanoparticles with sunlight for 8 hours led to the complete inhibition of the virus, but nanoparticles without irradiation also completely inhibited the virus (virucidal effect). In the control of the virus (without nanoparticles), the titer decreased by 1.2 $\log_{10}TCID_{50}$/ml. Two-hour irradiation of a mixture of virus and nanoparticles with a 5-watt land lamp at a distance of 10 cm led to complete inactivation of the virus in the NP-virus samples (regardless of irradiation). In the control of the virus, the titer decreased by 1.0 $\log_{10}TCID_{50}$/ml. Photoactivation of the NPs-virus mixture with a bactericidal lamp OBB15P (wavelength 254 nm) at a distance of 20 cm for 1-30 min also led to the complete inactivation of the virus. According to another scheme, photoactivation of only the

suspension of nanoparticles was carried out with a bactericidal lamp OBB15P (wavelength 254 nm) at a distance of 20 cm for 30 min and the addition of adenovirus after photoactivation. NPs in a propanediol-ethanol solution, irradiated with UV for 30 min, completely inhibited adenovirus reproduction. NPs in a glycine-water solution reduced the virus titer by 0.5 $\log_{10}TCID_{50}$/ml. The control with NPs without irradiation slightly reduced the virus titer (0.45 $\log_{10}$). **Conclusions**. It was shown that the non-enveloped HAdV5 virus could be efficiently inactivated by UV-induced TiO_2 photocatalysis.

Complex vaccination strategies prevent the emergence of vaccine resistance

Simon A. Rella[1], Yuliya A. Kulikova[2,3], Aygul R. Minnegalieva[3], Fyodor A. Kondrashov[3]
[1]*Institute of Science and Technology Austria, 1 Am Campus, Klosterneuburg, A-3400, Austria;*
[2]*International Institute for Applied Systems Analysis, Schlossplatz 1, Laxenburg, A-2361, Austria;*
[3]*Okinawa Institute of Science and Technology, Tancha 1919-1, Onna-son, Okinawa 904-0495, Japan*
(fyodor.kondrashov@oist.jp)

Vaccination is the most effective tool to control infectious diseases. However, the evolution of vaccine resistance, exemplified by vaccine-resistance in SARS-CoV-2, remains a concern. Here, we model complex vaccination strategies against a pathogen with multiple epitopes - molecules targeted by the vaccine. We found that a vaccine targeting one epitope was ineffective in preventing vaccine resistance. Vaccine resistance in highly infectious pathogens was prevented by the full-epitope vaccine, one targeting all available epitopes, but only when the rate of pathogen evolution was low. Strikingly, a bet-hedging strategy of random administration of vaccines targeting different epitopes was the most effective in preventing vaccine resistance in pathogens with low rate of infection and high rate of evolution. Thus, complex vaccination strategies, when biologically feasible, may be preferable to the currently used single-vaccine approaches for long-term control of disease outbreaks, especially when applied to livestock with near 100% vaccination rates.

Connecting virology towards a one-health approach

Ricardo Santos[1,2]

[1]*Laboratório de Análises do Instituto Superior Técnico, Universidade Lisboa, Lisboa, Portugal;*
[2]*Departamento de Engenharia e Ciências Nucleares, Técnico lisboa, Universidade Lisboa, Lisboa, Portugal*

Virus are the most abundant organism on earth. As one example, one milliliter of coastal water can contain up to 10 million bacteriophages. Environmental virology is a fast evolving field of research with new achievements every day.

This talk aims to emphasize different examples of environmental virology research that are thriving and making a difference nowadays: Related to human health, the detection of SARS-CoV-2 and other virus in wastewater and the future of wastewater-based epidemiology. In addition, the case of plant virus and their possible usefulness as normalization agents for human contamination will be focused. Furthermore, source tracking and the role that virus have in the determination of environmental sources of pollution will be discussed.

Water reuse, and its use for irrigation due to the lack of fresh water available due to misuse and climate change can be the solution to these increasing problems. However, as in the case of water for human consumption, care should be taken to prevent the presence of pathogens, namely virus that could pose a risk to human and animal health.

In all these cases viruses are major players for the human-animal-environment axis.

Epidemiological peculiarities of COVID-19 in Ukraine

N.O. Vynograd, and L.P. Kozak
Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
(vynogradno@ukr.net)

A pandemic of the novel disease COVID-19, caused by the SARS-Cov-2 virus, began in China, which was reported on December 30, 2019.

The purpose of the study was to summarize data based on the analysis of official reports and messages, own research on the COVID-19 epidemic. The first case of COVID-19 in Ukraine was registered on March 1, 2020 in the Chernivtsi region. The epidemic of COVID-19 lasted until July 1st, 2023, when the government of Ukraine announced its termination. According to official data, the incidence of COVID-19 in Ukraine was 128,681 per 1 million population, and the death rate was 2,603 per 1 million population. On average, the mortality rate during the epidemic was 2.1%. Quantitative manifestations of the epidemic process were characterized by five increases in morbidity, each of the next four being more intense than the previous one. Antigenic changes leading to the emergence of the Omicron genetic variant of the SARS-CoV-2 virus caused an increase in the contagiousness of COVID-19, so the highest incidence rates were during the circulation of this variant in early 2022. Reproductive number (Ro) of SARS-Cov-2 in 2021 year, it varied depending on the genetic variant from 0.78 to 1.29, and in the following year ones increased to 1.5. Much lower in intensity was thez fifth wave of the rise in morbidity, when milder clinical forms of the disease prevailed. Almost 416,000 children were involved in the epidemic process, the share of which grew during the epidemic. People over the age of 60 were at risk for severe disease and death. In the structure of mortality, patients with concomitant pathology prevailed: cardiovascular diseases, hypertension, diabetes. A difficult challenge during the pandemic was the protection of medical workers, of whom almost 150,000 fell ill, of which 0.8% died. The established epidemiological features of COVID-19 in Ukraine are the basis for further improvement of the system of surveillance and response to biological threats, including those caused by pathogens of infectious diseases new to humans.

Viral infections: medical aspects during the russian-Ukrainian war

N.O. Vynograd, Z.P. Vasylyshyn
Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
(vynogradno@ukr.net)

The active phase of the russian-Ukrainian war is going from February 24, 2022. Military operations affect the emergence and spread of viral infectious diseases.

The aim is to study the structure of viral infectious diseases during the russian-Ukrainian war on based of the Public Health Center data, others Department of the Ministry of Health of Ukraine, and professional Internet resources.

The data of sentinel and routine epidemiological surveillance in the 2022/2023 epidemic season indicated at 38.2% decrease of intensive incidence rates of acute respiratory viral infections in comparison with the previous epidemic season. In the group of influenza-like diseases, influenza viruses of type A dominated. In the group of acute respiratory infections, influenza viruses of types A and B were identified in 49.4%, the remaining 50.6% - SARS-CoV-2, para-, adeno-, RS-viruses, rhino-, metapneumoviruses. Increasing of the incidence of intestinal viral infections had been detected. In 2022, 3,533 cases of rotavirus enteritis were reported in Ukraine, of which 3,388 had been detected in children. During this period, 8 outbreaks of rotavirus enteritis were registered, as a result of which 49 people were injured, including 44 children. Outbreaks occurred most often in temporary accommodation centers for internally displaced persons — 6 (75.0%) and preschool institutions — 2 (25.0%). Serious problems arose regarding the provision of epidemiologic surveillance of HIV infection, the logistics of providing services for prevention, testing, treatment of HIV infection and clinical and laboratory monitoring of the course of HIV/AIDS among people living with HIV became complicated, primarily in South-Eastern regions. Thus, compared to the same period before the war, in the first three months of the war, the number of diagnosed cases of HIV infection decreased by 26.5%, particularly in the Eastern region of the country; more people with HIV infection were detected in the Central and Western regions. It was also difficult to implement the elimination strategy of the WHO, regarding 100% treatment coverage of HCV and HBV; the war led to the suspension of recruitment of new patients for treatment, and injuries led to an increase in cases. In Ukraine, over the past 30 years, the epidemic situation regarding the incidence of rabies in human has been unstable, sporadic cases of the disease have been registered (19 cases in 1988-1997, 27 in 1998-2007, 31 in 2008-2017, and 31 in 2018 year - 4). Military actions led to active changes in the habitats of wild animals, an increase in the number of feral domestic animals and contacts between them, and therefore with people. This can affect the change of the epidemic situation to an unfavorable one.

Modern state and prospects for the development of antiviral agents in Ukraine

Svitlana Zahorodnia
D.K. Zabolotny Institute of Microbiology and Virology NAS of Ukraine, Kyiv, Ukraine
(svetazagorodnya@ukr.net)

Viral infections are a global threat to human health. For about 60 years, significant efforts have been made to develop antiviral drugs and means of preventing their spread and prophylaxis. More than 90 antiviral drugs have been officially approved for use. However, a number of viral infections, such as epidemic influenza, are still spreading around the world, and new threats continue to arise from new and emerging viruses and viruses, and the development of drug resistance. The rapid development of in silico tools, chemical and genetic modification methods allows for the development of more antiviral drugs, while at the same time, today we face the threat of the emergence and re-emergence of infectious diseases, as there are no specific antiviral drugs available. To prevent a pandemic of these infections, it is also necessary to develop broad-spectrum antiviral drugs.

Ukrainian scientists, with the support of pharmaceutical companies, have many years of experience in the development and introduction of new antiviral drugs and in determining these properties in existing drugs. Repositioning of medicines is of growing interest, as it reduces the time and costs associated with drug development. A separate task facing our country's scientists is to restore the biotechnology of vaccine production in Ukraine to restore the production of domestic drugs for the immunoprophylaxis and treatment of infectious diseases. Relevant research work is carried out in many institutions, in particular in the Department of Virus Reproduction Institute of Microbiology and Virology of the NAS of Ukraine developed a model for the production of inactivated viral drugs on the influenza virus model, conducted numerous studies of compounds of different nature for their antiviral potential, and developed highly effective inhibitors of virus penetration and replication on influenza, adenovirus and herpes virus models.

Thus, new chemical compounds, natural products, nanoparticles and their composites with established antiviral activity give hope for the development of effective domestic drugs for the prevention and treatment of infectious diseases.

Nanoceria in virology

N.M. Zholobak[1,2], A.B. Shcherbakov[1]

[1]D.K. Zabolotny Institute of Microbiology and Virology NAS of Ukraine, Kyiv, Ukraine; [2]Kyiv National University of Technologies and Design, Kyiv, Ukraine

(n.zholobak2018@gmail.com)

We previously proposed and developed a fundamentally new direction – the nano therapy of viral infections, based on the involvement of ceria nanoparticles (CeNPs, nanoscale substances with antioxidant and regenerative properties) in the formation of antivirus resistance. What is nanoceria? On the one hand, these are just nanoparticles among many others that have long been known and/or recently synthesized. But if we analyze more deeply, then CeNPs are not at all simple, since the effects they cause in living objects are extremely wide and diverse. We will focus only on the role of CeNPs and their compounds in virology. First of all, CeNPs should be noted as virucides – disinfectants that destroy or irreversible inactivate viruses outside the living being. A very promising property since the application does not require in vivo clinical studies and the results can be quickly put into practice. As you know, antiviral agents include substances that can be used to prevent or treat viral infections in the living being (e.g., in the cell culture, animal and human body, etc.). CeNPs fully comply with this definition. We demonstrated in 2011 for the first time the antiviral activity of nanoceria and its compounds against RNA- and DNA-containing viruses in vitro. We associate the mechanism of this action with the wedging of CeNPs into almost all stages of the virus-cell interaction, as well as with its trigger-hysteresis properties. Separately, the antiviral activity of CeNPs and their compounds in vivo should be noted as a result of the effect of CeNPs on the cascade of reactions of a virus-infected organism. Nanoceria has an immunomodulatory effect and is involved in the ROS cycle. The role of CeNPs consists in its inclusion in the cascade of nonspecific protective reactions of the cell through interaction with biologically active molecules produced by the body under conditions of inflammation, and affect the activity of the kinase cascade and the NF-kB. Another component of the antiviral activity of CeNPs is its oxidoreductase activity and the ability to regulate the formation and destruction of reactive oxygen species. The ability of CeNPs shown by us to interact with biologically active molecules – interferon, tumor necrosis factor – is the basis for the use of CeNPs as a carrier and enhancer of the action of drugs in antiviral therapy. From the point of view of the prevention of viral infections, we have shown the promise of using CeNPs as a component of vaccines (adjuvant) using a model of influenza vaccine. Taking into account the features of CeNPs binding to biologically active molecules, as well as its ability to regulate the formation and destruction of reactive oxygen species, its use as a component of diagnostic test systems for viruses is shown to be promising. The described range of complex effects of CeNPs in virology indicates its multifaceted role in the implementation of the interaction between the virus and the sensitive object, which opens up prospects for its versatile application in the prevention, therapy, and diagnosis of viral infections.

Section 2

Veterinary Virology

Oncolytic potential of rhabdoviruses

Buchatskyi L.P., Zaloilo O.V., Rud Yu.P.
Institute of Fisheries of the National Academy of Agrarian Sciences

More than 40 viruses from 10 different families have oncolytic properties, many of them are undergoing clinical trials, and some are already approved for use. Since 2001 in Japan, the oncolytic vaccine DELYTACT, based on a modified herpes virus, is approved for the treatment of malignant gliomas; in China, in 2005, the Oncorine vaccine, based on a genetically modified Ad (H101) adenovirus, was approved for the treatment of nasopharyngeal carcinoma. In 2015, the oncolytic vaccine Talimogene laherparepvec (T-VEC) was approved in the USA for the treatment of post-operative melanoma recurrences. This vaccine was created with the help of genetic engineering on the basis of the herpes virus (HSV-1), in the genome of which the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene was inserted to enhance the immune response. In addition to the United States, T-VEC is currently approved for use in Israel, Australia, and Europe. Oncolytic rhabdoviruses of animals, especially those that are not pathogenic for humans, have attracted much attention of researchers in recent years.

Among animal rhabdoviruses, the largest number of works on oncolysis was performed on the model of viruses from the genus *Vesiculovirus.* such as vesicular stomatitis virus (VSV), Maraba, Morreton and Jurona viruses. VSV has been shown to be highly effective against malignant glioma, melanoma, hepatocellular carcinoma, breast adenocarcinoma, certain leukemias, and prostate tumors. To strengthen the oncolytic properties of the Maraba virus, two mutations in the sequence of proteins M and G were introduced into its genome using genetic engineering methods. This strain of the Maraba virus was named MG1. Compared to wild-type (wt) and other mutant strains of Maraba virus, it has faster replication and increased ability to kill tumor cells. With the help of genetic engineering methods, a chimeric virus was created on the basis of the VSV and the Maraba virus, which combined the capabilities of a wider range of hosts of the VVS and the safe properties of the Maraba virus, due to the peculiarities of the structure of the glycoprotein G of this rhabdovirus. Other animal rhabdoviruses, such as baraviruses BGV and MSV, also have oncolytic properties.

Spring carp viremia virus (SVCV), like vesiculoviruses, is not pathogenic for humans, accumulates in large quantities *in vitro*, therefore its use as an oncolytic agent is of great interest. Under the influence of this virus, CPD occurs 48 h after infection in cells obtained from carp epidermal tumor (EPC). Infected cells are rounded, then detached from the substrate and lysed. Among the SVCV isolates isolated by us in different regions of Ukraine, the highest infectious titers in EPC cells ($10^{6.0\text{-}7.5}$ $TCID/ml^{-1}$) were observed in the West, East and Cherkasy isolates.

Measures for containment of African swine fever in conditions of war

Dudnyk Ye.O., Fotina T.I.
Sumy National Agrarian University, Sumy, Ukraine

When considering the ways of spreading the virus, the impact of the armed aggression of the Russian Federation on the main risk factors for the spread of the ASFV was revealed. In our opinion, wild boars, the number of which has increased over the past year and which is not regulated by hunters due to the hunting ban, remain a particularly dangerous way of spreading the virus. Hostilities, shelling and the movement of military equipment through the ASF-affected regions directly affect the migration processes of disturbed wildlife, which can quickly spread the virus over long distances, spread it within the population and transmit it to the domestic livestock. An important anthropogenic factor in the spread of the disease is the chaotic contamination of military base areas with unprocessed food residues that may contain a viable virus and, together with other fomites, contaminate the environment. Therefore, state anti-epizootic measures and methods of monitoring infectious diseases should be updated to reflect the realities of today.

The main reason for our proposed additions to the Instruction on the prevention and control of African swine fever was the war in the country, which affected not only the indicators of the intensity of production in the pig industry but also the epizootic situation regarding infectious diseases. The impossibility of implementing several measures to prevent ASFV, specified in the Instructions, forces the creation of alternative ways of protecting pig farms and forestry from the risk of introducing the African swine fever virus and other dangerous diseases.

We developed an updated strategy to prevent the introduction of the African swine fever virus into the territory of pig farms, taking into account the risk factors that arose based on the full-scale military invasion of the Russian Federation into the territory of Ukraine. Having analyzed the Instruction, we identified a number of biosecurity rules, the observance of which has become much more difficult and set out the developed system of veterinary and sanitary measures in the conditions of war in several rules:

1. Treat roads with disinfectants in case of movement of military and civilian equipment past the private sector and the territory of farms.
2. Collect and dispose of food waste in military trenches.
3. In case of contact of servicemen with pigs, quarantine the animals.
4. When pigs run out of the territory of the farm in case of violation of the integrity of the premises, quarantine the animals before returning to the main herd and restore the fence as soon as possible.
5. Do not feed wild animals within the settlement.

Reproductive and antigenic properties of the *Teschovirus A* Dniprovskyi 34 strain under the influence of metal and non-metal nanoparticles

Anatoly M. Golovko, Stanislav V. Derevianko
State Scientific Control Institute of Biotechnology and Strains of Microorganisms, *Kyiv, Ukraine*
(admin@biocontrol.com.ua)

Modern scientific achievements in the field of nanotechnology open wide prospects for the production and use of nanoparticles (NPs) of nanometals and nonmetals in the field of veterinary medicine

The threshold limit value for bentonite NPs is 500 μg/cm^3, for Al_2O_3 and CeO_2 NPs – 100 μg/cm^3, Ni – 50 μg/cm^3, V – 25 μg/cm^3, Al – 20 μg/cm^3, for Ti NPs was 12.5 μg/cm^3, NPs Zn, Co and composition of nanoparticles of S and I – 5 μg/cm^3, composition of NPs Se + I – 0.5 μg/cm^3.

According to the prophylactic and treatment schemes, bentonite, Al_2O_3, CeO_2 and Ce nanoparticles showed the most pronounced antiviral activity against the strain of PTV-1 virus Dniprovskyi 34 among the studied NPs, reducing the virus titer by 1,50–2,00 lg TCD_{50}/cm^3. It has been found that bentonite, Al_2O_3, CeO_2 and Ce NPs have therapeutic index 2. According to the virucidal scheme nanoparticles of Ni, Ti and the composition of S and I NPs showed high virucidal activity against the strain of PTV-1 Dniprovskyi 34, significantly reducing its infectious titer in transplantable culture of porcine embryonic kidney cells by 2,46; 4,46 and 5,00 lg TCD_{50}/cm^3, respectively, with TI is 4, 8, and 8, respectively.

It has been established that NPs of Ce, Ti, S and I can adsorb on the surface of virions, while the shape and size of virions do not change. Under the influence of Ce, Ti, Ni, V, S and I nanoparticles, spherical virions of the icosahedral symmetry type with a size of 14–22 nm were found, which is much smaller than the sizes of native virions. In addition, virions of round irregular shape with dimensions of 26–28 nm, which by the nature of contrast can be destroyed virions or structural elements of virion capsids have been found.

Under the influence of nanoparticles, viruses retain their antigenic properties. The introduction of the studied nanoparticles into the antigen causes a decrease in the titers of virus-neutralizing antibodies, however, they remained at a high level.

Nanoparticles of Ni, Ti and the composition of nanoparticles of S and I are recommended for the development of antiviral drugs based on them. Based on the results of research, a database on the effects of nanoparticles on the biological and antigenic properties of *Teschovirus A* has been developed, which can be used in the development of antiviral drugs, which is a promising area for further research. Methods of inactivation of viruses by nanoparticles are protected by patents of Ukraine for inventions.

SARS-CoV-2 and expansion in animal species

Zinaida Klestova, Prof., Dr.
Institute for Medical Virology and Epidemiology of Viral Diseases, Germany
(zinaklestova@gmail.com)

The COVID-19 pandemic had a severe negative impact on human populations and national economies around the world. However, little is known about where the SARS-CoV-2 reservoir is located and how the virus infects wild and domestic animals. SARS-CoV-2 strains, their pathogenicity and infectivity are changing, but the ability to spread rapidly and harm human health remains. In addition, the virus is already circulating among animals, in different regions of the world. The range of the virus has expanded and there are sufficient conditions for its evolution. Some important questions remain unanswered: What are the natural reservoirs of SARS-CoV-2? Which animal species have transmitted the virus to humans? Today, since the outbreak of COVID-19, there have been numerous reports of SARS-CoV-2 infection in animals from 2021. The first case of SARS-CoV-2 in pets (in a dog) was officially reported to the WOAH (former OIE) (in report 1 31/05/2021 2021), where a total of 101 cats and 85 dogs were reported as outbreaks worldwide. In the latest WOAH report (30/06/2023), 29 animal species in 36 countries were reported to have SARS-CoV-2 (out of 775 outbreaks). Why has this happened? For all viruses, there is an optimal temperature at which they replicate efficiently in sensitive cells or when stored in the environment. SARS-CoV and SARS-CoV-2 show strong temperature-dependent differences in replication kinetics in cell culture. "Importantly, the significant increase in replication of SARS-CoV-2 at 33◦C likely confirms the enhanced upper respiratory tract replication and transmissibility of SARS-CoV-2 compared to SARS-CoV" (Herder et al., 2021). It is known that the normal body temperature of most mammals is 37-39◦C as the homeothermic temperature, according to other authors 36-38◦C (Hickman et al., 1984). We analyzed the range of body temperatures of animals in which SARS-CoV-2 was detected. It is noticeable that in some animals there are quite wide ranges of fluctuations in body temperature. Our review of the data showed that some animals have a low body temperature, below 37-39◦C. Low temperature is better for SARS-CoV-2 replication and interaction with ACE2 (Hoffmann et al., 2021) than higher temperatures. We can speculate that SARS-CoV-2 and related respiratory beta-coronaviruses can replicate and spread efficiently in animals with low body temperatures (around 33◦C). Low temperatures promote the interaction between SARS-CoV-2 Spike and ACE2, and ACE2 binding to the cell surface of SARS-CoV-2 Spike was higher at low temperature (4◦C) compared to 37◦C (Wan et al., 2020). the animals in which SARS-CoV-2 was detected, having a low body temperature between 30 and 37◦C and expressing a suitable ACE2 receptor, could potentially represent reservoirs of the virus. **Conclusion.** Many warm-blooded animals may be suitable hosts for the replication of SARS-CoV-2 and may transmit the virus to other susceptible animals or possibly to humans. We have outlined the possible environmental factor (temperature) and ways in which the virus can spread and circulate in wildlife. An important factor in the carriage or successful replication of the virus may be the animal's body temperature and its fluctuations.

The potential of metagenomics in studying fish viruses in Ukraine

Nataliia Matviienko, Viktoriia Shepelevych
Institute of Fisheries of NAAS of Ukraine, Kyiv, Ukraine
(mnarine73@ukr.net, vshepelevich@ukr.net)

Ukraine has a significant potential for the development of aquaculture for domestic market and in the future may become an important fish product exporter to Europe. The traditional objects of aquaculture in Ukraine are cyprinid fish, sturgeons, and rainbow trout. The military aggression and blowing up of the Kakhovka Hydroelectric Power Plant exacerbated the problems in the fishery. Metagenomics is a new field of study of fish viruses. This method is usually applied to study genomic multiformity, however, it also may be used to study fish disease pathogens. Compared to other methods, including PCR, metagenomics demonstrates higher efficiency and precision in deciphering of genomes of fish viruses (Yozwiak, 2012). Among viral diseases of fish in Ukraine were found: infectious pancreatic necrosis virus (IPNV), infectious hematopoietic necrosis virus (IHNV), viral hemorrhagic septicemia virus (VHSV), spring viraemia of carp virus (SVCV), piscine orthoreovirus (PRV), Acipenser iridovirus – European (AcIV-E), and carp edema virus (CEV) (Buchatskyi, Rud, Matviienko, 2020; Rud., 2021). Metagenomics is also important in genomic selection for the identification of potential genes-candidates for programs of selective breeding to form lines and breeds resistant to fish diseases (Bassi, 2022). Metagenomics may be used for diagnostics, prevention, and treatment of viral fish diseases and is an up-to-date sector in the Ukrainian fishery.

Fish viruses are a hidden threat to national aquaculture

Yu. Rud, L. Buchatsky

Laboratory of biotechnology in aquaculture, Institute of fisheries of the National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine

Today, aquaculture is the fastest-growing sector of livestock production, and according to FAO estimates, by 2028 the production of fish, crustaceans and mollusks will exceed the volume of its world capture. The development of modern aquaculture is driven by the profitability of the industry (over 180 billion USD annually) and the demand for high-quality protein. One of the main challenges in aquaculture is infectious diseases, especially viral etiology. Fish and shrimp viruses cause losses in aquaculture of more than 15 billion USD every year. In Ukraine, the epizootic situation regarding viral diseases on fish farms is challenging. The reasons for the spread of fish viruses are the uncontrolled importation of fish and their eggs, trade between farms within the country, poor water management, the lack of immunoprophylactic and therapeutic solutions on the national market, insufficient interaction between stakeholders and government organizations.

Fish viral diseases in Ukraine could be divided into three main groups depending on the areas of aquaculture: carp, salmon and sturgeon farming. The greatest diversity of viruses is found in the salmon group. Among the four trout viruses detected in Ukraine, two are the OIE-listed, namely Viral haemorrhagic septicemia virus (VHSV) and Infectious haematopoietic necrosis virus (IHNV), which belong to the *Novirhabdovirus* genus *Rhabdoviridae* family. Aquatic birnavirus Infectious pancreatic necrosis virus (IPNV), which mainly affects young salmonids, circulates on farms due to its properties of vertical transmission. An emerging disease for salmon farming in Ukraine is Pescine orthoreovirus (PRV), namely its third type PRV-3, detected in rainbow trout. In carp aquaculture, the Spring viremia of carp virus (SVCV), *Sprivivirus* genus of the *Rhabdoviridae* family, is traditionally the most devastating. Carp rhabdovirus is listed by the OIE, and in addition to common carp, it can infect other commercial carp species such as grass carp and silver carp. Carp edema virus (CEV), family *Poxviridae*, has been monitored in Ukraine since 2018. In 2023 CEV was included in the OIE list. The virus rarely leads to acute infection and high mortality, but causes a destructive effect on the fish immunity, contributing to its suppression and the development of secondary infections. In sturgeon farming, the iridovirus Acipenser iridovirus European (AcIV-E) has been detected from several sturgeon species in different geographical zones of Ukraine. The most sensitive species to AcIV-E are Siberian and Russian sturgeons.

A potential threat to carp farms can be widespread Cyprinid herpesvirus 3 (CyHV-3 or KHV) and Common carp paramyxovirus (CCPV), cases of which are increasingly being heard in Europe. Both viruses can be introduced into Ukraine together with imported fish stocking material.

Egg microbial environment influence on syncytin-like endogenous retroviral envelop ALV-*env* gene expression level during *Gallus gallus* early embriogenesis

I.Teplova[1], O.Zhuk[1,2], I.Skrypkina[1,2], V.Shablii[1,2]

[1]The Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine, 150, Zabolotnogo Str.,Kyiv, Ukraine, 03143

[2]Institute of Cell Therapy,3 Kosmonavta Komarova Ave.,03680 Kyiv, Ukraine

(i.teplova@kau.edu.ua)

Retrotransposons are genetic structures that originate from retroviruses and, after endogenization, remain in host genomes. Recent research (Janoušek et al., 2016) has shown that these structures play a crucial role in regulating various functions in Chordata species, ranging from the formation of the immune system to embryonic development. Furthermore, environmental factors, such as the local microbial influence on parental organisms and the breeding location, have a profound impact on transposon activity.

In our studies, we use *Gallus gallus* as a model organism. The chicken *env*-gene, we previously detected and identified, phylogenetically belongs to the ALVE branch of transposons and has an already described ORF in its genome.

Considering the common origin of other chicken LTR-structures and our identified env-gene, we conducted an investigation to determine whether it exhibits similar expression changes as other members of the *Gallus gallus* alpha-genera retrotransposon family during a reduction in bacterial levels within eggs. To achieve this, we performed decontamination of the eggs using broad-spectrum antibiotics, which had no discernible impact on chicken development. On the fifth day of embryo growth, we extracted total mRNA and conducted qRT-PCR for the ALV-*Env* gene using EIF3I as the reference gene. The results were statistically analyzed using the Kolmogorov-Smirnov test.

After data analysis we found no evidence of ALV-env gene expression dynamics when compared with the control group. This suggests that the structure is highly conserved in its role, and external biotic factors, such as bacteria, do not influence its activity.

Section 3
Viruses of Bacteria

Recombinant EPS-depolymerases of phage origin as promising tools against plant diseases caused by phytopathogenic bacteria

Yu. Faidiuk[1,2,3], M. Zlatohurska[2,4], N. Shenderovska[2,5], S.M. Moroz[2], T. Gorb[2], L. Romaniuk[2], A. Kushkina[1,2], and F. Tovkach[2]

[1] Laboratory of Phage Molecular Biology, Hirszfeld Institute of Immunology and Experimental Therapy, PAS, Wrocław, Poland; [2]Institute of Microbiology and Virology of the NAS of Ukraine, Kyiv, Ukraine; [3]Department of Microbiology and Immunology, ESC "Institute of Biology", Taras Shevchenko National University of Kyiv, Kyiv, Ukraine; [4]Group of Structural Virology, Central European Institute of Technology, Masaryk University, Brno, Czech Republic; [5]YuriaPharm LLC, Kyiv, Ukraine;

(i.v.faidiuk@gmail.com)

Bacterial exopolysaccharide (EPS), a major component of cell capsule and\or biofilm matrix, efficiently protects the bacteria against environmental factors, antibiotics, disinfectants, and phages, as well as allows bacteria to avoid host defense mechanisms. In the course of plant infection it can be heavily overproduced by a phytopathogen so eventually it obstructs plant vascular system vessels leading to wilting and plant death. Application of phage-coded enzymes, exopolysaccharide depolymerases (EPS-Depos), may be a promising approach for biocontrol in such cases. EPS-Depos are phage structural proteins that hydrolyse capsular or biofilm EPS allowing the phage to reach its susceptible receptor on the bacterial cell surface. Once devoid of EPS due to the natural activity of EPS-Depos, the pathogen decreases its virulence and reduces its resistance to external factors. Obtaining such recombinant phage-based products with targeted action and environmental safety is of specific interest nowadays. Recently we have sequenced the genomes of three broad-host-range bacteriophages E105, TT10-27 and KEY, active against plant-infecting bacteria of *Erwinia* and *Pantoea* genus, including a fire-blight disease causing agent, *Erwinia amylovora*. EPS-Depos genes were detected in all three genomes (coding for proteins of 93.50, 95.5 and 111.5 kDa, respectively). DepoE105, DepoTT10-27, and DepoKey were cloned into pET22b(+) vector. Due to the large size of the proteins, soluble forms were obtained only after testing a large set of expression conditions. These eventually were selected to be: co-transformation with pGro7 chaperone plasmid, induction with 0.05 mM IPTG at OD 0.4, 20°C for 17 hours; autoinduction media, and\or OverExpress(tm)C41(DE3) expression strain. When applied on the lawn of host bacteria in conditions that stimulate EPS-overproduction, all three EPS-Depo proteins were efficiently degrading the EPS of *E. billingae* and *P.agglomerans,* however, did not reveal a substrate specificity against *E.amylovora* EPS. This important observation will be used to improve strategy for selection of perspective candidates of EPS-Depo enzymes. Stability testing, structural studies, and substrate specificity testing against EPS of other closely-related species will be conducted in order to propose our recombinant EPS-Depos as antimicrobial formulations.

The prokaryotic viruses infect functional microorganisms in carbon cycle in anaerobic digestion of biowastes

Liping Hao[1], Zijun Li[2], Bihe Zhu[3]

[1]School of Earth System Science, Tianjin University,Tianjin, China.

(haoliping@tju.edu.cn)

Anaerobic digestion of food waste produces methane, which can be collected and used as clean energy, and can thus reduce carbon emission into the atmosphere. This process can help to achieve the “carbon peaking and carbon neutrality” goals, and has been used nationwide, especially after the mandatory municipal solid waste classification policy was implemented in China. In essence, microorganisms drive the carbon cycle through interaction between different species, which are mostly uncultured and unknown members in this system. Inadequate understanding of these uncultured microbial players hinders process innovation and breakthrough of the technical bottlenecks, such as process instability and low methane-production efficiency, especially for the transformation of acetate to methane as a rate-limiting step. These microorganisms are sensitive to the fluctuating environmental pressure caused by changes of organic loading rate, accumulated ammonia, VFAs, pH, temperature, as well as some unclear biological controlling factors, like bacterial and archaeal phages. In this work, the prokaryotic viruses involved in methanogenic conversion of food waste in industrial anaerobic digesters were studied. An optimized pipeline was established to simultaneously analyze the viruses and the bacterial and archaeal hosts, combing metagenomic, metatranscriptomic methods and stable isotope signature. It is shown that microbial players involved included diverse syntrophic acetate oxidizing bacteria and archaeal methanogens, forming an important functional guild in such systems. Various viruses infecting these microbial members were predicted from the virome, the abundance of which was one order of magnitude higher than that of the prokaryotes. And most of these viruses were novel. Several of them demonstrated similar shapes as members of *Acidianus* two-tailed virus (ATV, Bicaudaviridae), *Sulfolobus* spindle-shaped virus 1 (SSV1, Fuselloviridae). The auxiliary metabolic genes (AMG) involved in syntrophic acetate oxidation and methanogenesis were found in the their genomes, indicating a regulation of these metabolisms by AMGs, and an interaction between the prokaryotic hosts and the phages.

Pathogenic microbiota correlating with the purulent-inflammatory processes after gunshot wounds, bone fractures and mine-explosive injuries during the war activities in Ukraine

A. Kharina[1], H. Snihur[1,2], O. Andriichuk[1], I. Pozhylov[1], I. Budzanivska[1], N. Korniienko[1], O. Liutko[3], O. Linenko[4], I. Moysa[4], V. Poniatovskyi[5], O. Shevchenko[1]

[1]Virology Department, ESC 'Institute of Biology and Medicine', Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

[2]D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Ukraine

[3]The Laboratory of Microbiology and Chemotherapy of the State Institution 'ITO NAMNU'

[4]The Department of Bone and Purulent Surgery of the State Institution 'ITO NAMNU'

[5]Department of Microbiology, Virology and Immunology of the National Medical University named after O.O. Bogomolets

(alexshevchenko@ukr.net)

The problem of treatment of purulent-inflammatory processes after gunshot wounds, bone fractures and mine-explosive injuries of various nature, as well as other postoperative complications, has significantly worsened durting the last ~1,5 years of full-scale war activities in Ukraine following the russian aggression. Effective treatment of such injuries is complicated by the development of concomitant infectious pathological processes associated with a number of factors. Threatening rate of selection of antibiotic-resistant microorganisms prevents proper treatment if such patients requiring immediate activity. One of the most promising options is the use of bacteriophages as antibacterial agents. However, this entails prior dwelving into the composition of bacteria inducing such pathologies and complications. Here we report on the first outcomes of analysis of such associations of pathogenic microorganisms following the development of purulent antibiotic-resistant complications for patients with gunshot and mine-explosive injuries, and postoperative complications. Pathological material was collected from patients with bone-purulent pathology resulting from gunshot or mine-explosive trauma or surgical interventions. Our data indicate that *Staphylococcus aureus* remains the major causative agent inducing the development of purulent post-surgical complications and, most probably, is of endogenic origin. In parallel, large portion of gram-negative bacteria has also been noted including *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter baumannii*. These bacteria are considered to originate exogenously but are not unique in such microbial associations. These were mostly resistant to currently used antibiotics requiring further virological input in terms of selecting specific lytic phages.

Funding: This research has been funded by the National Research Foundation of Ukraine (grant No. 2022.01/0065 'Bacteriophages as biological agents for the control of bacterial complications in victims of gunshot and mine-explosive injuries').

Head assembly of phage phi812 in *Staphylococcus aureus* cells

M. Zlatohurska[1, 2], M. Procházková[2], T. Füzik[2], and P. Plevka[2]

[1]Department of Bacteriophage Molecular Genetics, D. K. Zabolotny Institute of Microbiology and Virology of the National Academy of Science of Ukraine, Kyiv, Ukraine;

[2]Group of Structural Virology, Central European Institute of Technology, Masaryk University, Brno, Czech Republic

(maryna.zlatohurska@ceitec.muni.cz)

The lytic phage phi812 from the family *Herelleviridae* has a broad host range, including antibiotics-resistant and biofilm-forming *Staphylococcus aureus* strains. This microorganism is an opportunistic pathogen causing a broad spectrum of human diseases. It has wide potential for acquiring antimicrobial resistance. Furthermore, biofilm-forming strains of *S. aureus* contaminate medical devices and cause chronic infections. Phage phi812 is a suitable candidate for the treatment of staphylococcal infections. However, it has not been approved for general clinical practice because of the insufficient understanding of many aspects of phage biology. To understand the mechanism of phage phi812 assembly, we inspected infected cells by cryo-electron microscopy. We used focused ion beam milling for the preparation of electron-transparent lamellas from infected *S. aureus* cells and cryo-electron tomography for three-dimensional reconstructions of the cell content, followed by sub-tomogram averaging of the phage assembly intermediates. The phage head assembly starts 15 minutes post-infection (m.p.i.) at the inner surface of the cytoplasmic membrane. In a short time (30 m.p.i.) the cell is fully packed with phage assembly intermediates structures, empty, filling, and genome-containing heads and tails connected to fully packed heads. Sub-tomogram averaging of the assembly intermediates revealed distinct classes of phage heads that differ in size and surface features. Our results provide *in situ* structural insight into the phage phi812 head formation in near-native conditions.

Section 4

Plant Viruses

Study of the potato gene pool for resistance to viral diseases

Bondus R.[1], Taran O.[2], Dashchenko A.[2], Boyko O.[2], Mishchenko L.[3]

[1]*Ustymivka Experimental Station of Plant Production of the Plant Production Institute Named after V.Ya.Yuriev of the National Academy of Agrarian Sciences of Ukraine);* [2]*Faculty of Plant Protection, Biotechnology and Ecology of the National University of Life and Environmental Sciences of Ukraine;* [3]*ESC "Institute of Biology and Medicine" of the Taras Shevchenko National University of Kyiv, Ukraine*

(bondus1971@gmail.com)

In the southern part of the forest-steppe of Ukraine, the most harmful for potato culture are viral diseases that cause rapid degeneration of varieties. It is obvious that in the future global warming of the climate will further complicate the situation. The collection of the Ustymivka Experimental Station of Plant Production of the PPI NAAS of Ukraine includes 675 cultivars that were tested for resistance to viral diseases. The methods of transmission electron microscopy (TEM), enzyme immunoassay DAS-ELISA and polymerase chain reaction (RT-PCR) were used. The 6 most common potato viruses (Potato virus X (PVX), Potato virus Y (PVY), Potato virus S (PVS), Potato virus A (PVA), Potato virus M (PVM) and Potato leaf roll virus (PLRV) and confirmed the data of many years of visual studies. Varieties resistant to wrinkled mosaic have been identified: Kardula, Pamir (Germany); Iskra, Galina (Czech Republic); Magura (Romania); Igor (Yugoslavia); Marijke, Desiree, Ostara, Debora, Radosa, Resy (Netherlands). Varieties with relative and high resistance (7.0-8.5 points) to potato leaf curl virus were also selected: Crystal, Ada, Bzura, Sprint, Aguila, Roxy, Shwalbe, Tempora, Turbella, Apta, Kardula, Iskra, Galina, Jaerla, Sante, Grata, Kardinal, Bintij, Ostara, Debora, Resy, Bintje, Buesa, Capella, Dryf, Gallina, Grata, Ilse, Leda, Lutetia, Mansour, Runo, Petrovsky, Stepnyak, Yagidka. Varieties with high and increased resistance to striped mosaic (7.5-8.5 points) were selected: Rakurs, Ada, Sprint, Apta, Agwila, Augusta, Carla, Eros, Grata, Lori, Luna, Oda, Ponta, Shwalbe, Feldeslohn, Desiree, Ostara, Radoza, Saturna, Electre, Maritta. The reason for the seed potato degeneration in the southern part of the forest steppe of Ukraine is unfavorable weather and climate conditions that occur during spring planting, as a result of which weakened plants are more likely to be depressed by a viral infection. The dependence between weather conditions, virus infection of plants, and their productivity was noted. Our research demonstrated that significant deviations from the average long-term temperature and precipitation rate increased the intensive manifestation of viral diseases. Under favorable soil and climatic conditions, viral diseases did not appear visually. As a result of the conducted research, it was established that the main factor in the resistance to degeneration of potato varieties is the genotype, and its reaction rate depends on the growing conditions. Even under unfavorable exogenous factors, the genotype of individual varieties was able to realize its potential and counteract viral diseases. For the implementation of breeding, scientific and other programs, a working reference collection of potato varieties based on resistance to viral diseases was formed, which includes 34 samples originating from 10 countries. This collection includes sources and donors of virus-resistance traits and is valuable for use in breeding programs.

Viruses of cucurbit crops: a complex and rapidly evolving situation

C. Desbiez
INRAE, UR407 pathologie végétale, 84140 Montfavet, France

Cucurbits, particularly melon, watermelon, cucumber and squash, are important crops worldwide and are grown in very diverse agrosystems, from traditional gardens to heated glasshouses. Viruses can severely affect cucurbit production, altering both the yield and quality of crop. Their efficient vector transmission and the lack of curative treatment of infected plants make them difficult to control. Whitefly-transmitted viruses, particularly begomoviruses, have emerged worldwide in the last decades and now have a major impact in numerous contries, particularly in tropical and Mediterranean climates. Even among the “old” aphid-transmitted viruses, frequent long-distance spread of strains are observed. They can result in strain replacements and the possible emergence of recombinants, what can be challenging for resistance breeding programs. Recent exemples of strain or virus emergence will be discussed to illustrate the possible drivers and consequences of evolution of cucurbit-infecting virus populations.

Cherry leaf roll virus infecting black elderberry plants in Ukraine

A.A. Dunich [1], A.V. Dashchenko [2], L.T. Mishchenko[1], O.O. Molodchenkova [3], L.A. Hlushchenko[4], H.V. Korotieieva [1]

[1] *ESC “Institute of Biology and Medicine”, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine;* [2] *Faculty of Plant Protection, Biotechnology and Ecology, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine;* [3] *Plant Breeding&Genetics Institute-National Center of Seed and Cultivar Investigation, Odesa, Ukraine;* [4] *Research station of medicinal plants, Institute of Agroecology and Nature Management, Ukraine*

(korenevochka1983@ukr.net)

Cherry leaf roll virus [CLRV, *Nepovirus, Secoviridae*] has diverse host range including shrubs, trees, and annuals. Our previous studies showed that infection of black elderberry with the CLRV Eld1MP isolate detected in 2019 (Ac. No. MW370509) leads to a significant reduction sugar content and overall yield (Mishchenko et al 2021). In 2021-2023 black elderberry leaves from wild-grown plants with leaf rolling and mosaic symptoms were collected in Poltava, Kyiv regions and Kyiv city of Ukraine. The presence of CLRV in the leaf samples was confirmed by DAS-ELISA and RT-PCR. Using DAS-ELISA with specific antibodies against CLRV (Loewe, Germany), CLRV was confirmed in all symptomatic leaf samples. Total RNA was extracted from symptomatic leaves using GeneJet PlantRNA Puntification Kit. The obtained RNA was tested by two step RT-PCR using specific oligonucleotide primers (Werner et al. 1997) for amplifying the 412 bp fragment of the 3' untranslated region (3'UTR) of CLRV genome as described previously (Mishchenko et al. 2021). RT-PCR showed DNA amplicons of expected size (412 bp). Sanger dideoxy sequencing was performed. In total, 3' UTR region of sequences 412 nt for 8 different CLRV isolates were obtained and were compared with CLRV sequences in the NCBI GenBank database with the BLAST program. Analysis showed that 8 CLRV isolates have the highest nucleotide identities (90.8-99.8%) with isolates belonging to the phylogenetic group E (Rebenstorf et al., 2006). The presence of CLRV in elderberry plants and the results of phylogenetic analysis suggest that wild plant species may serve as a reservoir for it, posing an epidemiological threat in the country and requiring testing of a wider plant species range for this virus.

1. Mishchenko L., Dunich A., Molodchenkova O., Hlushchenko L. (2021) First report of cherry leaf roll virus from *Sambucus nigra* in Ukraine. J Plant Pathol, 103: 1077.
2. Werner R, Mühlbach HP, Büttner C (1997). Detection of Cherry leaf roll nepovirus (CLRV) in birch, beech and petunia by immuno-capture RT-PCR using a conserved primer pair. European Journal of Forest Pathology, 27(5): 309- 318.
3. Rebenstorf K., Candresse T., Dulucq M.J., Buttner C. Obermeier C. (2006) Host species-dependent population structure of a pollen-borne plant virus, Cherry leaf roll virus. J Virol, 80 (5): 2453-2462.

Enhancement of antiviral RNAi mediated by host kinases subverting a viral virulence protein through phosphorylation

Huaibing Jin[1], Daowen Wang[2], and Xifeng Wang[1]

[1] *State Key Laboratory of Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China;* [2] *College of Agronomy, State Key Laboratory of Wheat and Maize Crop Science, Henan Agricultural University, Zhengzhou 450046, China*

(jinhuaibing@caas.cn)

Viruses often usurp host machineries for their amplification, but it remains unclear if hosts may subvert virus proteins to regulate viral proliferation. Here, we show that the 17K protein, an important virulence factor conserved in barley yellow dwarf viruses (BYDVs) and related poleroviruses, is phosphorylated by host GRIK1-SnRK1 kinases, with the phosphorylated 17K (P17K) capable of enhancing the abundance of virus-derived small interfering RNAs (vsiRNAs) and thus antiviral RNAi. Furthermore, P17K interacts with barley small RNA-degrading nuclease 1 (HvSDN1) and impedes HvSDN1-catalyzed vsiRNA degradation. Additionally, P17K weakens the HvSDN1-HvAGO1 interaction, thus hindering HvSDN1 from accessing and degrading HvAGO1-carried vsiRNAs. Importantly, transgenic expression of 17K phosphomimetics ($17K^{5D}$), or genome editing of SDN1, generates stable resistance to BYDV through elevating vsiRNA abundance. These data validate a novel mechanism that enhances antiviral RNAi through host subversion of a viral virulence protein to inhibit SDN1-catalyzed vsiRNA degradation and suggest new ways for engineering BYDV-resistant crops.

Characteristics of cucumber mosaic virus isolate from phlox plants

Hanna Korotieieva
Taras Shevchenko National University of Kyiv, 64/13, Volodymyrska Str., Kyiv, Ukraine, 01601
(korotyeyeva@ukr.net)

Industrial production of ornamental plants is one of the most popular and profitable business in many countries. In Ukraine this industry also shows positive dynamics. However, viruses can significantly affect both the decorative qualities of plants and quality of seeds. With the increase in the share of ornamental plants in the horticulture of Ukraine, the need for further study of viruses that can affect these crops and cause significant damage also increases. Screening of plants on virus diseases in the collections of M.M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine showed that plants of the genus *Phlox* were the most infected. Identification of viruses was carried out using electron microscopy, plant-indicator method, DAS-ELISA tests using commercial serums for CMV, TSWV, AMV, TRV, ArMV. It was confirmed the infection of *Phlox paniculata* plants by Cucumber mosaic virus. Phylogenetic analysis of the capsid protein gene revealed that the Ukrainian isolate of CMV collected from *Phlox* has a similarity with the isolates associated with vegetable and ornamental crops from France, Poland, the Netherlands, Canada and Japan and belongs to serotype II.

Liposomal strategy in growing, recovering and protecting plants against viral infections

O.G. Kovalenko

D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, 154 Acad. Zabolotny Str., Kyiv, 03143, Ukraine

Liposomes as artificial structures-vesicles, obtained by methods of supramolecular chemistry, have been known for a long time and are already quite successfully used in medical practice for targeted delivery of various drugs. For the formation of liposomes, diphilic substances such as phospho- or glycolipids, in particular rhamnolides, are used. In the latter case, cholesterol is usually used to neutralize carboxyl groups of fatty acids. In our work with lyosomes based on rhamnolipids, we used thiosulfonates for the first time (Kovalenko et al., 2023).

Although drugs in lyosomal form have been used in medical practice for quite some time, the liposomal strategy is almost unknown in the agricultural sector, in particular in crop production. At the same time, among foreign publications, our work was not the first to show the possibility of virus elimination from infected bean callus in the process of passage on a medium containing LS-forms of glycans (Kovalenko et al., 2019). And before that, we showed a fairly high effectiveness of the drugs during the natural spread of viral infection on soybeans and beans in field conditions (Kovalenko et al., 2017; 2023, work in this collection). In addition to the pronounced prophylactic and curative antiviral effect of the complex drugs, we noted an increase in the vital activity of the rhizobial microflora of leguminous crops. This was accompanied by the activation of the formation of "factories" of organic nitrogen - nodules as a result of the increase in plant productivity (Kovalenko et al., 2017). Phosphate-mobilizing bacteria introduced into the azome with glycine drug forms were similarly activated (our message in this book).

Thus, the liposomal strategy is quite promising for use in crop production, as it results, on the one hand, in the improvement of the ecological situation in the agrocenosisx and wildlife, and on the other hand, in the strengthening of metabolic processes in plants, which leads to an increase in the productivity of the latter.

Today, we can confidently say that we have the latest GLYCAPS nanotechnology, as well as drug forms developed on the basis of *Ganoderma adspersum, Candida maltosa, Tremella mesenerica* glycans and their mixtures, encapsulated in the liposomes we received and in combination with biological drugs, which are in effective doses are non-toxic to plants. GLYCAPS preparations do not pollute the environment and do not have a harmful effect on warm-blooded animals and humans. GLYCAPS products are recommended for the recovery of plants affected by viruses that reproduce vegetatively. It can be successfully used for microclonal propagation of plants, including potatoes, strawberries, grapes, and other crops. Glicapsis can be effective during the seedling period on tobacco, tomatoes, peppers, eggplants, etc. It can also be used to regenerate healthy plans, as well as as prophylactics to prevent secondary infections in regenerants.

Kovalenko O.G., Vasilev V.M. et al., ***Dopov. Nac. acad. nauk Ukr***, 2017, 1: 88-96.

Kovalenko O., Kyrychenko A.,Kovalenko O**.** ***J. Bot. Res.*** 2019, **1,** 02: 9-24.

Kovalenko O., Kyrychenko A.,. Lubenets V. ***Biol. plant***. 2023, **67**: 159-165.

Recovery of virus infected *Phaseolus vulgaris* L. by glycan liposomal forms and bacterisation by *Bacillus mesentericum* IMV b-7287

O.G. Kovalenko, A.M. Kyrychenko, N.A. Yamborko

D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, 154 Acad. Zabolotny Str., Kyiv, 03143, Ukraine

In the Forest-Steppe region of Ukraine, *Phaseolus vulgaris* L. crops can be affected by a number of viruses, the most widespread of which is the bean common mosaic virus (BCMV) (Kyrychenko and Kovalenko, 2018). The virus is transmitted from one generation to another through bean seeds with up to 80% efficiency, making seed quality improvement critical to growing beans as a valuable food crop. Additionally, adequate phosphorus nutrition is essential for the development of field resistance to viral diseases.

In our study, we have used two approaches to improve grain quality: application of antiviral substances with complex action, in particular liposomal forms of glycans (LF-glycans), and/or pre-sowing bacterization of seeds with *Bacillus megaterium* IMV B-7287, capable of dissolving soil-insoluble organophosphates and making these substances available to plants, individually and in combination.. In the experiment, seeds were sequentially treated with LF-glycans and/or bacterized. The experiments were conducted on the experimental fields of the National Scientific Center “Institute of Agriculture”, NAAS of Ukraine on the *Ph. vulgaris* L. cv. Mavka, a variety that is highly susceptible to BCMV infection. LF preparations containing glucan of *Ganoderma adspersum* and its mixture with *Candida maltosa* mannan and *Tremella mesenerica* glucuronoxylomannan (GXM) prepared according to our methodology were tested (Kovalenko et al., 2022). After a 6-hour soaking of the seeds with the mentioned LF-glycans (50 mg/l), the seeds were bacteriized with a suspension of B. megaterium IMV B-7287 cells (based on a bacterial load of 106 CFU/seed). Then the seeds were dried organically and then sown in the soil. We conducted phenological observations of plant development, symptoms of viral infection, and recorded yield in the variant.

Our studies have shown that pre-sowing treatment of infected seeds results in significant recovery of plants from BCMV. Decreasing the number of infected plants under the influence of LF-glycans was accompanied by an increase in bean yield. Moreover, the complex seed treatment (LF-glycans + bacterization) provided the maximum effect compared to other variants (45 % of yield increase). The preparation containing mannan and GXM in addition to glucan had lower efficiency on beans. Generally, it is worth pointing out that the combined application of antivirals and phosphate-mobilizing bacteria is an effective and reliable way of obtaining a high yield of beans and other legumes. Undoubtedly, the methods tested in our study can be practiced in organic farming.

Kyrychenko A.N., Kovalenko A.G., Bean common mosaic in the Kiev region: etiology disease and pathoden identivication. Microbial J., 2018, 80: 96 –107.

Kovalenko OG, Vasilev VM, Adamchuk-Chala NI, Tytova LV, Karpenko EV. Antiviral Agents and Biological Preparations for Agriculture Based on Artificial Glycan-Glycolipid Complexes. J. Ethol. & Animal Sci 2022, 4(1): 119-123.

Highly conservative stem-loop series contained in the genomic sequences of plant viruses of the *Virgaviridae* family

A.M. Kyrychenko, I.S. Shcherbatenko
Institute of Microbiology and Virology, NASU, 154 Academ. Zabolotny St., Kyiv, Ukraine
(ivanshch@meta.ua)

Conservative stem-loops of viral genomic RNAs are becoming increasingly important for fundamental research and also for the development of new antiviral drugs. In this study a computer search and comparative analysis of conservative stem-loops in genomic sequences of 132 plant viruses of Virgaviridae family was carried out. The genomic sequences were downloaded from the NCBI website http://www.ncbi.nlm.nih.gov/. To identify stable stem- loops, containing 5 pairs of complementary nucleotides in their stems, the unfolded D1S3L3 stem-loop of TMV-L strain, GGGAuucgaauUCCC, was used as a consensus sequence. We found that the majority (more than 70%) of viruses of all six genera of the Virgavidae family contain stable stems, in which substitutions of only one or two base pairs relative to consensus are the most often encountered. Nucleotide substitutions in conservative stem-loops are compensatory, which maintain the complementarity of nucleotide pairs required for provide the secondary structure. Higher variability is manifested in loops: up to four nucleotide substitutions are detected in their sequences. Conservative loops have one to three localization positions in viral genomes that vary considerably relative to the 5' end of the genomic RNA. Relative to the 3' end, the stem-loops are localized in one to three narrow or wide ranges such as -23…-67, -80 and -25…-41, -27…-43 and -63…- 65 and others. Identical or very similar sequences of stem-loops have groups of viruses of a certain genus, which include different species and strains isolated from different plant species in ecologically different regions. Despite the expected high variability of stem-loop sequences in large groups of different viruses, 35 strains of 19 tobamovirus species have only 3 variants of these sequences (GGGG...CCCC, GGGA...UCCC and GAGG...CCCUC) out of 256 possible variants ($2^8 = 256$). The groups of related viruses identified by us, which include representatives of different species and strains isolated in different regions of the planet from plants of different families, but have similar conservative nucleotide motifs and substitutions, resemble the N. Vavilov’s homologous series – the groups of related plants with similar morphological features. Such groups of viruses indicate the possibility of parallel variability of their genomes at the molecular genetic (nucleotide) level, which was found only in eukaryotes. Very high stability of the D1S3L3 stem-loops in various genera of plant viruses against the background of a high frequency of mutations in genomic viral RNAs convincingly indicates the important role of conservative sequences and secondary structures in regulating virus-host interaction. The properties and localization positions of stable stem-loops that we have established in six genera of virgaviruses can be used as reference material for development of specific RNA-bases therapeutic strategies. Our search for conservative viral RNA sites using nucleotide sequences of unfolded stem-loops is of interest as a simple and cheap method, an alternative to the Mfold and SHAPE (the selective 2′-hydroxyl acylation analyzed by primer extension) methods.

Barley yellow dwarf virus-PAV in Ukraine: influence on wheat yield and grain quality

A. Kyrychenko[1*], S. Stognii[2], L. Holyk[3], H. Snihur[1,2]

[1]D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, 154 Acad. Zabolotny Str., Kyiv, 03143, Ukraine; [2]Virology Department, ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, 64/13 Volodymyrska Street, Kyiv, 01601, Ukraine; [3]The National Scientific Center "Institute of Agriculture, National Academy of Agrarian Sciences of Ukraine, 2-B Mashynobudovnykiv St., village Chabany, Fastiv district, Kyiv region, 08162, Ukraine

(a.kyrychenko@imv.org.ua)

Barley yellow dwarf virus-PAV (BYDV-PAV) is one of the most economically important virus of cereals worldwide belonging to genus *Luteovirus* of the *Tombusviridae* family of plant viruses. The genus *Luteovirus* also includes species: *Luteovirus mavhordei* (MAV), *Luteovirus pashordei* (PAS), *Luteovirus kerbihordei* (kerII) and *Luteovirus kertrihordei* (kerIII) (Miller 2022). BYDV-PAV infect wheat, barley, oat and some other crop causing significant yield losses in susceptible cereal varieties. In 2020, an outbreak of wheat disease was recorded on the experimental fields of the National Scientific Center "Institute of Agriculture NAAS, with the disease most pronounced in winter wheat (*Triticum aestivum* L.) cv. Poliska 90. Yield loss of the infected wheat was 30% compared to the average crop yield over the last 5 years, while the yield of the wheat cv. Lisova Pisnia St., used in the research as a standard, changed slightly (about 1% losses). The spikes of affected plants were discolored, with immature, small and often deformed grains, the amount of grain was also detectably decreased. Wheat leaves, showing symptoms of virus infection were randomly collected and tested for the presence of BYDV-PAV, BYDV-MAV and CYDV-RPV by TAS-ELISA (Agdia, USA). As a result, BYDV-PAV was detected in all collected samples, that subsequently was confirmed by reverse transcription-polymerase chain reaction (RT-PCR) using BYcpF/BYcpR primers (Kundu 2008). Resulting 641 bp amplicon was Sanger sequenced and sequence named as BYDV-PAV_Ukr, was submitted to GenBank (OK338686). The phylogenetic analysis showed that the BYDV-PAV isolated from wheat has the highest homology of 96 % to Estonian isolate from winter wheat (GenBank: MK012650). BYDV-PAV has been identified in Ukraine on the basis of serological detection and here we provide for the first time the results of molecular detection of BYDV-PAV distributed in Ukraine. It was concluded that wheat yield and grain quality in 2020 were significantly reduced by BYDV-PAV infection.

1. Miller WA, Lozier Z. Yellow Dwarf Viruses of Cereals: Taxonomy and Molecular Mechanisms. Annu Rev Phytopathol. 2022. 60:121-141.
2. Kundu JK. First Report of barley yellow dwarf virus-PAS in wheat and barley grown in the Czech Republic. Plant Dis. 2008. 92(11):1587.

Diagnostics of viruses and phytoplasmas in peony plants

L. Mishchenko[1], L. Konup[2], A.Konup[2], A. Dunich[1], A. Dashchenko[3], V.Chistyakova[2], N. Kozub[4], V. Gorobets[5], and T. Shcherbakova[5]

[1]ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, Kyiv, Ukraine;[2]National Scientific Centre «V.Ye. Tairov Institute of Viticulture and Winemaking», Odesa, National Academy of Agrarian Sciences of Ukraine;
[3]National University of Life and Environmental Sciences of Ukraine,Kyiv, Ukraine;
[4]Plant Protection Institute of National Academy of Agrarian Sciences of Ukraine
[5] M.M. Gryshko National Botanic Garden
(lmishchenko(@ukr.net)

In 2021-2023, peony plants exhibiting severe symptoms of severe leaf rolling and deformation, stunting, severe plant dwarfism and rarely mosaics were observed in Kyiv city. Using TEM filamentous virus-like particles 1200±50×12 nm in size were obtained in peony leaves var. Zhemchuznyi Rozsyp. Grapevine leaf roll-associated virus-3 (GLRaV-3) beloning to the genus *Ampelovirus*, was detected in these plants by DAS-ELISA and real-time PCR (Mishchenko et al., 2023). In 2022, grapevine fanleaf virus (GFLV) was detected in peonies var. Skarbnytia i Zhemchuznyi Rozsyp by DAS-ELISA. In 2023, diagnostics of GLRaV-1, GLRaV-3, and GFLV by DAS-ELISA (Agritest, Italy) was performed in 9 peony samples (var. Zhemchuznyi Rozsyp, Nezabutnya, Rashoomon, Yukon with severe symptoms, Yukon with mild symtoms, Judy Ann; Varvara, Myr, Slavutich). GLRaV-1 was revealed in peony plants var. Zhemchuznyi Rozsyp and Yukon. We also did not exclude the possibility of peonies being affected by a phytoplasma infection. In 2023, real-time PCR was performed for diagnostics of phytoplasma. Amplification was carried out using test system for phytoplasma detection (Qualiplante, France), universal primers UniRNA Forward/UniRNA Reverse for amplifying 16S rRNA gene, and fluorescent probe UniRNA Probe (FAM) (Hren et al. 2007). Analysis showed the absence of phytoplasmas in all tested peony varieties. So, diagnostics of peony plants in 2021-2023 revealed the presence of three viruses, namely GLRaV-1, GLRaV-3, and GFLV.

Mishchenko L.T., Konup L.O., Dunich A.A., Gorobets V.F., Konup A.I., Zaimenko N.V., Kozub N.O., Dashchenko A.V., Chistyakova V.L., Shcherbakova T.O., Sovinska R.S. First report of grapevine leafroll-associated virus-3 on peony plants in Ukraine. Journal of Plant Diseases and Protection. 2023. Vol.130: 189–198.

Hren M., Boben J., Rotter A., Kralj P.,Gruden K. Real-time PCR detection systems for Flavescence dorée and Bois noir phytoplasmas in grapevine: comparison with conventional PCR detection and application in diagnostics. Plant Pathology. 2007. Vol 56(5): 785-796.

The influence of *Prunus necrotic ringspot virus* (PNRSV) and *Cherry virus A* (CVA) on the fruit composition of sour cherry (*Prunus cerasus* L.) cv. Łutówka

E. Paduch-Cichal [1 *], E. Mirzwa-Mróz [1], T. Krupa [2], K. Staniszewski [1], W. Wakuliński [1], M. Wit [1], M.S. Szyndel [1]

[1]Section of Plant Pathology, Department of Plant Protection, Institute of Horticultural Sciences, Warsaw University of Life Sciences (WULS-SGGW), ul. Nowoursynowska 166, 02-787 Warsaw, Poland, [2]Section of Plant Pathology, Department of Pomology and Horticultural Economics, Institute of Horticultural Sciences, Warsaw University of Life Sciences (WULS-SGGW), ul. Nowoursynowska 166, 02-787 Warsaw, Poland

(elzbieta_paduch-cichal@sggw.edu.pl)

A survey was carried out on a commercial sour cherry fruit orchard located in Lublin province in Poland to determine the influence of viruses on the fruit quality of sour cherry cv. Łutówka. Leaf samples from trees of sour cherry cv. Łutówka were tested for *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Little cherry virus 1* (LChV-2), *Little cherry virus 2* (LChV-2), *Cherry virus A* (CVA), *Cherry green ring mottle virus* (CGRMV), *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry rasp leaf virus* (CRLV) and *Cherry mottle leaf virus* (CMLV) using the RT-PCR technique. The results indicated that the samples were infected by PNRSV and PNRSV+CVA. PDV, LChV-1, LChV-2, CGRMV, CNRMV, CRLV and CMLV were not detected in any of the tested sour cherry trees. The effect of virus infection on the chemical composition of sour cherry fruits was investigated. The anthocyanin, total phenolic and vitamin C contents and the antioxidant activity were evaluated. The total phenolic compound and vitamin C contents and the antioxidant activity were significantly higher in PNRSV- and PNRSV+CVA-infected than in virus-free sour cherry fruits. The total anthocyanin content in PNRSV- or PNRSV+CVA-infected fruits was lower than in control plants. To our knowledge, this is the first report in the world about the effect of PNRSV or PNRSV+CVA infection on the anthocyanin compounds, total polyphenolic compound and vitamin C contents and the antioxidant activity of sour cherry fruits.

Grapevine Pinor gris virus (GPGV) infection in a Southern Hungarian vineyard

Réka Sáray[1,2], Erzsébet Szathmáry[3], Dóra Pinczés[1,2], Asztéria Almási[1], Tamás Deák[4], László Palkovics[5,6] and Katalin Salánki[1]

[1] Department of Plant Pathology, Plant Protection Institute, Centre for Agricultural Research, Eötvös Loránd Research Network, Budapest, Hungary; [2] Doctoral School of Horticultural Sciences, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary; [3] Department of Plant Pathology, Institute for Plant Protection, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary; [4] Department of Viticulture, Institute for Viticulture and Oenology, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary; [5] ELKH-SZE PhatoPlant-Lab, Széchenyi Isván University, Mosonmagyaróvár,, Hungary; [6] Department of Plant Sciences, Albert Kázmér Faculty of Mosonmagyaróvár, Széchenyi István University, Mosonmagyaróvár, Hungary

(palkovics.laszlo.amand@sze.hu)

Plant viruses are probably the most difficult group of plant pathogens to combat. To date, nearly 90 viral pathogens of grapevines have been identified worldwide, but their number is constantly increasing. *Grapevine Pinot gris virus* (GPGV) is a member of the *Trichovirus* genus of the *Betaflexiviridae* family. The virus was first described in Italy in 2012, and it was named 'Pinot gris', after the grape variety on which it was detected. Later, its presence was shown in many countries of the world, including Hungary. In the summer of 2021, we collected a total of 100 leaf samples from a vineyard in southern Hungary, including different traditional and innovative grape varieties, symptomatic and asymptomatic plants. We tested the samples for GPGV infection using various serological and molecular methods. Using the DAS-ELISA method, 92 samples out of 100 samples (92%) proved to be positive, i.e. infected, while the RT-PCR produced of the appropriate size in 94 samples (94%). Sequence analysis demonstrated the high variability of GPGV isolates.

Tomato mosaic tobamovirus survives anaerobic digestion of plant waste biomass in a lab-scale simulation

O. Shevchenko[1], A. Kharina[1], H. Snihur[1], T. Shevchenko[1], T. Kompanets[2], I. Budzanivska[1]

[1]Virology Department, ESC 'Institute of Biology and Medicine', Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

[2]Research Department, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

(alexshevchenko@ukr.net)

Reuse of waste resources (primarily, wastewater and plant biomass waste) nowadays become more and more important and even requirements in many countries aiming at the implementation of the closed cycle of production (including organic farming) and its general environmentalization. Plant biomass waste is nowadays processed mainly by composting or anaerobic digestion with the formation of compost or mulch, or digestate with a high content of ammonia and phosphorus, which is subsequently used for soil protection and fertilization, especially in organic production. There is an increasing body of evidence suggesting that many plant viruses can possibly survive the biomass treatment processes. The survival characteristics of model plant viruses during biomass waste processing and resource reuse in the context of biological safety were studied. For the first time, it was proven that model virus (ToMV) may preserve its infectivity during long-term incubation of plant biomass waste in anaerobic conditions, which requires further attention when using renewable resources.

Occurrence of viruses in black, red currants and gooseberry in Ukraine

K. Udovychenko[1], I. Ryaba[1], and L. Pavliuk[1]
Institute of Horticulture NAAS, Department of Virology, Plant Health and Propagation of Fruit and Berry Crops, Sadova Str., 23, Novosilky, Kyiv-27, 03027, Ukraine
(k_udovychenko@ukr.net)

Currants are widely cultivated in many countries. Today, Ukraine is among the top three world producers of currants. Main cultivation areas are planted with black currants (*Ribes nigrum*) and red currants (*R. rubrum*), while gooseberries (*R. uva-crispa*) are not as popular as currants but still attractive as a niche berry crops. *Ribes* are infected by more than 30 viruses, including Blackcurrant reversion virus (BRV), Strawberry latent ringspot virus (SLRV), Raspberry ringspot virus (RpRSV), Arabis mosaic virus (ArMV), Cucumber mosaic virus (CMV), Tomato ringspot virus (ToRSV) and Gooseberry vein banding associated virus (GVBaV).

To the date information on occurrence of *Ribes* viruses in Ukraine is limited. The goal of this study was to assess the occurrence and diversity of mentioned viruses by DAS-ELISA and RT-PCR. Totally 258 samples of 12 black currants, 6 red currants and 18 gooseberry cultivars were collected in spring-summer 2021 from a germplasm collection, commercial orchards and home gardens in Kyiv, Vinnytsya and Chernihiv regions. Visual observations revealed only one black currant plant with severe symptoms of virus infection such as deformed flowers and leaves, which were obviously induced by the R strain of BRV. However, DAS-ELISA demonstrated the presence of CMV, SLRV, RpRSV and ArMV from 0,5 to 4,3% of tested samples depending on virus and species. RT-PCR analyses revealed high infection rates by BRV and GVBaV in black and red currant samples (up to 17%). No viruses were detected in any of the gooseberry samples. The sequence identities of studied fragments of several isolates of BRV and GVBaV were estimated by comparisons with corresponding sequences from GenBank. Phylogenetic analysis also was conducted for the isolates. As a result virus-free clones of currants cultivars were selected for further testing and propagation.

Molecuar transmission mechanism of wheat dwarf virus by vector leafhopper

Xifeng Wang

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Yuan Ming Yuan West Road, No.2

(wangxifeng@caas.cn)

Hemipteran insects that transmit plant viruses in a persistent circulative manner acquire, retain, and transmit viruses for their entire life. The mechanism enabling this persistence remains unclear for many years. Here, to determine how wheat dwarf virus (WDV) persists in its leafhopper vector *Psammotettix alienus*, we found that WDV caused the upregulation of actin-depolymerizing factor (ADF) at mRNA transcription and protein expression level in the midgut cells of leafhoppers after experiencing 6-, 12- or 24-h of WDV acquisition access period (AAP). Experimental inhibition of F-actin depolymerization by jasplakinolide and dsRNA injection led to lower virus accumulation level and transmission efficiency, suggesting that depolymerization of F-actin regulated by ADF is essential for WDV invasion of midgut cells. Exogenous viral capsid protein (CP) inhibited ADF depolymerization of actin filaments in vitro and in *Spodoptera frugiperda* 9 (Sf9) cells because the CP competed with actin to bind ADF and then blocked actin filament disassembly. Interestingly, virions colocalized with ADF after a 24-h AAP, just as actin polymerization occurred, indicating that the binding of CP with ADF affects the ability of ADF to depolymerize F-actin, then inhibits WDV entry. Similarly, a luteovirus also induced F-actin depolymerization and then polymerization in the gut cells of its vector Schizaphis graminum. Thus, F-actin dynamics are altered by the nonpropagative viruses in midgut cells to enable virus persistence in vector insects.

Section 5

For Students and Young Scientists

“The viruses are Might”

Isolation of bacteriophages and their application to control genus of gram-negative bacteria Pseudomonas

M.Bilous[1], V.Holovan[2], O. Andriichuk[2]

[1] PZO "KLARIS VERBIS HIGH SCHOOL" LLC

[2]Taras Shevchenko National University of Kyiv, Educational-Scientific Center "Institute of Biology and Medicine"

(mykhailo.v.bilous@gmail.com)

Viruses are the most common form of life in the biosphere. In water and soil, the number of viral particles is at least 10 times bigger than the number of cells. This study focused on phages that can infect plant bacteria It is important to find ways to protect plants from bacteria because about 80% of food for humans comes from agriculture.

To search for phages, 10 samples of plants of the *Orchidaceae* family with signs of bacterial infection were selected in the A.V. Fomin Botanical Garden. On the indicator bacterium *Pseudomonas savastanoi pv. phaseolicola* 4013, four samples formed negative colonies: sample *Phalaenopsis philippinensis* 0.2 ± 0.02 mm, sample *Cymbidium iridioides*, 0.3 ± 0.02 mm, *Dendrobium delicatum* 0.2 ± 0.02 mm and *Synadenium grantii* 0.1 ± 0.02 mm.

Increasing antibiotic resistance numbers force both scientists and politicians to tackle the problem, and preferably without any delay. The application of bacteriophages as precision therapy to treat bacterial infections, phage therapy, has received increasing attention during the last two decades. The results obtained in the work can be used in the future to develop antibacterial drugs to combat phytopathogenic bacteria.

Lytic bacteriophages are capable of persisting in plant tissues and limiting *Pseudomonas syringae* propagation

Oleksandra Bondarenko[1,2], Nataliia Korniienko[1,2], Alla Kharina[2], Iryna Budzanivska[2], Nikita Zrelovs[3], Lenka Burketova[1] and Tetiana Kalachova[1]

[1] *Institute of Experimental botany, Czech Academy of Sciences, Rozvojova 263, Prague, Czech Republic;* [2] *ESC "Institute of Biology and Medicine" Kyiv;* [3] *BMC Riga*

(bondar.vir@gmail.com)

Plant bacterioses cause economic losses due to crop damage at different stages of vegetation. Following the low-pesticide and non-GMO farming guidelines, the new approaches to fight pathogenic bacteria are needed. We conducted a study of the effectiveness of Eir4 (recently identified and described lytic bacteriophage, which infects bacteria of *Pseudomonas syringae* group, including strain DC 3000) in planta, using microbiological, virological, and biochemical methods and various approaches to the introduction of phages into the plant organism, in particular: MS medium inoculation and infiltration. We discovered that Eir4 was more effective in the case of pretreatment, while the treatment was ineffective. This suggests that Eir4 circulates in plant tissues and can potentially be used for biocontrol. We are currently trying to determine this phage’s effect on plant immunity. One of the questions is whether the immune response is induced by phage particles themselves, or whether the cellular debris in the phagolysate plays the main role in this process.

Human intestinal enteroids as a model system for human norovirus infection

Maryna Chaika[1], Carmen Mirabelli[1], Christiane Wobus[2], Stefan Taube[1]
[1] University of Lübeck, Lübeck, Germany, [2] University of Michigan, Ann Arbor, USA

Human noroviruses (HuNoV) are non-enveloped viruses with a positive single strand RNA genome that belong to the *Caliciviridae* family. They are a major cause of acute gastroenteritis worldwide. To this day, our knowledge about HuNoV biology is limited, as there have been no susceptible cell lines available to cultivate HuNoVs.

Human intestinal enteroid (HIEs) culture is an emerging tool to study enteric viruses. HEIs represent the only efficient cell culture model to cultivate HuNoV. Derived from biopsies, HIEs are primary cell cultures that form three-dimensional spheroids derived from intestinal stem cells. Infection of HIEs by HuNoV requires differentiation by the removal of the Wnt3A growth factor. HIEs can be infected in both two-dimensional and three-dimensional culture.

The HEI model has become a widely accepted and robust model to study infection of HuNoV from multiple stool isolates. Quantification of the virus in HIEs is achieved by measuring genome equivalents per milliliter (GE/ml) using RT-qPCR. Average increase in genome titers can be observed between 2 hours and 2 days post infection was found to increase 100-1000 fold. HuNoV infection in HEIs supports screening of anti-HuNoV compounds such as the inhibitor 2-chloromethcathinone (2-CMC) blocking polymerase function and replication. A concentration of 75 μM of 2-CMC resulted in a 10-100 fold decrease in virus titers, depending on the stool isolate.

HEIs serve as a versatile model that maintains spatial integrity of the intestinal epithelium and encompasses multiple cell types. These unique characteristics make HEIs an ideally suited tool for investigating the still unknown cellular tropism of HuNoVs. As part of our ongoing project, we are developing method to induce increased numbers of enteroendocrine cells (EECs), which are hypothesized to be a key susceptible cell type for HuNoVs. Our approach will provide a prospect to investigate infection of EECs by HuNoV.

To induce differentiation of EECs, HEIs were treated with the FOXO1 inhibitor AS1842856 for 12 days. The treatment was initiated in the presence of the Wnt3A growth factor for the first 5 days and continued for an additional 7 days in its absence. The induction of EECs were assessed using a chromogranin A marker, which is specific for cells of endocrine lineage. Immunofluorescent staining was performed on fully differentiated spheroids with visualization by 2-photon microscopy. Our microscopy data revealed successful inducement of EECs with increase in both number of positive cells per sphere and positive spheres per sample. Further, EECs-stimulated HEIs were infected with two HuNoV stool isolates. EECs-induced HEIs supported infection of HuNoV to similar level as non-induced spheroids, meaning that system can used to study human norovirus infection of enteroendocrine cells. Our investigation will lead to a better understanding of the HuNoV tropism and provide an efficient platform for developing and testing urgently need anti HuNoV interventions.

‘*Candidatus* phytoplasma rubi’ detection in blackberries (*Rubus plicatus*) and raspberries (*Rubus idaeus*) in Lithuania

M.Dėlkus; M. Zizyte; D. Valiunas
Nature Research Centre, Laboratory of Plant Pathology; Vilnius, Lithuania
(martynas.delkus@gamtc.lt)

Blackberries (*Rubus plicatus*) and raspberries (*Rubus idaeus*) are defined as functional food due to their protective and health enhancing effects. These berries have strong antioxidant, anti-inflammatory, antidiabetic, and anticarcinogenic effects against various cancer types. Plant yellows diseases cause huge damage to beneficial plants and berry crops. Certain plant yellows diseases, previously classified as viral diseases, are caused by prokaryotic organisms - phytoplasmas. This pathogen has no cell wall and is restricted to the phloem. Typical phytoplasma is pleiomorphic and so far, these microorganisms are not cultivated in a cell free medium. Phytoplasmas are common worldwide and capable of infecting more than 700 plant species. Phytoplasmas, like some plant viruses, can cause plant yellowing, dwarfing and similar diseases and cause huge economic losses. These pathogens are spreading rapidly between plants by insect vectors. Plant grafting and vegetative plant propagation are also the primary ways to spread phytoplasmas in agriculture. The prevalence of these diseases in Eastern Europe are less pronounced as compared to the southern regions of Europe, thus it is more difficult to detect and control the infection in the early stages. ‘*Candidatus* Phytoplasma rubi’ was found in blackberries (*Rubus plicatus*) and raspberries (*Rubus idaeus*) in naturally occurring forests in Lithuania. Diseased plants usually show symptoms that indicate a disturbance in the normal balance of plant hormones. *‘Candidatus* Phytoplasma rubi’ is characterized by leaf yellowing, stunting, shoot proliferation, virescence (loss of normal flower colour), enlarged sepals, phyllody (abnormal development of floral parts into leafy structures), flower proliferation, and fruit malformations, resulting in the decline of the host plant and subsequent economic losses. Samples of plants with possible phytoplasma infection were collected and genomic DNA was extracted using CTAB protocol. ‘*Candidatus* phytoplasma rubi’ was found using routine method by amplifying 16S rRNA region using nested-PCR assay with P1/P7 and R16F2n/R16R2 primer pairs. Other genetic markers were analysed for better sub-group identification and molecular descriptions of the phytoplasma found in berry plants. Restriction fragment length polymorphism (RFLP) analysis of nested PCR products revealed that detected phytoplasmas belonged to 16SrV-E subgroup. Further research should aim to develop efficient and environmentally friendly methods to control phytoplasma- related diseases and the insects that spread them.

Mycoviroids infecting both fungi and plants have potential to control crop fungal diseases

K.L. Dong [1,2,3,4], L.H. Kong[1,2,3,4], and W.X. Xu[1,2,3,4*]

[1]*National Key Laboratory for Germplasm Innovation & Utilization of Horticultural, Wuhan 430070, China;* [2]*Hubei Hongshan Laboratory, Wuhan 430070, China*; [3]*College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China*; [4]*Key Lab of Plant Pathology of Hubei Province, Wuhan 430070, China*

(xuwenxing@mail.hzau.edu.cn)

A novel class of viroid-like RNAs naturally infecting a filamentous fungus *Botryosphaeria dothidea* were isolated from apple, and tentatively named Botryosphaeria dothidea circular RNAs (BdcRNAs) 1 to 3, which have been characterized and termed as mycoviroids referring to viroid-like RNAs naturally infecting fungi besides its original definition. BdcRNAs 1 to 3 in size of 450 to 221 nt display no detectable nucleotide identity to known RNA sequences, but share different identity levels with each other, replicate autonomously in the nucleus via a rolling-circle mechanism following a symmetric pathway. BdcRNAs significantly affect the biological traits of *B. dothidea* by regulating the gene expression and metabolic pathways related to important cellular processes of the fungal host. More importantly, BdcRNAs 1 and 2 can significantly attenuate or even erase the fungal virulence, while enhance the growth (for BdcRNA1) and increase the tolerance to some osmotic stress of the host fungus. To determine whether these circular RNAs can infect and replicate in plants, RNA dimers of BdcRNA 1, BdcRNA 2 and BdcRNA 3 were inoculated on cucumber, tomato, *Chenopodium quinoa, Chenopodium amaranticolor*, tobacco and tea, new leaves 21 days post innoculation were collected for RT-PCR detection. The results showed that BdcRNA 1 could be detected on all inoculated plants, and the positive ratio was 4/8 (cucumber, Chenopodium quinoa and tea), 6/8 (tobacco) and 8/8 (tomato and *C. amaranticolor*); BdcRNA 2 was only detected in tomato and *C. quinoa,* and the positive ratio was 1/8 and 3/8 respectively; BdcRNA 3 was not detected in all inoculated plants. These features provide an important alternative candidate to serve as a biocontrol tool for attenuation of fungal diseases similar to some mycoviruses that cause hypovirulence.

Bacteriophage–derived double-stranded RNA (Larifan) causes specific metabolic shift in human monocytes *in vitro*

R. Dovhyi[1], M. Rudyk[1], Ye.Hurmach[2], T. Serhiichuk[1], Yu.Yumyna[1], D.Pjanova[3] and L. Skivka[1]

[1] *Taras Shevchenko National University of Kyiv, Kyiv, Ukraine;* [2] *O.O.Bogomolets National Medical University, Kyivl, Ukraine, ;* [3] *Latvian Biomedical Research and Study Centre, Riga, Latvia*

(roman_dovhyi@knu.ua)

COVID-19 is a complex disorder, and its manifestations can range from mild upper respiratory illness to severe bilateral pneumonia, acute respiratory distress syndrome, disseminated thrombosis, multi-organ failure and death. Lung macrophages (resident alveolar and blood monocyte-derived interstitial) are the first immune cells that contact alveoli-invading viruses, and can be directly infected. Direct infection of phagocytes with SARS-CoV impairs type I IFN response in these cells, which is crucialy important in dampening antiviral defence. In addition, SARS-CoV promotes pro-inflammatory metabolic shift of phagocytes (both, tissue resident and monocyte-derived), which is involved in the development of cytokine storm. Bacteriophage-derived double-stranded RNA (dsRNA), also known as Larifan (Larifan Ltd., Riga, Latvia), is a nationally well-known antiviral medication. Larifan can inhibit SARS-CoV-2 replication *in vitro* and reduce the viral load in the lungs of infected hamsters alongside the improved lung histopathology. This study was aimed to examine the effect of Larifan on metabolic characteristics of human monocytes *in vitro*. Treatment with Larifan causes slight decrease of phagocytic activity, potent inhibition of reactive oxygen specie generation along with increase of NO release. NO can selectively regulate M1 macrophage dedifferentiation, leading to the control of innate immune responses and preventing cytokine storm.

Antiviral properties of modified nanoceria

I.V. Dubova[1], N.M. Zholobak[1,2], A.B. Shcherbakov[1]

[1]D.K. Zabolotny Institute of Microbiology and Virology NAS of Ukraine, Kyiv, Ukraine; [2]Kyiv National University of Technologies and Design, Kyiv, Ukraine

(ira04071999@gmail.com)

Two types of cerium dioxide nanoparticles (CeNPs) were synthesized and characterized: CeNPs pristine and doped with different concentrations of Pt (0-4% w/w), bare and functionalized with the amino acid histidine. The toxicity, antioxidant, and antiviral activity of CeNPs sols (according to prophylactic and therapeutic schemes) was studied using MA104 cells and Vesicular Stomatitis Virus (VSV, *Rhabdoviridae*) *in vitro*. The toxicity was determined 24 hours after contact cells and CeNPs using MTT-test and the crystal violet staining (CV-test), then the integral indicator – cellular Index of Metabolic Activity (IMA) was calculated for each type of CeNPs. The oxidative stress was induced by addition of 0.1 mM H2O2 to the wells. The antiviral activity of the samples in the prophylactic scheme was determined by adding different concentrations of the CeNPs to the cells 2.5 h before VSV infection. In the therapeutic scheme, different concentrations of CeNPs samples were introduced to the cells 1 hour after viral infection. Results: According to the integral indicator of toxicity (IMA), bare CeNPs showed significantly higher toxicity (IMA in the concentration range of 0.06-0.5 mM was higher than the value of control cells). The presence of Pt in the CeNPs significantly reduced their cytotoxic effect, which decreased inversely to their concentration. Functionalization of CeNPs with histidine was accompanied by a significant sharp decrease in their toxicity. The antioxidant activity of the both types of CeNPs was detected only at the maximum investigated concentrations of 0.25-0.5 mM, where the cell protection was higher than 50%, compared to the control of the cytotoxic effect of peroxide on intact cells. For histidine-functionalized CeNPs, an increase in the Pt content led to a decrease in their prooxidant effect. The antiviral activity of bare CeNPs in both schemes studied was less pronounced than that of CeNPs functionalized with histidine. The presence of Pt in the histidine-functionalized CeNPs in both prophylactic and therapeutic regimens led to a higher antiviral effect in the MA104/VSV model system. Thus, the functionalization of CeNP with the amino acid histidine can significantly change the cytotoxic, antioxidant, and antiviral effects of CeNPs, both pristine and containing different concentrations of Pt. The results obtained are a promising tool for the creation of effective complex functionalized and biocompatible CeNPs as targeted antiviral objects.

Novel lytic phages isolated from antarctic soil

V.Holovan[1], O. Andriichuk[1].

[1]Taras Shevchenko National University of Kyiv, Educational-Scientific Center "Institute of Biology and Medicine"

(holovanviktoria@gmail.com)

Two lytic *Pseudomonas* specific bacteriophages, provisionally named UAntarctica and UAVern, were isolated from Antarctic soil samples collected from the low-temperature terrestrial environment near the Ukrainian Antarctic Station ‘Academician Vernadskiy’, Argentina Islands, Antarctica. Based on their virion structures, UAntarctica and UAVern phages were classified as siphovirus and myovirus, respectively. Both phages showed a low-temperature plating profile, an ability to form plaques at 4°C, with an optimum plating temperature of about 18°C. The UAntarctica phage was found to possess a dsDNA genome of 81.1 kbp with a G+C content of 58,4%, and included 119 open reading frames (ORFs) and three tRNA genes predicted. The UAVern myophage possessed a 101,6 kbp dsDNA genome with a G+C content of 51,26%, and included 176 ORFs and 19 tRNA genes. The majority of predicted ORFs (~81% for both phages) were encoded for proteins with unknown functions. The genes with assigned putative functions were clustered together into functional modules presumably responsible for virion morphogenesis as well as for DNA replication, recombination, and metabolism. Genome comparison revealed that both phages had low similarity to other known phages. Phylogenetic analysis confirmed that the UAntarctica and UAVern phages may represent novel genera.

Structural analysis of Nuclear shuttle protein (NSP) on sida virus sequences of the Geminiviridae family

M. Hourshad[*], S. Seddigh[2] , M. Shouri[1] , M. Zolfaghari[1]

[1]Ph.D student, Department of Plant Pathology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

[2]Assistant professor Department of Plant Protection, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

(hourshad.mahnaz@gmail.com)

Begomoviruses (Geminiviridae) are major nuisance to agriculture worldwide. Because ssDNA viruses replicate in the nuclus of infected cells, the resulting viral DNA must translocate to the cytoplasm and then to the adjacent cell to cause disease. The nuclear shuttle protein (NSP) of begomovirus helps to transport the virus intracellularly. In this survey, the NSP protein reference sequences (RefSeq) five sida virus species of gemniviridae family including Sida bright yellow mosaic virus, Sida ciliaris golden mosaic virus, Sida golden mottle virus, Sida yellow golden mosaic virus, Sida chlorotic leaf virus, were retrieved from NCBI in FASTA format. Structural analyses of this protein were done online on some servers. The Primary and secondary structures of the NSP proteins were deduced for each species ProtParam and SOPMA, respectively. Also SignalP 4.0 server was used for prediction of the presence and location of signal peptide NSP protein in all insects ranged from 200 to 300 amino acids in length. The theoretical isoelectric points and molecular weight were calculated at a range of 9.65–9.90 and 29719.95–29121.26 kDa, respectively. The GRAVY values of this protein designated NSP viruses species that to be globular (hydrophilic protein) in nature. The Instability index analysis revealed that all five sida virus NSP samples were unstable proteins. Secondary structural analysis showed that extended strand and random coils, are the most abundant structural elements, while beta turns and alpha helixeswere occasionally distributed in the protein. Furthermore, SignalP analysis of all samples showed that NSP is not a secretary protein.

Structural analysis of Nuclear shuttle protein (NSP) in some species *Begomovirus* genus viruses

M. Hourshad[1*], S. Seddigh[2], M. Shouri[1], M. Zolfaghari[1]

[1] Ph.D student, Department of Plant Pathology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran; [2] Assistant professor Department of Plant Protection, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.

(hourshad.mahnaz@gmail.com)

Begomoviruses are a group of plant viruses with a wide variety of host range, infecting dicotyledonous plants. Begomoviruses use two proteins to transport their DNA from cell to cell. The nuclear shuttle protein (NSP) transports it between nucleus and cytoplasm and the movement protein (MP) can transport the DNA-NSP complex to the cell periphery and facilitate movement across the cell wall. In present analysis, the seventeen NSP protein reference sequences (RefSeq) of different species of Begomovirus genus, were downloaded from NCBI in FASTA format. Structural analyses of this protein were carried out online on Protparam and SOPMA websites to predict primary and secondary structures, respectively. NSP protein in all viruses ranged from 200 to 300 amino acids in length. The theoretical isoelectric points and molecular weight were calculated at a range of 8.83–10.01 and 30943.29–23830.18 kDa, respectively. Secondary structural analysis of Tobacco leaf curl Cuba virus as the representative of viruses revealed that this protein was composed of 17.58% α-helix, 25% extended β-strand, 3.12% β-turn, and 54.30% random coil. These results showed that random coils and extended β-strand are the most plentiful structural elements, while α-helix and β-turns were intermittently distributed in the protein.

Modulation of the CRISPR/Cas13 gene editing system to stimulate plant antiviral resistance

N. Iksat[1], Z. Masalimov[1], R. Omarov[1], and A. Shevchenko[2]

[1] *Department of Biotechnology and Microbiology, L.N. Gumilyov Eurasian national university, Astana, Kazakhstan;* [2] *Virology Department, ESC “Institute of Biology and Medicine”, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine*

(nurguliksat@gmail.com)

In planta inhibition of wild type Tomato bushy stunt virus (TBSV) and RMJ-1 mutant in the presence of the CRISPR/Cas13 system was investigated. For this purpose, pk2GW7-pCas13 and crRNA constructs targeting the P19 suppressor protein and the GFP reporter protein were constructed. The constructs were delivered by *Agrobacterium* infiltration into *N. benthamiana*. The efficiency of formation of the Cas13-crRNA complex was estimated by liquid-liquid phase separation (LLPS). The optimal temperature was estimated by determination of the accumulation of ROS and the production of heat shock proteins Hsp-90, Hsp-70. The performance of the CRISPR/Cas13 system was evaluated by conducting assays using P19 and GFP specific antibodies. Amounts of co-precipitated virus-derived siRNAs were estimated in presence and absence of an editing system without heat treatment and in the presence of heat stress. When compared to controls, all crRNAs displayed lower levels of GFP expression in inoculated leaves under UV light. Furthermore, at P19, lower, undetectable levels of GFP signal decrease were reported in GFP crRNA targets, whereas larger levels of reduction were observed with crRNA. The ability of crRNA complementary to the P19 region of the TBSV genome to be more targeted than other targets may be related to P19's function in the viral genome as a suppressor of the host's RNA interference defense mechanism. Any dose-dependent change in P19 levels can influence virus transmission in plant cells. Higher GFP signals, on the other hand, were found in the non-specific crRNA control and in *N. benthamiana* plants infected with the wild type, indicating the efficiency of the CRISPR/pCas13a system for viral interference. This study also demonstrates the significance of temperature (+37, +40 C) in modifying the activity of the CRISPR/Cas13 system in eukaryotes and proposes a express method for increasing targeted degradation of foreign RNAs in plants utilizing the CRISPR/Cas13 gene editing system. Overall, our data demonstrated successful targeting and intervention against TBSV and its mutants. These results indicate the effectiveness of CRISPR/pCas13a in targeting and interacting with RNA viruses for potential plant biotechnology applications.

Identification of potato samples with the potato virus Y resistance genes Ry_{chc} and Ry_{adg}

S.O.Kyrychenko, N.O. Kozyb, T.I. Bondar, R.O. Bondus

Institute of Plant Protection of NAAS, 33, Vasylkivska str., Kyiv, 03022, Ukraine

Ustymivka Experimental Station of Plant Production of the PPI NAAS, 15 Akad. M.I. Vavilova Str.Poltavska Obl., Hlobynskyi Raion, Ustymivka,

E-mail:s.a.kyrychenko@gmail.com, natalkozub@gmail.com

Viral diseases of potato cause damage to the crop, which may be equal to 30-80% of the gross harvest, reducing the quality of the harvested crop. Potatoes are an important food, technical and fodder crop, with total global potato production amounting to 354.3 million tons in 2022. The prevalence of Potato virus Y (PVY) in the potato fields poses a severe threat to efficient production. So far, three resistance genes have been identified that confer extreme resistance (ER) to PVY to potato plants. This means that they are able to provide a high level of resistance to a wide range of virus strains. These genes originate from *S. tuberosum ssp. andigena* (Ry_{adg}), *S. stoloniferum* (Ry_{sto}) and *S. chacoense* (Ry_{chc}) and are located on chromosomes XI, XII and IX, respectively . Ry_{chc} was mapped from the *S. chacoense* 40-3 sample. Ry_{chc} encodes the TIR-NLR protein and provides extreme resistance of potato plants to PVY^{O}, $PVY^{N:O}$ and PVY^{NTN} strains. The first PVY resistance gene which had been mapped, was Ry_{adg}. It originated from the cultivated *S. tuberosum* of the Andigenum group. The locus containing Ry_{adg} is used in programs to develop resistant varieties and is effective against all known PVY strains, including PVY^{NTN} . Molecular markers for these genes are of great importance to breeders, as they allow them to select resistant progeny from PVY-resistant parents at the early stages of breeding, when there is not enough breeding material to test the virus in the field for PVY infection. To control viral diseases of crops, it is necessary to create a database of resistant varieties for further selection for the desired traits. In our study, we investigated the frequency of *Potivirus* Y resistance alleles in 70 potato samples that have not yet been registered yet. The study of such plant material at the early stages of introduction can help to understand the potential disease resistance of a future variety.

The genetic markers Ry_{o}*-186* and *RYSC-3* were used to determine the allelic state of the resistance genes Ry_{chc} and Ry_{adg}, respectively. For the polymerase chain reaction, a mixture of reagents for DNA amplification PCR MIX 2x HOT (Neogen™, Ukraine) was used. PCR was performed in an Applied Biosystems 2720 Thermal Cycler.

In this study, the Ry_{chc} resistance gene was identified in 53 varieties (75.72%). The resistance gene Ry_{adg}, in turn, was identified in 7 accessions (10%). The following four samples: P.17.36-8, G.10.3/11, P.16.21-8, P.17.21/36 had both of the genes, which indicates their potential to virus Y resistance.

Harnessing Plant Viruses as Versatile Tools for Protein Overexpression, Gene Silencing and Gene Editing using stabdardised GoldenBraid parts

T. Moravec, H. Plchová, H. Hoffmeisterová, J. Dušek, N. Ceřovská, R. Vítek, J. Kumar
Laboratory of Virology-Centre for Plant Virus Research, Institute of Experimental Botany of the Czech Academy of Sciences, Rozvojová 263, 165 02 Prague, Czech Republic
(moravec@ueb.cas.cz)

Plant viruses have proven to be versatile tools for numerous applications in biotechnology and molecular biology. Here we present an innovative approach for the construction of infectious virus clones. Using the GoldenBraid/MoClo convention, we systematically converted several plant viruses into standardised genetic parts, creating a comprehensive toolbox for multiple applications. The viral vectors were developed not only to streamline the assembly of viral constructs, but also to ensure compatibility with existing synthetic biology standards.

By taking advantage of the unique properties of different plant viruses, we can fine-tune protein expression levels and subcellular localization, silence endogenous genes, or introduce gene editing reagents into plant cells. To date, we have edited *Potato virus* X (a potexvirus), *Bean yellow dwarf Mastrevirus, Tobacco mosaic virus* U1 and Cg tobamoviruses, *Tobacco rattle tobravirus*, and *Apple latent spherical cheravirus*.

We hope that this toolbox will help researchers manipulate plant genomes, produce high-value proteins, and unravel gene functions, making an important contribution to the advancement of sustainable agriculture and biotechnological innovation.

Biological activity of phages during long-term storage at different temperature variations

O. Naumenko, O. Andriichuk

Taras Shevchenko National University of Kyiv, Educational-Scientific Center "Institute of Biology and Medicine"

(manasashana@gmail.com)

Plant diseases caused by phytopathogenic bacteria are a major cause of production losses worldwide, as well as an important cause of economic, social and environmental impacts. Bacteriophages, as modern treatment methods, have gathered attention on themselves as promising biocontrol measure. Phage specificity can help control bacterial infections in crops and during product storage. Creating phage collection is going to allow their use as preventive, diagnostic or therapeutic agents.

Experimental statistical work was carried out to check the biological activity of the studied isolates during long-term storage at different temperature variations. The obtained results made it possible to explore phage stability and to better assess the possibilities of their adaptability to the conditions of the surrounding environment.

Thus, at +4°C and + 20°C, the titers of each of the studied bacteriophages (Ser/1, 8573/B, 8573/M and 4013) remained stable during each subsequent passage for a month. At +28°C, a slight decrease in the lytic activity of phages was observed starting from the sixth day until the thirtieth. Phage isolate 8573/M proved to be the most stable among all used phages, because, in comparison with others, its biological activity remained quite stable and decreased slower than that of other isolates. The lytic spectrum of phages against 7 bacterial strains was determined. It was established that all phage isolates showed lytic activity against 5 bacterial strains and are polyvalent.

Hence, it was found that phage isolates, used in the research, can be promising for practical use as biological plant control products due to their ability to maintain biological activity in different temperature variations and broad lytic activity spectrum.

Novel potential inhibitors against ZIKV infection

K. Naumenko[1,3], L. Ivanova[2], E. Žusinaite[1], M. Karelson[2], A. Merits[1]
[1]Institute of Technology, University of Tartu, Nooruse 1, 50411 Tartu, Estonia
[2]Institute of Chemistry, University of Tartu, Ravila 14A, 50411 Tartu, Estonia
[3]Zabolotny Institute of Microbiology and Virology NAS of Ukraine, St. Akad. Zabolotnogo, 154 03143, Kyiv, Ukraine
(krystyn.naumenko@gmail.com)

The *Flaviviridae* family includes high-profile mosquito transmitted viruses such as dengue (DENV) and Zika (ZIKV), for which no antiviral treatments are currently available. ZIKV is responsible for an acute febrile illness with rash, arthralgia and conjunctivitis, and microcephaly and neurological disorders in newborns to ZIKV-infected mothers and represents an ongoing public health emergency of international concern.

Our research here focuses on the eight chemical compounds with previously described antiviral effects against the DENV-2 and DENV-4; these compounds were tested for their ability specifically inhibits ZIKV infection.

Vero E6 cells were infected with ZIKV encoding nanoluciferase reporter at a multiplicity of infection of 0.1. Prominent inhibition was observed for four compounds out of eight with effective concentration 50 (EC_{50}) values ranging from 0.1 to 10 μM. In order to assess the selectivity index (SI) of the identified potential dual ZIKV/DENV inhibitors, the range of the cytotoxic concentration 59 (CC_{50}) was determined using a real-time cytotoxicity assay. According to the results, only two compounds have SI >3 and could be selected for further study as potential dual inhibitors of DENV and ZIKV. The rest of compounds were either ineffective or highly cytotoxic. As selected compounds were effective against two different flaviviruses it can also be assumed that they have a potential in further development of efficient and targeted inhibitors against multiple flaviviruses.

Investigation of the Epstein-Barr virus infection in untreated patients with chronic lymphocytic leukemia in Latvia

M. Nazarenko[1,2], Laura Zvejniece[1], Svetlana Kozireva[1], Olga Kornilova[3], Valērija Gorbačova[1], Alla Rivkina[3,4], Sandra Lejniece[3,4], Irina Kholodnyuk Holodnuka

[1] Institute of Microbiology and Virology, Riga Stradiņš University, Riga, Latvia; [2]Educational and scientific center "Institute of Biology and Medicine" of Taras Shevchenko Kyiv National University, Kyiv, Ukraine; [3] Clinic of Chemotherapy and Hematology, Riga East University Hospital, Latvia; [4] Department of Internal Diseases, Riga Stradiņš University, Latvia

(mariia.nazarenko.22@gmail.com)

Chronic lymphocytic leukemia (CLL) is a B-cell malignancy according to the WHO classification (Alaggio, 2022). CLL is the most common adult leukemia in the United States and Europe; about 4.7 new cases of CLL per 100,000 individuals are diagnosed annualy (Swerdlow, 2016). Based on clinical behavior, CLL can be divided into two main types: an indolent slowly progressing (low-risk) and an aggressive rapidly progressing (high-risk) disease. For determination of the disease clinical stages, the Rai and Binet systems are the most commonly used (Rai, 1975 and Binet, 1977). But none of them can predict the development of the disease in the early stages. The high-risk form of CLL is characterized by unmutated immunoglobulin heavy chain variable region genes (IGHV) and often by CD38 expression on CLL cells.These two indicators are also considered in the modern treatment of CLL patients (Agathangelidis, 2022). Epstein-Barr virus (EBV) infection induces CD38 expression on B cells of healthy donors (Kholodnyuk, 2017). The virus is a companion of many diseases, complicating their course (Houen, 2021). EBV resides in B cells and a high EBV DNA load in peripheral blood (PB) cells of CLL patients was associated with poor overall survival (Visco, 2015; Grywalska, 2015). In this study, the presence and the quantity of EBV in PB mononuclear cells of 62 untreated CLL patients were analyzed, aiming to associate them with low-risk and high-risk CLL. The study involved the analyzes of the IGHV sequence somatic mutations, using multiplex PCR and sequencing methods. Quantitative real-time PCR was used to detect EBV DNA copy numbers. The results showed a positive association between the PB lymphocyte counts and the disease stages. While the majority of CLL patients (61%) did not have detectable EBV DNA, the vitus presence was highest in patients at the advanced stage of the disease (in 50% of patients). The impact of EBV on CLL development is not clear, and further research with a larger number of patients is needed to investigate the role of EBV infection in CLL disease progression. re-activation of EBV may influence disease progression and worsen patient outcomes. The results of such studies could improove the current personalized treatment selection for CLL patients.

Alaggio R, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Rewiew. Leukemia. 2022 Jul;36(7):1720-1748. doi: 10.1038/s41375-022-01620-2.

Agathangelidis A, et al., ERIC, the European Research Initiative on CLL. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: the 2022 update of the recommendations by ERIC, the European Research Initiative on CLL. Leukemia. 2022 Aug;36(8):1961-1968.

Kholodnyuk I, Rudevica Z, Leonciks A, Ehlin-Henriksson B, Kashuba E. Expression of the chemokine receptors CCR1 and CCR2B is up-regulated in peripheral blood B cells upon EBV infection and in established lymphoblastoid cell lines. Virology, 2017, 512:1-7. PMID:

Visco C, Falisi E, Young KH, Pascarella M, Perbellini O, Carli G, Novella E, Rossi D, Giaretta I, Cavallini C, Scupoli MT, De Rossi A, D'Amore ES, Rassu M, Gaidano G, Pizzolo G, Ambrosetti A, Rodeghiero F. Epstein-Barr virus DNA load in chronic lymphocytic leukemia is an independent predictor of clinical course and survival. Oncotarget. 2015 Jul 30;6(21):18653-63.

Grywalska E, Roliński J, Pasiarski M, Korona-Glowniak I, Maj M, Surdacka A, Grafka A, Stelmach-Gołdyś A, Zgurski M, Góźdź S, Malm A, Grabarczyk P, Starosławska E. High Viral Loads of Epstein-Barr Virus DNA in Peripheral Blood of Patients with Chronic Lymphocytic Leukemia Associated with Unfavorable Prognosis. PLoS One, 2015;10(10):e0140178.

Trying to make sense of many thousands of RNA viruses - insights from genome secondary structure

Uri Neri, Rima Sghayer, Uri Gophna

The Shmunis School of Biomedicine and Cancer Research, George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel

(uri.neri@gmail.com)

Decreasing nucleic sequencing cost, advances in data analysis, the increasing need to monitor potential zoonotic pathogens, and the growing understanding of the role of viruses in ecological systems, have all led to the emergence of the new research field of virus discovery. Yet, the vast majority of these viruses are not likely to be isolated under laboratory conditions, as the very host they depend on are also likely not amenable to simple cultivation. As a result, the life cycle and characteristics of these viruses remains largely unknown. This issue increases as the virus analysed is more divergent than those already known and studied - such as those outside established taxa and familiar lineages, or with small genomes containing only a handful of proteins each, many of unknown function. In such cases, key traits of the virus biology are left unresolved, e.g. the genome polarity (-\+ss or ds RNA/DNA), the genome topology (linear, circular), the genome architecture (mono/poly - segmented), the virion structure (helical, icosahedral, rod, enveloped…), the packaging machinery (Multi/mono partite) and most notably the host range information. RNA viruses (Orthornavirae) compound this further, due to their generally smaller genome size and lack of host integration. However, many RNA viruses display “genome gymnastics”; employing a variety of RNA structure related mechanisms for temporal regulation of protein synthesis (e.g. ribosome access to the initiation codon), as packaging signals (binding their own genome within their proto-capsid) or to enable polymerases to preferentially replicate their genome. Recently, we were able to uncover tens of thousands of novel RNA viruses and other infectious agents, yet our understanding of them is lacking. Here, we quantified and featurized the predicted RNA secondary structures, and performed statistical analysis and classifier construction attempting to characterise the viral genomes.s

Phylogeographic reconstruction of High Plains wheat mosaic virus isolates

I. Pozhylov[1], H. Snihur[1,2], O. Anopriyenko[3], I. Budzanivska[1]

[1]Virology Department, Educational and Scientific Centre «Institute of Biology and Medicine» at Taras Shevchenko National University of Kyiv, Ukraine; [2]Laboratory of Plant Viruses, D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, 03143 Kyiv, Ukraine; [3]Laboratory of Biosynthesis of Nucleic Acids, Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine

(illiapozhylov@gmail.com)

High Plains wheat mosaic virus (HPWMoV, genus *Emaravirus*; family *Fimoviridae*) poses a severe threat to worldwide agriculture, infecting a wide spectre of *Poaceae* species. HPWMoV genome is represented by 8 single-stranded negative-sense segments. The virions are spherical or pleomorphic in shape and ca 100nm in diameter. The virus was reported in the US, Argentina, Australia, Canada, and, recently, Ukraine and Iraq. In this work, phylogenetic analysis with the molecular clock method was performed on 50 RNA3 and 20 RNA6 sequences of HPWMoV isolates available in the GenBank database. Judging by the analysis of the RNA3 sequence fragment of HPWMoV, despite the high genetic variability of the sequences of the Ukrainian isolates of this emaravirus, the isolates that were collected in the USA (especially from the states of Colorado, Kansas, and Nebraska) have a common evolutionary past with the isolates that were collected in Ukraine, which may be evidence of the origin of this virus from one common location. Interestingly, the results of the phylogenetic analysis for different segments of the same HPWMoV isolate are slightly different. According to fragments of the RNA3 segment, Ukrainian isolates form a separate clade that has a common origin with group A isolates. The separation occurred only 14 years before collection, while according to the analysis of the RNA6 segment, the Ukrainian isolate is still in group A, and the separation from a part of the isolates in this group happened 23 years before the collection. Otherwise, samples from Australia and Canada were collected just a few years after the divergence from the closest isolates from the US. Also, it was found that a couple of the US isolates may have evolved from Australian isolates, which indicates an intense exchange of HPWMoV between Australia and the US. Thus, it can be seen that, firstly, the evolutionary history of different segments is inconsistent, therefore, to obtain a more complete picture of the evolution of emaraviruses, information about all segments is necessary, and secondly, the rate of accumulation of evolutionary changes for different segments is not the same.

Association of the Epstein-Barr virus (EBV) with the pathogenesis of multiple sclerosis

S.P Prilutskiy
Department of Human Biology and Ecology, Melitopol State Pedagogical University named Bohdann Khmelnytskii, Melitopol, Ukraine
(priluckijsergej356@gmail.com)

Multiple sclerosis is an autoimmune, chronic pathology, which is characterized by damage to the myelin sheath of the centers of higher nervous activity and neurons of the spinal cord. The disease was first described in 1868 by Jean Martin Charcot. In the herpesvirus family, representatives are one of the most dangerous pathogens in nature, capable of affecting the central nervous system. If Epstein-Barr virions (EBV) are present in the body reservoir, they provoke the development of such neurotropic pathologies: arachnoiditis, arachnoencephalitis, disseminated encephalomyelitis, encephalitis, arachnoencephalitis, encephalomyelitis and others (Rudenko A.O., Muravskaya L.V. et al., 2017). In a recent retrospective study by scientists from Harvard University in the USA, it was determined that EBV infection is directly associated with the pathogenesis of multiple sclerosis as neuronosology. Statistics show that almost 100% of patients with multiple sclerosis (MS) of various forms were infected with EBV or had infectious mononucleosis. Compared with uninfected patients, the risk of developing a tendency to develop MS is higher in those who have been infected with the Epstein-Barr pathogen in the juvenile and pubertal period of postembryonic ontogenesis. The virus initiates immune responses of Th1 cells, and is also able to encode proteins responsible for modifying the host's immune responses in favor of the infectious agent. EBV is able to remain in a latent state in B cells throughout the ontogeny of the carrier organism. Some patients with MS may have a deficient function to inhibit the latent feature of the Epstein-Barr virus, thereby forming copies of infected B-cells in the manner of oncogenic viruses. It is certain that most of the B-cells that infiltrate the higher nervous activity of patients with multiple sclerosis increase the likelihood of the virus, due to its peculiarity of interfering with the differentiation of B-lymphocytes to their dysregulation in the body of a patient with MS (Asaulyak I.O., Dyachenko A.A. et al., 2018).

SARS-CoV-2 convalescence and hybrid immunity elicits mucosal immune responses

Olha Puhach[1], Mathilde Bellon[1], Kenneth Adea[1], Meriem Bekliz[1], Krisztina Hosszu-Fellous[2,3], Pascale Sattonnet[1], Laurent Kaiser[2,3], Isabella Eckerle[1,2], Benjamin Meyer[4]

[1]Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, Geneva, Switzerland; [2]Geneva Centre for Emerging Viral Diseases, Geneva University Hospitals, Geneva, Switzerland; [3]Division of Infectious Diseases, Geneva University Hospitals, Geneva, Switzerland; [4]Centre of Vaccinology, Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland

(olha.puhach@unige.ch)

Mucosal antibodies play a key role in the protection against SARS-CoV-2 infection in the upper respiratory tract, and potentially in limiting virus replication and therefore onward transmission. While systemic immunity to SARS-CoV-2 is well understood, little is known about the antibodies present on the nasal mucosal surfaces. In this study, we evaluated SARS- CoV-2 mucosal antibodies in response to infection, vaccination, or a combination of both. Paired nasal fluid and serum samples were collected from 136 individuals, which include convalescent, vaccinated, or breakthrough infections. We detected a high correlation between IgG responses in serum and nasal fluids, which were higher in both compartments in vaccinated compared to convalescent participants. Contrary, nasal and systemic SARS-CoV-2 IgA responses were weakly correlated, indicating a compartmentalization between the local and systemic IgA responses. SARS-CoV-2 secretory component IgA (s-IgA) antibodies, present exclusively on mucosal surfaces, were detected in the nasal fluid only in a minority of vaccinated subjects and were significantly higher in previously infected individuals. s-IgA binding antibodies showed significant correlation with neutralizing activity of nasal fluids against SARS-CoV-2 ancestral B.1 and Omicron-BA.5 variant, indicating that s-IgA is the crucial contributor to neutralization in the nasal mucosa. Neutralization against both SARS- CoV-2 strains was higher in the mucosa of subjects with previous SARS-CoV-2 infections compared to vaccinated participants. In summary, we demonstrate that currently available vaccines elicit strong systemic antibody responses, but SARS-CoV-2 infection generates more potent binding and neutralizing mucosal antibodies. Our results support the importance to develop SARS-CoV-2 vaccines that elicit mucosal antibodies.

mRNA transfection approach to transient gene expression in selected mammalian cell lines.

K. Rokush, Y. Faidiuk, P. Miernikiewicz, K. Dąbrowska
LBMB, Hirszfeld Institute of Immunology and Experimental Therapy, PAS, Wroclaw, Poland
(kostiantyn.rokush@hirszfeld.pl)

Accurate and fine-tuned expression of target genes in eukaryotic cells is crucial for the development of gene function study models, effective introduction of gene therapy and novel types of vaccines. The transfection efficiency, protein expression rate and duration as well as undesirable side effects for lipid-based (Lipofectamine) transfection with mRNA were assessed on selected cell lines. Pharynx epithelial cells (FaDu) and murine macrophages (RAW 264.7) were transfected with different types of reporter gene (EGFP) mRNA molecules and analyzed in a time-dependent manner. mRNA molecules produced by in vitro transcription contained various UTRs, Cap0 or Cap1 structures, uridine or 5-methoxyuridine substitution. Results were compared with plasmid DNA transfection (Lipofectamine) with the same reporter under CMV promoter. Both cell lines are hard to transfect with DNA and RAW 264.7 could not be transfected with DNA at all, while FaDu showed up to 20% transfection efficiency. In both cases, obvious cytotoxic effects were observed. mRNA approach provides a possibility to transfect both these lines with very high efficiency: up to 80% for RAW 264.7 and up to 90% for FaDu without any visible side effects on cells. The fluorescent signal appears from 5 hours post-transfection and increases gradually until it reaches its maximum around 24-36 hours post- transfection. Staying at this level for up to 48 hours it turns to decline. The presence of UTRs and their structures strongly correlates with translation efficiency. Cap-1 structure and 5- methoxyuridine substitution are crucial to avoid cytotoxic side effects on cells, especially on immune cells RAW 264.7. mRNA transfection technique allows the accurate introduction of the target gene into a broad range of cell types without additional impact on the model system and expands the spectrum of gene function study models.

Peculiarities of the development of systemic viral reactions in *Nicotiana bentamiana* plants expressing the heterologous *ZRNase* II gene

A.Y. Serazhym[1], A.O. Potrohov[2], O.O. Ovcharenko[2]

[1] Biology and Medicine Institute Science Educational Center of Taras Shevchenko National University of Kyiv; [2] Institute of Cell Biology and Genetic Engineering NAS Ukraine.

(angelinkathegreat@gmail.com)

Heterologous ribonucleases are involved in plant antiviral responses. Influence of heterologous plant RNase genes on the spread of viruses in plants has an obvious interest. *N. bentamiana* is widely used in plant virology as a host for plant-virus interactions investigations due to its amenability to a great amount of plant viruses and its systemic response to viral infection. The aim of our study was to obtain transgenic *N. bentamiana* plants expressing the heterologous gene *ZRNase* II of extracellular ribonuclease from *Zinnia elegance* L. and to compare the systemic movement of viral based vector in transformed and wild type plants.

Transgenic plants were obtained by *Agrobacterium*-mediated genetic transformation of leaf discs. Stable integration of the *ZRNase* II gene was confirmed by PCR analysis. Control and transgenic plants were further agro-infiltrated with PVX-based vector system pICH27566 with genes coding the viral RNA polymerase, the triple gene block proteins, the coat protein, and the reporter green fluorescent protein (GFP) (in GV3101 strain of *Agrobacterium tumefacience*) to investigate transient expression of recombinant reporter and its systemic movement. The spread of the viral particles through the plant was detected by GFP fluorescence.

GFP fluorescence appeared at the sites of agro-infiltration in 3-4 days after injection. We observed GFP fluorescence in the central and lateral veins in the leaves of the lower and middle level, and the local fluorescent areas along the edges of the young leaves of the upper layer in the control non-transgenic plants. While in the transgenic plants, GFP fluorescence was detected only in lateral and central veins of lower leaves. In the leaves of the middle level, the GFP fluorescence was occasional, and in the young leaves of the upper level, it was absent.

Thus, the spread of viral particles differed in the transgenic and wild type plants. Although viral vector was not completely localized in the transgenic plants, its systemic spread delayed.

CRISPR Cas9-mediated deletion of intact SMRV proviral copies from the BLaER cell line

D. Shestopal

Nucleic Acid Immunity and Genome Defense, The Institute of Clinical Chemistry and Clinical Pharmacology (ICCCP), Bonn, Germany

(danylo_shestopal@icloud.com)

Studying innate immune signaling pathways has informed the development of new targeted therapies to treat autoimmune diseases and cancer. To achieve this, cell culture models that are easy to genetically engineer are instrumental. Such a model is the BLaER cell line, which is genetically engineered human B cell lymphoma cells that express the transcription factor C/EBPa fused to the estrogen receptor binding domain. Upon exposure to estradiol, BLaER cells transdifferentiate into macrophage-like cells. This system allows to rapidly introduce genetic alterations when BLaER cells are in the B cell stage and proliferate fast and subsequently study innate immune signaling in the relevant after transdifferentiation into myeloid cells of mutated clones. However, in the original BLaER1 cells, the endogenous retrovirus SMRV is actively replicating, potentially confounding results obtained with these specific cells. The SMRV status of recently a sister cell line BLaER2 cells is currently unclear. In this work, we tested whether BLaER2 cells are infected and detected the presence of intact proviral copies of the endogenous retrovirus SMRV using DNA and RNA extraction methods and subsequent PCR analysis. Successful amplification of SMRV genes was demonstrated, confirmed by analysis of amplicons with appropriate length and 100% identity to the SMRV reference genome. Next, we devised a CRISPR/Cas9-based genome editing strategy to remove proviral copies of SMRV from BLaER 1/2 cells, including the design of specific guide RNAs, electroporation, and analysis of process efficiency by flow cytometry and quantitative PCR. The results showed successful deletion of SMRV proviral copies of the cell genome. In summary, we showed that BLaER cells are infected with active SMRV. We successfully established a strategy to remove SMRV proviral copies from the genome of BLaER cells which can be used to create a clean experimental cell cultures system to study innate immune signaling.

Isolation and characterization of bacteriophages effective against *Aeromonas* spp. pathogenic to fish

K. Svanberga[1], J. Avsejenko[2], J. Jansons[1], D. Fridmanis[1], T. Kazaka[1], A. Berzins[2], A. Dislers[1], A. Kazaks[1], N. Zrelovs[1]

[1]*Latvian Biomedical Research and Study Centre, Riga, Latvia;* [2]*Institute of Food Safety, Animal Health and Environment BIOR, Riga, Latvia*

(karina.svanberga@biomed.lu.lv)

Aeromonas spp. outbreaks are increasingly common in freshwater-farmed fish and are associated with high mortality leading to severe economic losses (Mzula et al. 2019). The emergence of multidrug-resistant strains stemming from reckless antibiotic usage raises global public health concerns and has negative socioeconomic impacts (Dien et al. 2022). While there are several methods available for controlling *Aeromonas*-caused diseases in fish, the effectiveness of these measures has been inconsistent. Consequently, it is crucial to explore alternative treatment options such as revisiting phage therapy. In this study, *Aeromonas* spp. strains (n=19) previously isolated from diseased fish retrieved from local aquaculture settings were obtained from BIOR collection of bacterial strains. The identity of the bacterial strains was validated by 16S rRNA gene sequencing using conventional 27F and 1492R primers. These isolates represent 7 versatile species - *A. allosaccharophila*, *A. hydrophila* subsp. *hydrophila*,
A. rivipollensis, *A. salmonicida* subsp. *salmonicida*, *A. sobria*, *A. media,* and even tentative *Aeromonas* sp. nov (among them are pigment-producing non-motile psychrophilic and motile mesophilic strains). These strains were then used to screen the wastewaters of Latvian cities with the goal of isolating and characterizing lytic *Aeromonas*-infecting phages to select potential candidates for prospective use as biocontrol agents for local aquaculture settings. Up to date, more than 30 presumably different *Aeromonas*-infecting phages were isolated and purified by CsCl gradient ultracentrifugation, some of them already sequenced on the Illumina MiSeq platform and subjected to transmission electron microscopy. Sequenced phage genomes are currently undergoing an in-depth functional annotation to make sure that the selected candidates would be genomically safe to use in biocontrol. It is expected that the well- characterized phage collection that is being accumulated during this study shall serve as a basis for the formulation of a phage cocktail prototype that would open possibilities for an environmentally safe and effective phage-based biocontrol of *Aeromonas* spp. in Latvian aquaculture.

Phylogenetic analysis of LYSV and OYDV isolated from garlic and onion crops in Ukraine

K. Taher[1], H. Snihur[1,2], T. Shevchenko[1]

[1]Virology Department, ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, 64/13 Volodymyrska Street, Kyiv, 01601, Ukraine; [2]D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Ukraine

(kirilltager@gmail.com)

Garlic and onion are crucial agricultural crops in Ukraine. However, they are threatened by viral pathogens, specifically Leek yellow stripe virus (LYSV) and Onion yellow dwarf virus (OYDV). Comprehensive understanding of the genetic diversity of these viruses, in relation to their host specificity and geographical distribution, is vital for developing robust strategies for their control and prevention (Katis et al., 2012).

In this study, samples of garlic, onion, and other types of Allium plants from Vinnytsia, Kyiv, Cherkasy, Zaporizhzhya, and Poltava regions were analyzed using enzyme immunoassay and RT-PCR methods, employing primer sets to coat protein gene as described by Parrano et al., (2012).

Our phylogenetic analysis revealed that the Ukrainian OYDV isolates OYDV_31 and OYDV_32 formed a distinct clade, closely related to Italian isolates and specifically infecting onions, indicating the possible existence of a unique Ukrainian strain or variant of OYDV. The LYSV isolates LYSV_B and LYSV_112 identified were found to belong to the geographically diverse N-type LYSV, which is commonly associated with garlic crops. Interestingly, these LYSV isolates were more closely related to isolates from geographically distant countries like China, USA, Italy, and Serbia than to those from neighboring regions, potentially indicating virus transmission from other continents via extensive trade (Santosa et al., 2023).

The study highlights the presence of LYSV and OYDV in garlic and onion crops across multiple regions in Ukraine, revealing potential geographical and host-specific variations of these viruses. These findings will serve as a foundation for future studies, aiding in the development of effective strategies to control and prevent these diseases. They will also contribute to preserving the productivity of Ukraine's critical Allium crops, supporting economic stability and food security in the country.

Monitoring of sunflower crops for the presence of viral infections in Ukraine

V. Tsvigun, S. Mazur, I. Gumeniuk, and A. Levishko

Institute of Agroecology and Environmental Management of NAAS of Ukraine, National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine

(vika-natcevich@ukr.net)

Ukraine exports sunflower oil and grist to nearly 90 countries worldwide and remains a leader in this sector, with 55% of global sunflower oil exports. On sunflower crops, around 70 pathogens have been identified, resulting in an annual yield loss of 20-25%, deterioration in product quality, and reduced seed viability. The study aimed to determine the presence of viral infections in sunflower crops under different soil and climatic conditions in Ukraine. We collected plant samples from sunflower crops under different soil and climatic conditions (Steppe - Odesa and Mykolaiv oblasts; Forest-steppe - Kyiv, Vinnytsia and Cherkasy oblasts). Visual diagnosis confirmed the presence of various viral symptoms on sunflower plants, with their manifestation significantly varying even within the same plant. The most common virus-specific symptoms found were leaf mosaic, necrotic spots, blistering, wrinkling, enations, leaf twisting, and stunting of plants. The ELISA results revealed positive findings for CMV (cucumber mosaic virus), TMV (tobacco mosaic virus), and TSWV (tomato spotted wilt virus) among the investigated sunflower plant samples. The analysis of the obtained results uncovered the presence of CMV in all research areas. TMV was observed in the Forest-steppe zone (Kyiv and Vinnytsia oblasts), while TSWV was characteristic of the southern region of Ukraine (Odesa oblast). Drawing upon the research results, one can conclude that the spread of various viruses and their epiphytotic depends not only on the biological characteristics of the viruses and their optimal conditions for existence but also on the unpredictability and instability of weather and climatic conditions. These manifestations increasingly result from climate change on the planet.

Tobacco mosaic virus isolated from Norway maple (*Acer platanoides*): symptoms, diagnostic and propagation species

V. Tsvihun[1], N. Sus[1], and A. Orlovskyi[2]

[1]Institute of Agroecology and Environmental Management of NAAS of Ukraine, National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine; [2]Institute of Horticulture of NAAS of Ukraine, National Academy of Agrarian Sciences of Ukraine, Novosilky, Kyiv Region, Ukraine

(email@nazariy-sus.com)

Until now, we have only known about two virus species that infect Norway maple (*Acer platanoides* L.): maple leaf perforation virus and alfalfa mosaic virus (Šubíková, 1973 and 1994; Sutakova, 1984; Jerković-Mujkić, 2012). Here, the authors reported a third virus species that infect *A. platanoides* – the tobacco mosaic virus (TMV). Therefore, this study aimed to describe the symptoms of the disease caused by TMV in *A. platanoides* and to identify diagnostic and propagation species for it. Researchers identified the virus using enzyme-linked immunosorbent assay (ELISA) and transmission electron microscopy (TEM). To describe the symptoms and signs of the disease caused by TMV in *A. platanoides*, the authors observed infected plants for 3 years. As a result, the authors found that the disease symptoms change throughout the growing season. The first symptoms appear in April-May and manifest as barely noticeable chlorotic spots between the leaf veins. In May-June, these chlorotic spots develop into chlorotic mosaics. In July, necrosis of the leaf edges in infected plants occurs. These necrotized leaf edges curl up in August-September. In October, there is intense defoliation of the leaves in infected plants. The authors also found that peanut (*Arachis hypogaea* L.) and the pricklyburr (*Datura innoxia* Mill.) are the most suitable diagnostic species for this TMV isolate. In *A. hypogaea* inoculated with TMV isolated from *A. platanoides*, chlorotic patterns develop on the leaves. Simultaneously, necrotic reactions manifest on the leaves of *D. innoxia* inoculated with this TMV isolate. The researchers also identified a range of propagation species for this TMV isolate. This range of propagation species included the European black nightshade (*Solanum nigrum* L.), lamb's quarters (*Chenopodium album* L.), thorn apple (*Datura stramonium* L.), and Aztec tobacco (*Nicotiana rustica* L.). These propagation species manifested various dissimilar necrotic reactions. In conclusion, the authors recommend using this data for further studying this pathogen.

Identification of viruses infecting *Zea mays* L. in Ukraine

T. Vlasova[1], H. Snihur[1,2], T. Shevchenko[1]

[1]Virology Department, Educational and Scientific Centre 'Institute of Biology and Medicine' Taras Shevchenko National University of Kyiv, 01601 Kyiv, Ukraine; [2]Laboratory of Plant Viruses, D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, 03143 Kyiv, Ukraine
(t.vlasova3101@gmail.com)

Maize (*Zea mays* L.) is an annual plant of the *Poaceae* family, one of the most important cereal crops in the world, and is a major food commodity in many countries. During 2018-2022, Ukraine was producing approximately 34 million tons of corn per year. Viral diseases of corn are one of the reasons for the decrease of corn yield and deterioration of grain quality. According to available literature, only maize dwarf mosaic virus (MDMV) (Naumenko, 1973), High Plains wheat mosaic virus (HPWMoV) (Snihur, 2020) and sugarcane mosaic virus (SCMV) (Snihur, 2021) were found on corn in Ukraine up to day. Considering that more than 20 viruses are known to infect this crop, maize samples from different regions of Ukraine have been analyzed for presence of eleven common maize viruses including MDMV, HPWMoV, SCMV, maize chlorotic mottle virus (MCMV), maize streak virus (MSV), maize mosaic virus (MMV), maize white line mosaic virus (MWLMV), wheat streak mosaic virus (WSMV), barley stripe mosaic virus (BSMV), barley yellow dwarf virus-PAV (BYDV-PAV) and brome mosaic virus (BMV).

In 2021-2022, maize plants grown commercially and in home gardens were examined, and plant samples with typical viral symptoms were collected from three regions of Ukraine: Vinnytsia, Kyiv, and Kharkiv. Double or triple antibody sandwich (DAS or TAS) enzyme-linked immunosorbent assay (ELISA) confirmed the presence of SCMV in plants sampled in Kyiv region (28% of affected plants), as well as BYDV-PAV in samples collected in Vinnytsia (6%) and Kyiv (11%) regions. Overall, SCMV was detected in 12,5% of symptomatic plants, whereas BYDV-PAV was found in 7,5% of the maize samples. It should be noted that SCMV-infected plants developed different symptoms of streak and stripe mosaic.

Although the maize plants collected in Kharkiv region had clear symptoms of virus infection, we were unable to identify above-mentioned pathogens which may suggest the occurrence of other virus(es).

1. Naumenko L.A. (1973). Identification of maize mosaic virus. Mikrobiolohichnyi zhurnal, 35(4), 468-472 (in Ukraine).
2. Snihur H., Pozhylov I., Budzanivska I., Shevchenko O. (2020). First report of occurrence of High Plains wheat mosaic virus on different hosts in Ukraine. Journal of Plant Pathology, 102(2), 545-546.
3. Snihur H., Kharina A., Kaluzhna M., Chumak V., Budzanivska I. (2021). First report of Sugarcane mosaic virus in *Zea mays* L. in Ukraine. Mikrobiolohichnyi zhurnal, 83(5), 58-66.

Antiviral activity of silver nanoparticles coated with natural resins

Zaichenko O.V.[1], Baranova G.V.[2], Holovan A.V.[2]

[1] *ESC “Institute of Biology and Medicine”, Taras Shevchenko National University of Kyiv, Ukraine;* [2]*D.K. Zabolotny Institute of Microbiology and Virology NAS of Ukraine, Kyiv, Ukraine*

(olegzaychenko@ukr.net)

Viruses are capable of cell transformation, which leads to negative consequences. The Epstein-Barr virus is a human tumor virus and is etiologically linked to various malignancies. Although conventional antiviral drugs are useful for eliminating viral infections, they can lead to side effects or physiological toxicity. Silver nanoparticles and nanocomposites are known to have inhibitory properties against pathogenic microbes, including archaea, bacteria, fungi, algae, and viruses. Given the growing interest in nanotechnology, it is important to deepen knowledge about the interaction of nanoparticles with various viruses.

The aim of the study was to determine the cytotoxicity and antiviral effect of silver nanoparticles on EBV-associated cell culture models B95-8 and P3HR1. A runoff-stable experimental aqueous dispersion containing 20 mg/ml of silver nanoparticles coated with natural resins (AgNPs), manufactured by Noble Elements LLC, USA, was studied. The study was carried out with nanosilver particles in a polysaccharide shell using trypan blue dye, MTT test, neutral red, and PCR.

It was found that nanosilver at a concentration of 2000-200 μg/ml is toxic to both cell cultures by all three cytotoxicity assays, with less toxicity to human B-lymphocytes, in which the virus strain has no transforming effect, and greater cytotoxicity to tamarin monkey B-lymphocytes. The PCR method was used to determine the effect of the drug on virus replication. It has been shown that nanoparticles in non-toxic concentrations effectively inhibit EBV replication under conditions of chronic and lytic EBV infection in lymphoblastoid cells at a concentration of 2 μg/ml. Microscopy of nanoparticle and virus samples was also performed and it was found that incubation of the virus with NPs for 2 hours led to damage to EBV virions. Thus, the ability of silver nanoparticles in a polysaccharide shell to inhibit Epstein-Barr virus replication and to exhibit virulent effects upon prolonged contact was demonstrated.

Virucidal activity of polymer silver-containing nanocomposites obtained by the green synthesis

P. Zaremba[1], V. Demchenko[2], S. Zahorodnia[1]

[1]*Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine, Kyiv, Ukraine;* [2]*Institute of Macromolecular Chemistry of the NAS of Ukraine, Kyiv, Ukraine.*

(p.zaremba@imv.ogr.ua)

In the list of causative agents of acute respiratory diseases, influenza viruses have a permanent place, but the problem of the efficient drugs lack still remains unsolved, in particular due to the peculiarities of RNA-viruses. The approach to solving this issue should be comprehensive. We need not only etiotropic antiviral agents, but also virucidal, to reduce the spread of viruses. Silver nanoparticles have the ability to directly interact with virions, and special attention is paid to nanocomposites that improve the properties of this metal. In this research, the cytotoxicity and virucidal activity of silver-containing film materials based on low molecular weight chitosan (LMWC) and sodium carboxymethylcellulose (Na-CMC) polyelectrolyte complexes against influenza A virus were investigated. The effect of polymer silver-containing nanocomposites (LMWC-Na-CMC-Ag) obtained by the laser ablation method and the green synthesis using the green tea (GT) extract was compared. Polymers without Ag were also investigated (LMWC, Na-CMC, LMWC-Na-CMC, LMWC-Na-CMC-GT) as well as the green tea extract (w=8.2%) on an ethanol-glycerol basis. Experiments were performed using MDCK cell culture and influenza A (H1N1) virus strain A/FM/1/47. The cytotoxicity of the investigated nanocomposites was determined using the MTT assay. Virucidal activity was determined by the method of pre-incubation with the virus for 1 hour at 37°C followed by determination of the infectious titer (TCID50/ml). The results of the toxicity study show that of all the tested samples, only the green tea extract reduces cell viability by more than 50%. CC50 index was calculated for the exctract, it is 1:34.83±1.74 in dilution or 0.23% in mass fraction of dry matter. Polymers LMWC, Na-CMC, LMWC-Na-CMC, LMWC-Na-CMC-GT do not demonstrate virucidal activity against the influenza A virus. The green tea extract reduces the infectious titer of the virus by 106.06±0.3 compared to the virus control. The nanocomposite LMWC-Na-CMC-Ag-GT showed the best virucidal activity with complete inhibition of the infectious titer (≥109.3), on the other hand, LMWC-Na-CMC-Ag obtained by the laser ablation method was not effective. Thus, the polymer silver-containing film nanocomposite obtained by the green synthesis LMWC-Na-CMC-Ag-GT considering the lack of cytotoxic effect on MDCK cells and the effective virucidal effect against influenza A virus is promising for further research. Therefore, it is the green synthesis that makes it possible to obtain highly effective silver-containing composites with high virucidal activity against enveloped viruses. Accordingly, this may be important for many viruses that have airborne and fecal-oral routes of transmission.

Physiological and morphological changes in tomato plants infected with tomato leaf curl virus

Muhammad Ahmad Zeshan

Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha. Pakistan. 40100.

(muhammad.ahmad@uos.edu.pk)

The main objective of the study was to evaluate the effects of TLCVD on growth and yield parameters of tomato plants including their height, no. of fruits, intermodal distance, weight of healthy and infected leaves, no. of leaves and no. of branches. The presence of virus was confirmed by the biological assays such as whitefly mediated inoculation and leaf patch grafting. Growth and yield parameters of both healthy and diseased plants were compared to assess the losses. Leaf area was measured by using leaf area meter while vernier caliper was used for the fruit size. Leaves and fruits were weighed by using electric balance. Plant height was taken by measuring tape. Membrane stability index and electrolyte leakage was calculated by the method of Sairam (1994) and Sullivan and Ross, 1979, respectively. All the aforementioned growth and yield parameters were significantly reduced in infected plants as compared to healthy plants. Plant height, no. of fruits/plant, no. of branches, intermodal distance, no. of leaves/plant etc. decreased in diseased plants. Average electrolyte leakage of healthy leaves was 23% while 94% EL leakage recorded in infected leaves. The membrane stability index of healthy leaves was 61% that reduced to 44% in infected leaves. Average leaf surface area of infected leaves reduced to 5.23 cm^2 as compared to 6.33 cm^2 of healthy plants. TLCV infection interrupted the plant physiology considerably due to which all plant parts and physiological factors that contribute towards yield and quality of produce were greatly affected resulting in heavy yield losses.

Apple chlorotic leafspot virus genetic diversity in wild and cultivated hosts

N. Zuļģe[1], A. Gospodaryk[1] and I. Moročko-Bičevska[1]
[1] *Institute of Horticulture, Graudu str. 1, Ceriņi, Krimūnas par., Dobele reg., LV-3701, Latvia*
(neda.zulge@llu.lv)

Apple chlorotic leaf spot virus (ACLSV) has a wide distribution around the world in a wide range of economically important plants. It infects apples, pears, plums, and cherries, but has been found in many other fruit trees and ornamental plants of the *Rosaceae* family, which are increasingly used in landscaping or have significant importance in the horticultural industry. Although the only proved transmission pathway of the virus is a vegetative propagation, it has also been found in several wild species, indicating a much wider range of putative host plants and other potential ways of the virus transmission. To determine the genetic diversity of ACLSV, samples were collected from hosts in wild habitats and genetic resource collections during 2008 to 2021. Overall, 262 samples were collected, of which 216 were from different *Rosaceae* species, mainly from cultivated apples, pears, plums, apricots. Forty-six samples were collected from *Amelanchier, Cotoneaster, Crataegus, Malus, Prunus* and *Sorbus* plants in wild. Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania) was used to extract nucleic acids from leaf samples. Nucleic acid mixture was analysed by RT-PCR with Qiagen OneStep RT-PCR kit. ACLSV genome-specific primers (Menzel et al., 2002) were used for virus diagnostics in the plants. The second primer pair CP1/CP2 (developed in this study) were used for amplification of ACLSV CP gene. Among the fruit crops included in this study, 80% of ACLSV positive samples were from *Malus* plants. ACLSV was not detected in apricots and in wild *Amelanchier, Cotoneaster, Crataegus, Prunus* and *Sorbus* plants. The virus was detected in cultivated apples, pears, plums and wild *Malus*, including native species *M. sylvestris*. Totally, 24 ACLSV CP sequences from *Malus* hosts obtained in this study, and 267 homologues sequences from GenBank were used for phylogenetic reconstruction by maximum parsimony with 1000 replicates. The phylogenetic reconstruction showed that the two genetic types, B6 and P205 are common among the ACLSV isolates on *Malus* hosts. The clustering of sequences had no relation to the origin of hosts.