With this data we can come to a conclusion about the circulation of the virus in 19 regions of Ukraine. In the herds of Poltava and Ternopol regions, there was detected one PRRSV positive serum. We believe more research is needed to monitor dynamic changes of PRRSV antibodies in serum for the establishment of the epizootic situation in the farms in these areas. PRRSV was not detected only in herds from Lugansk, Rivne, Kherson regions, and AR Crimea (Table1). However, it should be noted that the number of samples that we examined in Lugansk, Rivne and Kherson regions were insufficient for analysis of epizootic situation and therefore, more research is needed.

Within a herd, PRRSV can rapidly spread through nose-to-nose contact and inutero infections [3, 12]. There are a number of factors to the contribution of PRRSV such as animal welfare, increasing herd size, carriers from purchased animals, and sperm [3, 12]. Infection can also be spread through PRRSV-contaminated clothing via injection [3]. Active animal trade, the purchase of pigs, and semen from different farms in Ukraine and the world, non-technology holding of animals, and a high variability of the pathogen can cause the spread PRRSV in Ukraine.

Conclusions and Prospects for Further Research. 1. The results suggest the circulation of the porcine reproductive and respiratory syndrome virus in 28 farms in 19 regions of Ukraine. 2. The results significantly complete the existing information about spreading PRRSV and indicating the spreading trend of the pathogen through Ukrainein comparison with studies epizootic situation 2006-2007. 3. It is important to create a bank of PRRSV isolates to make their sequencing for further analysis of the situation with PRRSV in Ukraine.

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SELECTION OF BACTERIOPHAGES, SPECIFIC TO *SERRATIA MARCESCENS*

Представлены результаты скрининга бактериофагов специфичных к возбудителю гнили лука. Высокостабильный литический фаг, специфичный к S. marcescens выделенный из окружающей среды и обозначен как бактериофаг Smd. У выделенного фага изучена морфология вирионов и состав белков. Показана способность фага Smd1 подавлять развитие бактериальной инфекции на чешуйках лука.

The results of screening bacteriophages specific to causative agent of onion decay are presented. Highlystable lytic phage specific for ***S.marcescens was isolated from the environment and denoted as phage Smd1. The virion morphology and protein content of isolated bacteriophage was studied. There was shown the ability of phage isolate Smd1 to reduce the development of bacterial infection on an onion scales.***

Introduction. Modern society focuses on ecologically pure future. Guarantees of that are "naturalization" of production, management of natural resources and energy.The preservation of crop losses caused by bacterial pathogens is significant problem for Ukraine and international community. Nowadays pesticides and herbicides, that in chemical nature are antibiotic substances, are widely used for prophylactic and therapeutic purposes. Only the last 20-30-years scientists began to pay special attention to the processes of resistance formation of pathogenic bacteria to the antibiotics. Therefore people faced the problem of search and development of new protective forms that would be characterized by high efficiency, safety and would not cause rapid adaptation of bacteria. The hypothesis of the possibility of using bacteriophages (viruses of bacteria) for therapeutic purposes was nominated in the beginning of the 20^{th}century. Nowadays, this direction develops rapidly and expands the range of phage usage [2,3]. Significant development as acquired agricultural phage therapy that is engaged in elaboration of phage preparations to deal with pathogenic bacteria (for example, *Erwinia carotovora*, *Pseudomonas sp.*, *Serratia marcescens*).

Due to the importance and attractiveness of the area of research, the aim of our study was the selection and characterization of lytic phages, specific to the pathogen *Serratia marcescens*, which causes an onion decay and is considered to be one of the most harmful phytopathogens for agriculture in Ukraine.

Materials and methods.

Bacterial strains. Studies were carried out on bacterial culture *Serratia marcescens* IMBG291 [5], generously provided by colleagues from the laboratory of microbial ecology, Institute of Molecular Biology and Genetics (National Academy of Sciences of Ukraine). Working with phages we used an overnight culture of bacteria, in which bacteria was in exponential phase of growth. The concentration of bacteria cell culture was 10^8-10^9 c.f.u./ml. Bacteria was cultivated on plate count agar or in PC-broth. Incubation temperature was 25 ^{O}C.

Sample collection. During laboratory studies a wide range of samples was analyzed. Samples were selected from vegetable washouts (including onion, potatoes, cab-

bage, carrot, sugar beet, etc.) and fruits with symptoms of rot processes and from waste water and water from open water reservoirs. Investigated samples were centrifuged and handled by chloroform. The objects of research became phage, isolated from samples of carrot with symptoms of rot processes.

Spot-titer assay. The samples or serial dilutions of samples were applied dropwise on the plates with seeded bacterial culture. Following 20 minutes they were kept at room temperature in order to samples diffused into agar medium. Then plates were overturned and incubated in a thermostat at 37^0C for 12 hours. After that the plates were analyzed for the presence of phages. Results were recorded as the reciprocal of the highest dilution at which clearing the lawn was evident.

Double agar layer method.0.2 ml of overnight bacterial culture (10^8 c.f.u./ml) was put together with 2.5 ml of 0.7% agar (the temperature of agar was 46-49^0C). Then 1 ml of the studied sample was added. The resulting mixture was accumulated on the bottom layer of 1,4% agar [1]. According to the results of spot-test, the concentration of phage particles in a sample of carrot was very high, so we diluted the phage lyzate to the 10^{th} degree in order to get separated plaques. After exposure within 15 minutes at the room temperature, plates were inverted and incubated at 37^0C for 12 hours. After incubation all resulting plaques were counted. Separate phage plaques were then picked. Isolated bacteriophages were purified by serial propagation of single plaques and amplified.

Electron microscopy. Morphology of virions was investigated using the electron microscope. Formvar films placed on 400-mesh copper grids were dipped into sample for 2 min and contrasted in 2% uranyl acetate. The preparations were dried and viewed under the electron microscope at an instrumental magnification of 20,000.

SDS-PAGE. Structural proteins of phage isolates were analyzed by SDS-polyacrylamide gel electrophoresis [4]. Phage particles purified by ultracentrifugation were mixed with the sample buffer and then heated in a boiling water bath for 3 min, followed by separating the proteins in the gel (12%). Protein bands were visualized by staining the gels with Coomassie brilliant blue.

Investigation of bacteriophages influence on the expression of pathogenic properties of the bacteria.

In laboratory conditions the infectious process was modeled with the defeat of onions by investigated bacteria *Serratia marcescens*. Onion bulbs were disinfected with a solution of potassium hypermanganate for 20 minutes. Onion bulbs were divided on scales in sterile conditions and placed in plates on sterilized filter paper discs. Then 10mkl of phage mixture (titer 10^{10}) and bacterial suspension (concentration of cells 10^8 c.f.u./ml) were applied on the scales. For controlling the initiation of pathogenic process with bacteria a drop of bacterial suspension was applied on the scales of onion, placed in other plate. Onion scales with deposited drop of physiological solution served as a control of the experiment. Plates with the studied materials were incubated in a thermostat at 28^0C during the week. For statistical significance of data each experiment was conducted in three repetitions.

Results and discussion. Plant material and water from two regions of Ukraine were screened for bacteriophages active against *Serratia marcescens* using a spot-test and double-agar technique. The agents capable to case the lysis of investigated bacteria were detected in carrot, sugar beet, onion, apples, water from career (tabl. 1).

***Table* 1. The research results of presence of *S. marcescens* phages in samples of different origins**

Sample	Thepresenceof lysis zones	Thepresenceplaques after re-seeding
Carrot *Vitaminna*	+	+
Potato*Vesta*	-	-
Cabbage Caporal	-	-
Sugar beet Cylindra	+	-
Sugar beet Bordo	-	-
Pepper Diabolo	-	-
Onion Snow Ball	+	-
Onion Buran	-	-
Apple Gloster	+	-
Apple Goldstar	+	-
RaddishMantagong	-	-
Cauliflower Moravia	-	-
Waterfromthesettlersewage	-	-
Water from well	-	-
Water from lake	-	-
Water from river	-	-
Water from spring	-	-
Waterfromcareer	+	-
Water from pond	-	-
Mule	-	-
Beetpulp	-	-
Silos	-	-

Then lytic spots were picked from agar and re-seeded on relevant strain of bacteria *Serratia marcescens* by the double agar layer procedure. It was observed that the majority of samples did not form negative colonies, with the exception of sample of carrot with symptoms of rot processes. The further work was carried out with this sample. After seeding the described sample on the *S. marcescens* lawn separate plaques with a clear edge 0,2-0,4 cm in diameter were received (fig. 1).

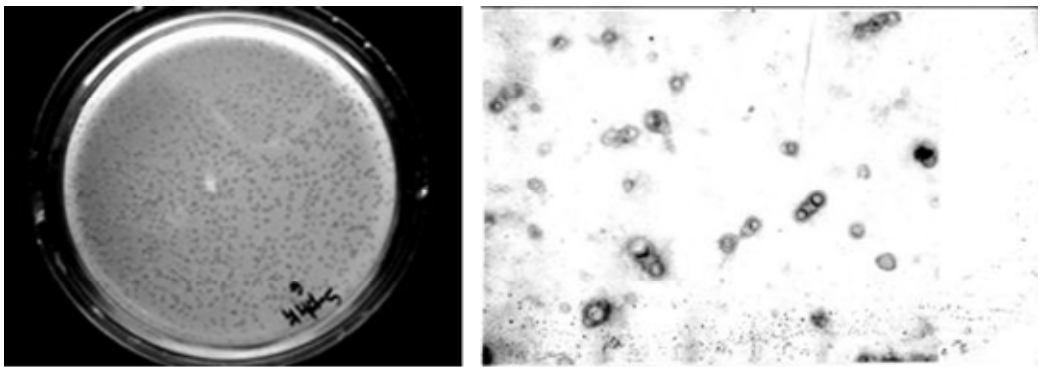

Fig.1. Phage plaques and electron micrographs of *Serratia marcescens* phages

The presence of phages in carrot samples was conclusively confirmed by TEM examination of concentrated phage filtrates (Fig. 1). Electron microscopy has revealed spherical phage particles without tails (fig. 1). According to the literary data it have not been identified viral particles of similar morphology for *S. marcescens* earlier. Therefore that is a problem of establishing their taxonomic affiliation. Newly isolated bacteriophage of *Serratia marcescens* was denoted as phage Smd1 and accumulated in high titer (10^{12}).

Six major protein bands (86,6 kDa, 45 kDa, 41,9 kDa, 35 kDa, 30,6 kDa, 21,4 kDa,) were identified after SDS-polyacrylamide gel electrophoresis of phage Smd1 proteins (Fig. 2).

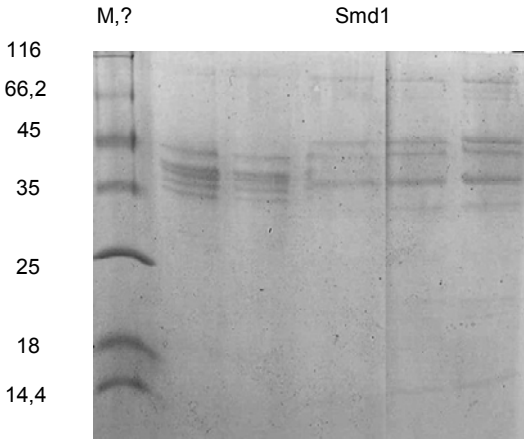

Fig.2. Protein gel profiles for purified virions of bacteriophage Smd1

As part of the experiments an impact of phage Smd1 on the manifestation of pathogenic properties of the investigated phytopathogenic strain of *S.marcescens* was modeled. Retrieved results showed that on control onion scales with deposited physiological solution and the scales with deposited phage and bacteria the processes of deterioration and rotting were not observed. On control scales with deposited overnight bacteria culture the gradual development of rotting processes was observed (fig. 3)..

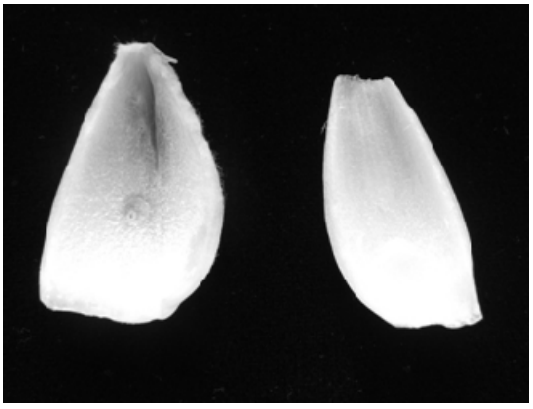

Fig. 4. An Impact of phage Smd1 on manifestation of pathogenic properties of bacteria *Serratia marcescens*:
A – onion scales with deposited suspension of bacterial culture;
B – onion scales with deposited suspension of bacteria and phage Smd1

Conclusion. Bacteriophage Smd1 was isolated from carrot and characterized. This phage is spesific to bacteria *Serratia marcescens* IMBG291 that case an onion diseases. Newly isolated phage morphologically and for it's proteins profiles differ from another known for today phages of *Serratia marcescens.* Taxonomy affiliation of bacteriophageSmd1 should be established. This phage can be useful for rapid diagnosis of *Serratia marcescens.* Obviously, the researched phage suppressed the growth of bacteriain a modelsystem. This leads us to suggestthat newly isolated phage can be used effectively as part of integrated onion disease management strategies.

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PECULIARITIES OF PHYLOGENY AND EXPRESSION OF TAS3-LIKE SMALL RNA PRECURSOR GENES IN PLANTS BELONGING TO FAMILY *ASTERACEAE*: EXPERIMENTAL APPROACH AND BIOINFORMATICS ANALYSIS

Используя разработанную ранее нами методику для детекции и клонирования генов малых транс-действующих интерферирующих РНК (TAS3 генов), мы показали, что во взрослых растениях трех видов родов Curio и Senecio происходит экспрессия только одного типа TAS3 генов. Более того, анализ баз данных последовательностей кДНК у других родов в семействе Asteraceae также показал преимущественную экспрессию только одного TAS гена, названного нами TAS3-Sen1 и проявляющего значительную межвидовую гомологию в пределах этого семейства. Более того, при транскрипции гена TAS3-Sen1 у растений рода Senecio образуется не единственная РНК (первичный транскрипт ta-siRNA), а набор РНК за счет преждевременной терминации транскрипции внутри гена TAS3-Sen1. Обнаружение нами в составе наиболее протяженногоTAS3-Sen1 РНК-транскрипта повторяющейся последовательности (GA)n, учитывая данные литературных источников, позволяют предположить ее участие в синтезе недостроенных транскриптов.

In this paper, we have studied the presence of tasiARF RNA genes in the representatives of genus Seneciohaving either unifacial or true bifacial leaves. It was found that both types of species encode such RNA precursors principally similar to those found in Arabidopsis. We used primers mimicking miR390 and oligo (dT)-based oligonucleotides. One of the TAS3 species (TAS3-Sen1) in Senecio representatives was actively transcribed and distributed among many Asteracea plants and found to be similar to AtTAS3a. The data obtained may contribute to understanding of expression regulation of Senecio TAS3-like genes and the potential involvement of these RNAs in development of plant organ abaxial-adaxial symmetry.

Introduction. In eukaryotes, small RNAs (sRNAs) exert transcriptional and post transcriptional control of genome expression to modulate development and responses to environmental stimuli [1–3]. Generally, sRNAs are inhibitors of gene expression that guide bound nuclease proteins to target nucleic acids via base-pairing interactions [3]. In the model plant *Arabidopsis thaliana*, sRNA biogenesis is catalyzed by homologues of the ribonuclease Dicer-like, DCL, that use double-stranded (ds) RNA as a substrate [4]. In plants, sRNAs can be broadly classified as microRNAs (miRNAs) and small interfering RNAs (siRNAs) [2, 3]. The genes encoding miRNA are transcribed by RNA polymerase II into primary transcripts containing a local stem-loop structure that provides the substrate for DCL1 cleavage into mature miRNAs of 21–22 nucleotides [1, 3]. miRNAs have a big impact as they negatively target cognate mRNAs for destruction or translational arrest [3]. Vascular plants including angiosperms (eudicots and monocots), gymnosperms and pteridophytes contain a repertory of miRNAs that are evolutionary conserved and control a large set of fundamental processes in plant development and homeostasis [5].

The various classes of plant 21- to 24-nt siRNAs derive from long dsRNA precursors that are processed by DCL2, DCL3 and DCL4 [1-3, 5]. The biosynthesis of these long dsRNA precursors usually entails the activity of one of several cell RNA-dependent RNA polymerases (RDRs) as well as plant viral RDRs [5]. In addition to virus-specific replicative-related double-stranded RNAs, sources of siRNAs include repetitive sequences, transposons, centromeres, convergent mRNA transcripts and other natural sense-antisense pairs, duplexes involving pseudogene-derived antisense transcripts and the sense mRNA from their cognate genes, hairpin RNAs as well as trans-acting siRNA (ta-siRNA)-generating transcripts (TAS) [1-6]. Endogenous siRNAs inactivate homologous sequences by a variety of mechanisms that include canonical post-transcriptional gene silencing as well as chromatin-dependent gene silencing [7].

The species of ta-siRNA were originally discovered in *Arabidopsis* [8]. Four gene families have been identified in *Arabidopsis* that each produce a number of ta-siRNAs: TAS1, TAS2, TAS3 and TAS4. Within the TAS1 family, TAS1a, TAS1b and TAS1c are very similar in sequence and all produce the siR255 ta-siRNA among others [6, 8]. One of the most important ta-siRNAs is the trans-acting short-interfering RNA-auxin response factor (tasiR-ARF). Auxins are a class of signaling molecules that play a central role in plant development. TasiR-ARF targets the mRNA of three Auxin Response Factor (ARF) genes (ARF2, ARF3/ETT and ARF4) for degradation [8]. TasiR-ARF is derived from the TAS3 gene. While *Arabidopsis* contains several ta-siRNAs not found in other plants, tasiR-ARF is highly conserved in all of these systems including mosses [6, 9]. This indicates that, like miRNAs, ta-siRNAs (and tasiR-ARF in particular) have been used to regulate gene expression in plants since before the separation between the seed plant and moss lineages [6, 8].

Each higher plant TAS gene so far identified produces a non-protein coding transcript that contains multiple ta-siRNAs within it. Each of these ta-siRNAs are lined up one after the other in both sense and anti-sense orientations. After transcription of the TAS gene, specific miRNAs pair with certain members of the Argonaute (AGO) protein family and bind to the single stranded RNA (ssRNA) at miRNA recognition sites [6, 10]. This specifies site specific cleavage of the primary TAS gene transcript at the beginning of the first ta-siRNA in the series and sets the phase for future processing [2, 6-8]. TAS3 gene transcript contain two miRNA recognition sites. Nevertheless only second (3'-terminal) site is cleaved AGO7 and downstream