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DESTABILIZATION OF THE PHAGE-BACTERIA SYSTEM DURING BACTERIAL INFECTIONS OF TREE PLANTS

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Despite significant progress in the understanding of the bacteriophage genome organization, the issues of their ecology and enormous diversity in nature remain under-explored. ***The aim*** *of the work was to study the destabilization effect of witches' broom disease symptom development in plant tissues on the pseudolysogenic endophytic bacterium Pantoea agglomerans resulting in accumulation of various lytic bacteriophages.* ***Materials and methods****. The subjects of the research were witches' broom disease affected parts of willow plants that were collected at the Nizhne-Dnestrovsky National Nature Park (Odesa region) as well as in the suburb of Kiev (Goloseevsky district) in the summer and autumn periods. Endophytic bacteria were isolated from the crushed plant material by extraction into Luria-Bertani broth. The bacterial and bacteriophage quantity was estimated according to the standard techniques. Purification and TEM imaging of phage particles were performed with using of common approaches.* ***Results.*** *Observations show that witches' broom disease of willow has massively epidemic character in Ukraine. For the first time for this disease, the main features of a three-component system: plant – epiphytic bacterium – bacteriophage were considered. It has been established that the transformation of plant tissues of Salix alba and Salix babylonica results in significant changes in the endophytic bacterial consortium with predominance of yellow-pigmented bacteria P. agglomerans (Pag). This in turn leads to the phage accumulation due to destabilization of the pseudolysogenic state of Pag in the binary system bacterium - phage. The number of viruses and their diversity depends on the bacterial host location in relation to the site of infection. Phages are less common in transformed willow tissues than at the periphery of the affected branches. In this work, the universal indicator system Pag g157 was proposed which determines up to 50% of specific bacteriophages. The morphology of negative colonies together with inability to lysogenize the sensitive bacterial cultures suggested that all isolated phages have an obligatory lytic lifestyle. A detailed study of six phage lines classified them as belonging to the Siphoviridae morphotype B1. The phage AF6 particles have the heads with the average diameter of 67,11 ± 1,87 nm and the tails of 168,96 ± 9,97 nm in length.* ***Conclusions.*** *Thus, a significant quantitative prevalence of P. agglomerans in the endophytic consortium together with the activation of pseudolysogeny in this bacterium can be a reliable indicator of local as well as systemic infections or mechanical damages of tree plants. In this work, approach for studying of the three-component system: plant – bacterium – bacteriophage has been proposed that may be helpful for our understanding of infectious processes occurring under interaction of biological objects with different complexity orders.*

Keywords: Salix alba, Salix babylonica, Pantoea agglomerans, "witches' brooms", phage-bacterium system, destabilization of phage system, new lytic phages.

To date, bacteriophages as well as other viruses are considered as the representatives of the peculiar world of living beings which differ from cellular life forms and included in a separate Akamara domain [1, 2]. They are characterized by an original genetic organization designed basically to parasitize effectively on their hosts. The general trend of studying viruses within a separate domain of life has intensified due to the accelerated development of genomics and DNA sequencing by "new generation methods" [3]. In this regard, phages are rarely considered in the context of a single two-component system of virus and cell that exists in the conditions of a permanently changing external environment. Meanwhile, phage-bacteria systems imply a wide variety of interactions developing during lytic, lysogenic and pseudolysogenic (the phage carrier state) types of infections.

Under natural conditions the phage-bacteria system predominantly is in an equilibrium state, namely in the state of pseudolyzogeny. Uncontrolled environmental changes cause the phage-carrier state to transform into persistent or lytic infection; classical lysogeny is possible only in the case of bacterial population carrying temperate phages [4]. However, conditions and delicate mechanisms for moving from the carrier state to persistent infection or true lysogeny still remain unknown.

As it has been previously found massive infection of tree plants caused by phytopathogenic bacteria *Erwinia amylovora* destabilizes the phage-bacteria system [5]. At the same time, there is the emergence of viral populations that mainly affect the epiphytic microorganism *Pantoea agglomerans*. Based on the results obtained, it was made a conclusion about *P. agglomerans* as a universal indicator of phage production during fire blight disease development. In addition, the original causative agent of phytopathogenic infection is not affected by phages. Probably, it is plant bacteriosis that provokes destabilization of the *P. aglomerans* pseudolysogenic state leading to the development of persistent phage infection.

Thus, based on the obtained data, we can assume that tree plants and their permanent endophytic satellite, *P. agglomerans*, are a convenient model for the study of the pseudolysogenic state and its destabilization with subsequent phage accumulation. In this regard, the current work aimed to study the phage's ability to multiply within *P. agglomerans* that appear under conditions of tree damage by various phytopathogenic factors.

Materials and methods. Sampling was carried out in Kiev and in the Nizhne-Dnestrovski National Natural Park (Odesa region) in the summer and autumn. Willow tree parts (*Salix babylonica* and *Salix alba*) with galls ("witches' brooms") and poplar leaves with insect infestation symptoms were collected.

To isolate *P. agglomerans* bacteria, the affected plant parts were treated with 70% alcohol. The crushed plant material was placed into 4 ml of LB medium and incubated at 4°C for 18 hours. Next, bacteria were plated on solid LB medium and incubated at 28°C. The appearance of colonies was observed after 2 days. Yellow pigmented, shiny colonies of *P. agglomerans* were selected for further work. Preliminary identification of *P. agglomerans* strains was performed by gas chromatography analysis of the fatty acid composition, based on the ability to ferment sugars, spectrophotometric determination of carotenoids [6] and, in some cases, phage typing with *P. agglomerans*-specific phage KEY and its variant (KEY/25) [7].

Phage isolates were obtained from 1 ml of the initial samples, to which 100 µl of chloroform was added and the samples were then centrifuged for 10 minutes at 11,000 rpm in an Eppendorf centrifuge. The collected supernatant was taken for the original phage isolate.

The isolated phages were cloned (up to 6 times) on the sensitive strains of *P. aglomerans*. All phage isolates were active against the tested *P. agglomerans* strains g157 (isolated from fish, England) and g157/RI (phage-resistant variant of strain g157).

Bacteria and their accompanying bacteriophages were isolated similarly from unaffected parts of plants and together with the collection strains of *P. agglomerans* (*Pag*) g157, g157/RI, g150, 9/7-2 and 28/2-1 were used in control experiments.

For phages from primary phage isolates, phage host range and efficiency of plating on the *Pag* strains were determined using the standard double layer agar plate method.

The preparations of viral particles were obtained by the confluent lysis after phage cloning on two strains of *P. agglomerans* g157 and g157/RI, with the highest phage sensitivity. The obtained earlier preparation of phage KEY/25 [7] was used for comparison.

For electron microscopic studies, phage particles purified in a CsCl gradient were used. The phage samples were adsorbed to carbon-coated nitrocellulose grids for 20-40 minutes and stained with 2% uranyl acetate. The studies were performed using the JEOL JEM 1400 transmission electron microscope. Phage particles were measured on microphotographs using ImageJ and Adobe Photoshop CS5 software, the schemes were built in Adobe Illustrator CS5, and statistical analysis was performed in Excel.

Results and discussion. The main material for the research was the parts of *S. babylonica* and *S. alba* affected by “witches’ brooms” (Fig. 1). The plant material with disease symptoms was collected in Kiev and on the Turunchuk River bench within the Nizhne-Dnestrovsky National Natural Park (Belyaevka town, Odesa Region) in the summer and autumn period.

Despite the considerable distance from each other (about 500 km), the willow tree damage did not differ in both regions of Ukraine. Initially, “witches’ broom” damage looks like a proliferation of elongated threads from the plant bud that acquires a spherical shape over partial differentiation (Fig. 1, 1 – 4).

To date, the symptomatology of this massive willow disease is unknown and requires a detailed study. However, it can be stated unequivocally that the result of the disease is an extensive transformation of plant tissues.

By analogy with fruit trees affected by a fire blight disease [5], we suggested that “witches’ brooms” could be a source of bacterial viruses. As it was previously shown, the endophytic bacterium *P. agglomerans* can be use as the universal indicator for phages of various phytopathogens [8].

Thus, it may be appropriate to consider the phage and *P. agglomerans* isolates selected from the same local lesion as a binary, involved phage-bacterium system.

Fig. 1. The gall-lesions («witches' brooms») of willows (*Salix alba*). 1 – 3 – general view of affected plants and 4 – the separate lesion of *S. alba*

Further, we have assumed that this binary system may be in a state of pseudolysogeny, when the plant or its parts are healthy, whereas "witches'broom" damages should generally destabilize this state. Therefore, the number of endophytic bacteria was primarily compared between the tissues of different stems (affected and without visible disease symptoms). In this case, initial recognition of representatives of *P. agglomerans* is not a challenging task, since it can be done through determining the quantity of well-marked yellow-pigmented colonies in contrast to unstained colonies of other endophytic and contaminant microorganisms.

Our observations have shown that the development of «witches' brooms» symptoms in two willow species (*S. babylonica* and *S. alba*) takes place without visible differences and consists of four stages. The initial stage (stage 1) is associated with the appearance of separate stem-shaped thin and long enough growths in the internodes of plant branch. These growths have highly reduced leaf blades (Fig. 2, a).

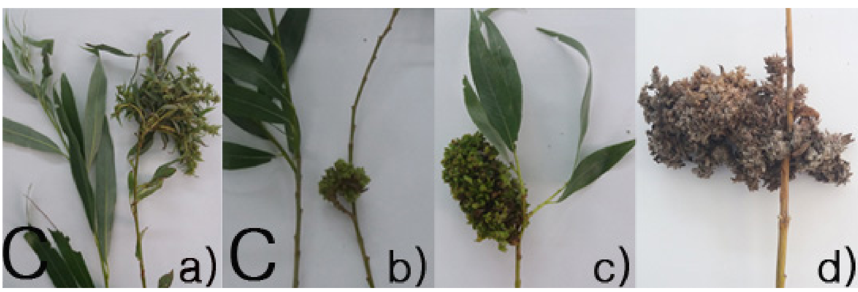

Fig. 2. Witches' brooms on willow: a – stage 1, the separate stem-like thin and and long enough growths in the internodes of plant branch, b – stage 2, the spherical or ellipsoid dense growth , c – stage 3, d – stage 4, the wilting of growths that acquire yellow or gray-black color; C – the control samples are the unaffected parts of the plants

In the second stage, branching is significantly enhanced leading to the formation of a spherical or ellipsoid dense growth (Fig. 2, b). At these two stages, the growth actually reminds the "broom" with rich green color. Next, the "broom" begins to turn yellow and the peripheral part of the affected branch loses leaves (stage 3). The last (fourth) stage is characterized by the complete wilting of growths with acquisition of a yellow or gray black color. The affected branch dries out completely and becomes brittle. The peak development of the witches' broom disease clearly correlates with the increasing of metabolic rate in gall formations. It can be assumed that the process described above may destabilize the microbial community at the site of symptoms formation. In order to verify this hypothesis, isolates of endophytic bacteria were extracted directly from the galls with the second or third symptoms formation stage.

The same order willow branches without visible witches' broom disease symptoms were used as a control (Fig. 2, a and b – C). Our numerous studies show that any bacterial association isolated from the lesions of the any plant contains pigment producing bacteria. The results of current study have fully confirmed this position. In contrast to control samples, all three isolates contain an increased number of yellow-pigmented bacteria (Table 1). Although one control sample (sample No. 2) appeared to be an outlier, since the fraction of yellow-pigmented bacteria was 0.15. This number is 4.7 times less than in the test sample isolated from affected plant with the second symptoms development stage (Table 1).

Table 1

Characteristics of bacterial isolates from *S. alba* with symptoms of "witches' broom" disease

Characteristic	Bacterial isolate from control (C) and test samples (O)							
	№1		№2		№3		№4*	
	C	O	C	O	C	O	C	O
CFU ** / cup / 3 μL	0	110	80	100	35	102	31	277
The proportion of yellow-pigmented colonies	0	0,22	0,15	0,70	0	0,23	0,16	0,50
Place of selection / the stage of damage***	I	II/2	I	II/2	I	II/3	I	II/2

* – Insect-affected *Populus alba* bacterial isolate (with visible thin bites) was used as a positive control. ** – CFU colony forming unit. *** – sites of sampling of the stem (leaf) without visible damage (I) or transformed/infected tissue (II) at the intermediate (2) or late stage (3) of disease (see Fig. 2).

In the case of a positive control study, the poplar leaves with and without signs of multiple insect bites were used (Fig. 3). The number of yellow-pigmented bacteria was considerably higher in the damaged leaf extract than in the extract obtained from unaffected leaf (Table 1).

Thus, the presented results show that the transformation of *S. alba* tissues leads to significant changes in the endophytic bacterial consortium. It is easily detected by the ratio of unstained and yellow pigmented bacteria; the last microorganisms noticeably prevalent in the experimental plant extracts. This phenomenon is most likely characteristic of many lesions of various plant parts (see sample number 4, Table 1, and also [9]).

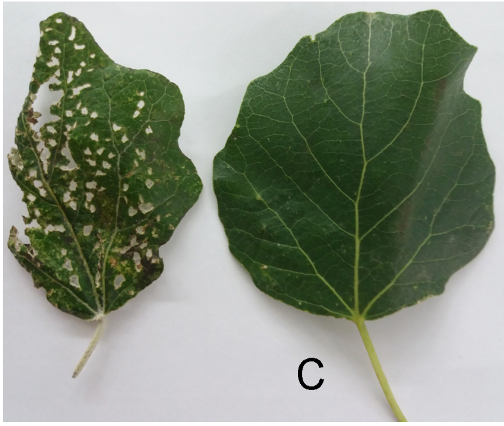

Fig. 3. Insect- damaged leaf of silver poplar (*Populus alba*): C – control sample

The following criteria were used for the preliminary genus and species identification of yellow pigmented bacteria: the ability to ferment sugars, the qualitative and quantitative composition of cellular fatty acids, the presence and type of carotenoids, and the plasmid content of bacterial cells (data not shown, private communication Gorb T.E.). The tested bacteria were classified as *P. agglomerans* (*Pag*) (Fig. 4), these criteria were applied in subsequent studies.

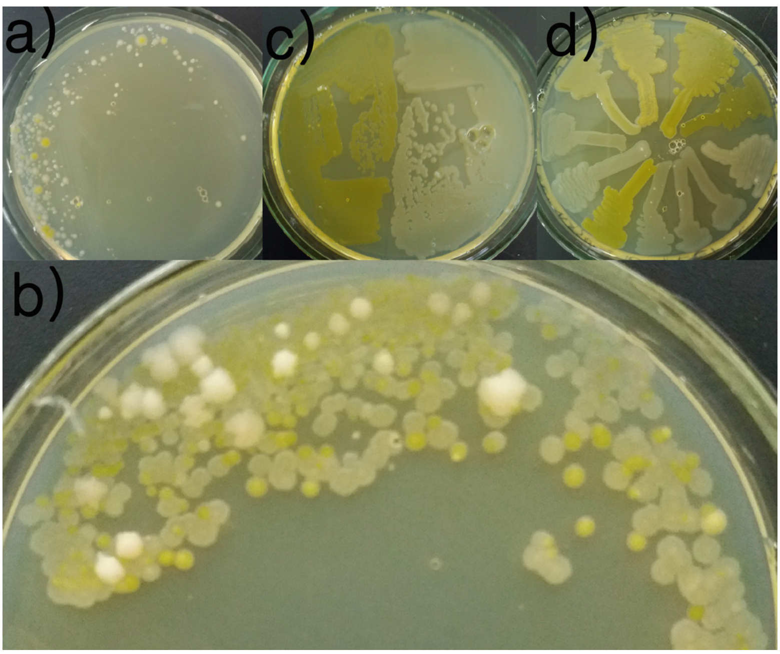

Fig. 4. Microbial diversity of the gall-lesions of *S. babylonica* (a and b): c and d - *P. agglomerans* strains (pigmented and depigmented variants)

We further suggested that there should be processes in affected tissues changing not only the endophytic consortium, but also phage populations, which typically accompany the individual bacterial community members.

In order to verify this assumption, chloroform-threated and centrifuged isolates (No. 1–4, Table 1) were applied onto agar plates with lawns of the *Pag* 9/7-2, g157, g157/RI, g150 collection strains and the *Pag* strains obtained in this work (No. 2O, No. 4O and 1, 2, 3, see below). In a group of indicator strains consisting with 4 collection strains and strains No. 2O, No. 4O (Table 1), the control phage KEY/25 forms clearly distinguishable plaques except for strain No. 4O. Under application of isolates to the lawns of 6 different indicators, the spots of lysis were observed in about half of the cases (21 of 48), but subsequent passages did not result in individual plaque formation and the spots of lysis disappeared. Perhaps the cells of indicator strains are responding to the presence in samples of phytoncides or other similar factors. As can be seen from the table 2, only sample I-3O contains viable phage particles which propagate on the *Pag* 9/7-2 strain.

Thus, despite the significant prevalence of *P. agglomerans* in transformed tissues of *S. alba* in comparison with other endophytes (Table 1), the *P. agglomerans*-infecting phages are extremely rare in the places of general virus-host localization: their frequency of occurrence is only 0.002.

For more detailed studies, a set of indicator cultures was supplemented by three additional *Pag* strains I, II, and III (Table 2). It turned out that the increase in the number of indicators raises the frequency of phage detection more than 3 times and is about 7 %. This trend may indicate that bacteriophages appear with the changing of bacterial consortium and represent the great diversity.

On the other hand, the number of phages may also depend on the bacterial host location relative to the infection site. Therefore, we studied the sample taken at the periphery of affected brunch and at the considerable distance from "witches' broom", with the third stage of symptoms development (see above). This sample has demonstrated clear positive results on the *Pag* g157, g150 and II indicators (Table 2). Taking this into account, the frequency of phage detection was 9 % in the group of 9 indicator cultures and 9 phage isolates.

The phage-sensitive strains chosen as indicators can be divided into 4 groups (Table 2). In this group the I-2O and I-4O strains isolated from *S. alba* and *P. alba*, respectively, were the least sensitive to tested phages. The phage sensitivity of both strains was 6.3 %.

The phage sensitivity of the *Pag* laboratory strains 9/7-2 and g150 as well as in strain III was at the level of 12.5 %. It is interesting that strains I and II isolated from *S. babylonica* was found to have sensitivity of 25 %. Two lysogenic strains, g157 and g157/RI, were the most sensitive strains, while the *P. agglomerans* strain g157 can be considered as a universal indicator for *P. agglomerans*-infecting as it have been previously shown in our studies [9].

With this indicator, the phage presence was determined in 7 out of 16 isolates that is close to the 50 percent sensitivity. For this reason, it is strain g157 that was used for obtaining of pure phage lines through six. *P. agglomerans*-infecting viruses isolated from witches' broom disease affected willow plants have lytic phages plaque morphology.

Each negative colony of all six cloned lines consists of an absolutely clear center and a wide halo (Fig. 5).

Purified phage particles obtained after differential centrifugation and a step gradient were used for electron microscopic studies.

Table 2

The sensitivity of the *P. agglomerans* strains to phages during primary titration of isolates from infected and control plants

Strains of *Pantoea agglomerans*	Isolates*															
	KEY/25	I-1K	I-1O	I-2K	I-2O	I-3K	I-3O	I-4K	I-4O	I-O	II-1	II-2	II-3	II-4	II-5	II-6	Phage sensitivity (%)
9/7-2	ph	+	+	+	+	+	ph	+	-	-	-	+	+	+	-	-	12,5
g157	ph	-	+	+	+	-	+	-	+	ph	ph	ph^3	ph	ph	-	ph	13,8
g157/RI	ph	+	+	+	-	-	-	-	-	-	ph	ph	ph^2	ph^1	-	ph^1	37,5
g150 yellow**	ph	+	+	+	-	-	+	-	-	ph	+	+	+	+	-	+	0,5
№2O	ph	-	+	-	-	+	-	+	-	+	-	-	-	+	-	+	6,3
№4Oyellow**	+	-	-	+	-	-	-	-	-	-	-	+	ph	+	+	+	6,3
I	ph	+	+	-	+	+	+	+	ph^1	+	ph	+	-	-	ph	+	25,0
II	+	ph^1	+	-	-	ph^1	+	-	+	ph^1	-	ph	+	+	-	-	25,0
III	+	+	-	+	-	-	-	-	+	+	-	ph	ph	-	-	-	12,5

* The phage isolates were obtained in geographically separate regions of Ukraine (Odesa region and Kiev) from affected plants *S. alba* (I) and *S. babylonica* (II), respectively.

** - “yellow” - means that the colonies turn yellow when exposed to visible light during 3-5 days;

“+” - the presence of lysis spots;

“-” - the absence of lysis spots and phage plaques;

ph - phage plaques (5 µl suspension);

ph1, ph2 and ph3 - one plaque, large plaques within the spot and the separate large clear plaques (3 mm).

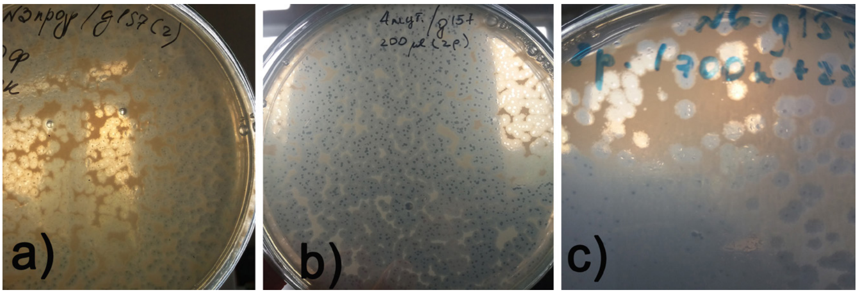

Fig. 5. Negative colonies of phage clones on the lawns of *P. agglomerans* g157. Initial phages were obtained from isolates 3 (a), 4 (b) and 6 (c)

All six phages had a typical B1-morphology and were assigned to the *Syphoviridae* family. The peculiarity of these viruses is the special structure of the basal plate close to that of the KEY phage [10]. The sizes of the heads (the distance between the opposite apexes) of the AF6 phage are 67,11 ± 1,87 nm, and the length of their tail processes is 168,96 ± 9,97 nm, respectively (Fig. 6).

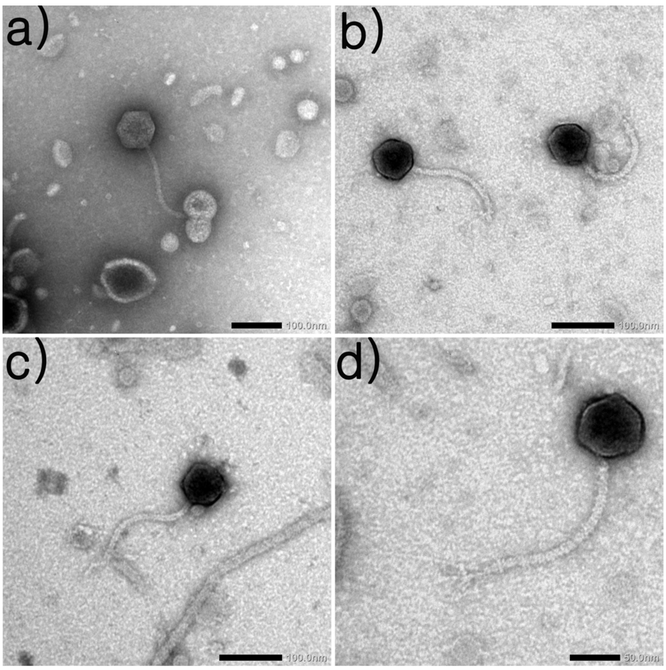

Fig. 6. Electron micrographs of AF6 phage (a, b, c and d) contrasted with 2% uranyl acetate. The scale bars correspond to 50 and 100 nm

Discussion. Our observations show that willow damage by witches' broom disease reached epidemic proportions in Ukraine independently of the natural and climatic conditions as well as geographical region (Fig. 1). In this regard, the disease is of interest for environmental studies.

In this work, the main features of a three-component system: plant – epiphytic bacterium – bacteriophage was examined for the first time. As a model, willow plants of two species *S. alba* and *S. babylonica* were collected from two extreme regions of Ukraine, the northern (Kiev) and the southern (Odesa region), respectively. This approach allowed us to note two basic patterns. It has been shown that the transformation of willow tissues caused by witches' broom disease leads to significant changes in the quantitative composition of endophytic bacteria with predominance of yellow-pigmented bacteria *P. agglomerans* (Table 1). And at the same time, there is pseudolysogeny in this three-component system which activation provokes accumulation of *P. agglomerans*-specific bacteriophages.

On the basis of these observations, *P. agglomerans* and its lytic bacteriophages can serve as indicators of local as well as systemic plant infections. As we have shown early, *P. agglomerans*-infecting phages together with phage KEY of *E. amylovora* accumulate in fruit tree tissues possessing the symptoms of fire blight disease [9, 11]. Thus, we assumed that the detected phenomenon can be widespread. In this work, we also found that insect caused mechanical damages of poplar leaves leads to an increasing the absolute number of *P. agglomerans* and the appearance of significant number of associated bacteriophages in the plant tissue (Tables 1 and 2).

In this report, we introduced the concept of a "universal indicator bacterium" which means the *P. agglomerans* strain with ability to determine the greatest number of phages, typical for tree tissues with local or systemic infection. A certain set of such strains with maximum phage sensitivity can be used for the maximum phage screening in perspective.

To date, the ecology of bacterial viruses is more studied for marine phage-bacterial communities [12]. Ecological studies of plant associated bacteria are rare [13]. Therefore, with a view to the future, the proposed new three-component system: plant – bacterium – bacteriophage will expand general knowledge about infectious processes occurring during complex interaction of various biological objects.

ДЕСТАБІЛІЗАЦІЯ СИСТЕМИ ФАГ–БАКТЕРІЯ ПРИ БАКТЕРІАЛЬНИХ ІНФЕКЦІЯХ У ДЕРЕВНИХ РОСЛИН

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Резюме

Незважаючи на значні успіхи в розумінні геномної організації бактеріальних вірусів, невирішеними залишаються проблеми їх екології і величезного різноманіття в природі. **Мета** роботи полягала у вивченні дестабілізації псевдолізогенії ендофітної бактерії *Pantoea agglomerans* при розвитку в рослинних тканинах верби симптомів хвороби «відьмина мітла», яке призводить до накопичення різноманітних літичних бактеріофагів. **Матеріали і методи**. Об'єктами досліджень були частини рослин верби, уражені хворобою «відьмина мітла», відібрані в Нижнєдністровському національному природному заповіднику (Одеська область) і на околиці м. Києва (Голосіївский район) влітку та восени. Ендофітні бактерії виділяли після подрібнення матеріалу і його екстракції в рідке средовище LB. Облік кількості бактерій і бактеріофагів проводили стандартно. Для очистки і електронно-мікроскопічної візуалізації фагових часток використовували загальноприйняті підходи. **Результати.** Спостереження показують, що враження верби «відьминими мітлами» на Україні носить масовий епідемічний характер. Для цієї хвороби вперше розглянуто основні особливості трьохкомпонентної системи: рослина – епіфітна бактерія – бактеріофаг. Встановлено, що трансформація тканин рослин *Salix alba* і *Salix babylonica* призводить до суттєвої зміни ендофітного бактеріального корсорціуму, в якому спостерігається надлишкова кількість жовто-пігментованої бактерії *P. agglomerans* (*Pag*). В свою чергу це призводить до накопичення бактеріофагів за рахунок дестабілізації псевдолізогенного стану *Pag* в бінарній системі бактерія – фаг. Кількість вірусів і їх різноманіття залежить від місця локалізації бактерії-хазяїна по відношенню до вогнища інфекції. В трансформованих тканинах верби фаги зустрічаються не так часто, як на періферії уражених гілок. В роботі запропоновано універсальну індикаторну систему *Pag* g157, за допомогою якої може бути визначено 50 % специфічних бактеріофагів. По морфології колоній і по неспроможності до лізогенізації чутливих культур визначено, що всі виділені фаги є строго літичними. Детальне дослідження шести фагових ліній показало, що вони представлені бактеріофагами родини *Syphoviridae*, які відносяться до B1-морфотипу. Середній діаметр фагових капсидів для фага AF6 складав 67,11 ± 1,87 нм, а довжина його хвостового відростка – 168,96 ± ± 9,97 нм. **Висновки**. Таким чином, значне кількісне превалювання *P. agglomerans* в ендофітному консорціумі, а також активація псевдолізогенії цієї бактерії може слугувати надійним показником не тільки локальних, а й системних інфекцій або механічних уражень деревних рослин. Запропонований в роботі підхід для дослідження трьохкомпонентної системи рослина – бактерія – бактеріофаг може в перспективі розширити загальне уявлення про інфекційні процеси, які протікають на рівні взаємодії при участі біологічних об’єктів різних рівнів складності.

Ключові слова: *Salix alba, Salix babylonica, Pantoea agglomerans*, «відьмині мітли», система фаг–бактерія, дестабілізація фагової системи, нові літичні фаги.

ДЕСТАБИЛИЗАЦИЯ СИСТЕМЫ ФАГ–БАКТЕРИЯ ПРИ БАКТЕРИАЛЬНЫХ ИНФЕКЦИЯХ У ДРЕВЕСНЫХ РАСТЕНИЙ

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Резюме

Несмотря на значительные успехи в понимании геномной организации бактериальных вирусов нерешенными остаются проблемы их экологии и огромнейшего разнообразия в природе. **Цель** работы состояла в изучении дестабилизации псевдолизогении эндофитной бактерии *Pantoea agglomerans* при развитии в растительных тканях ивы симптомов болезни «ведьмина метла», которое приводит к накоплению разнообразных литических бактериофагов. **Материалы и методы.** Объектами исследований были части растений ивы, пораженные болезнью «ведьмина метла», отобранные в Нижнеднестровском национальном природном заповеднике (Одесская область) и на окраине г. Киева (Голосеевский район) в летний и осенний периоды. Эндофитные бактерии выделяли после измельчения материала и его экстракции в жидкую среду LB. Учет количества бактерий и бактериофагов проводили стандартно. Для очистки и электронно-микроскопической визуализации фаговых частиц использовали общепринятые подходы. **Результаты.** Наблюдения показывают, что поражение ивы «ведьмиными метлами» на Украине носит массовый эпидемический характер. Для этой болезни впервые рассмотрены основные особенности трехкомпонентной системы: растение – эпифитная бактерия – бактериофаг. Установлено, что трансформация тканей растений *Salix alba* и *Salix babylonica* приводит к существенному изменению эндофитного бактериального корсорциума, в котором наблюдается избыточное количество желто-пигментированой бактерии *P. agglomerans* (*Pag*). В свою очередь это приводит к накоплению бактериофагов за счет дестабилизации псевдолизогенного состояния *Pag* в бинарной системе бактерия – фаг. Количество вирусов и их разнообразие зависит от места локализации бактерии-хозяина по отношению к очагу инфекции. В трансформированных тканях ивы фаги встречаются не так часто, как на периферии пораженных веток. В работе предложена универсальная индикаторная система *Pag* g157, с помощью которой может быть определено 50 % специфических бактериофагов. По морфологии колоний и по неспособности к лизогенизации чувствительных культур определено, что все выделенные фаги являются строго литическими. Детальное исследование шести фаговых линий показало, что они представлены бактериофагами семейства *Syphoviridae*, относящимися к В1-морфотипу. Средний диаметр фаговых капсидов для фага AF6 составляет 67,11 ± ± 1,87 нм, а длина его хвостового отростка – 168,96 ± 9,97 нм. **Выводы.** Таким образом, значительное количественное превалирование *P. agglomerans* в эндофитном консорциуме, а также активация псевдолизогении этой бактерии может служить надежным показателем не только локальных, но и системных инфекций или механических поражений древесных растений. Предложенный в работе подход для исследования трехкомпонентной системы растение – бактерия – бактериофаг может в перспективе расширить общее представление об инфекционных процессах, протекающих на уровне взаимодействий с участием биологических объектов разных порядков сложности.

Ключевые слова: Salix alba, *Salix babylonica, Pantoea agglomerans,* «ведьмины метлы», система фаг-бактерия, дестабилизация фаговой системы, новые литические фаги.

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