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M.A. Zlatohurska[1], F.I. Tovkach[2]

[1] Mechnykov Odesa National University,
2 Dvoranska Str., Odesa, 65082, Ukraine
[2] Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine,
154 Acad. Zabolotny Str., Kyiv, 03143, Ukraine

MORPHOLOGICAL HETEROGENEITY OF TEMPERATE ERWINIOPHAGE 59

This paper is devoted to the phenomenon of morphological heterogeneity within the population of the temperate erwiniophage 59, which does not have analogies among other bacterial viruses. ***Aim.*** *To investigate the basic properties of erwiniophage 59 heterogeneous population obtained from different isogenic strains of amilovora-like bacterium Erwinia "horticola" (Eho).* ***Methods.*** *Erwiniophage 59 was obtained by propagation on its traditional host Eho 450, as well as on its three isogenic strains and a related bacterium E. "horticola" 60. Physical and chemical properties of the phage particles were studied using centrifugation in CsCl-gradients, electrophoresis in agarose gels, electron microscopy, restriction analysis of DNA and SDS-PAGE of the virion polypeptides.* ***Results.*** *It was shown that the pool of temperate phage 59 is a heterogeneous population consisting of two phage types when propagated on the mentioned hosts. These types are discrete and have buoyant-density difference of 0.02 g/cm^3, that allowed to separate subpopulations for a detailed investigation. The subpopulation with the higher density was determined as the authentic bacteriophage 59 (subpopulation II with capsid diameter of 55.36 nm). The capsid diameter of the subpopulation I particles equals 51.16 nm. Both types of particles do not differ by DNA size and have identical restriction patterns. Based on the SmaI-restriction analysis it may be concluded that the DNA packaging remains unchangeable and is carried out according to the headfull packaging mechanism. However, the subpopulation I differs from the original one by a relative content of some polypeptides. Curiously, the subpopulations I and II have different values of lysogenization and spontaneous induction frequencies.* ***Conclusions.*** *An unusual type of morphological heterogeneity of the phage 59 particles was observed for the first time; this heterogeneity is associated with the presence of two equimolar subpopulations with different physical and chemical parametrs of the virions. Morphological heterogeneity of temperate erwiniophage 59 significantly differs from such of the classical coliphages as T4, P1 and the phage system P2–P4.*

K e y w o r d s: temperate erwiniophage 59, morphological heterogeneity of phage population, physical and chemical properties of particles, restriction analysis of DNA, lysogeny.

It is widely known that natural phage populations are characterized by diversity of viral particles. There are two basic types of such heterogeneity. In the first case, the lysates contain separate structural components along with the intact particles – capsids, tails, baseplates and fibers; they are not able to assemble into mature virions due to morphopoetic deviations. As a rule, these components constitute a small percentage of the lysate, however, their quantity rises under an abortive infection [13]. The second case is directly associated with defects of such a stage of the virion assembly as morphogenesis. As a result, in addition to normal virions, the infection processes is accompanied by formation of nonviable phage variants with a changed structure and size of capsid. Since the mentioned changes are related to the virion morphology, such diversity of a phage population can be defined as

morphological heterogeneity. The "classical" examples of morphological heterogeneity were described for coliphage T4 [9], P1 [18] and the helper-satellite system of phages P2–P4 *Escherichia coli* [11]. The reason of the populational heterogeneity of these phages are related not only to accidental, but also to targeted changes on various stages of phage head morphogenesis. Capsid morphogenesis is affected by structural changes in the phage genome as well as by the functioning of the host chaperone systems [5]. Therefore, morphological heterogeneity should be considered as an expression of the dynamic phage-bacterial interaction system.

Investigating temperate erwiniophage 59 [3] we revealed the phenomenon of morphological populational heterogeneity which has no analogies among other viruses of bacteria. When propagated on various sensitive strains, the phage has produced two equimolar subpopulations with different capsid sizes, buoyant density, protein contents but with the same size of genome. Morphological heterogeneity also influences the phage life cycle changing its lysogenization and lysogenic induction frequencies.

Thus, the aim of this research was to characterize the populational heterogeneity of erwiniophage 59 obtained on different isogenic strains of the host microorganism.

Materials and methods. The objects of the research were: different variants of bacteriophage 59 propagated on the original strain *E. "horticola"* (*Eho*) 450 [1], its auxotrophic mutant *Eho* 450 His3 [16], its isogenic strains *Eho* 450(49) [14] and *Eho* 450(P1)$^-$ [2], and a related bacterium *Eho* 60 [14]. The nutrient medium № 1 (SRCAMB, Russia) was adapted to the cultivation needs of phytopathogenic *Erwinia* species by the addition of the horse chestnut fruit extract (20 ml/l). The peeled and granulated fruit (200 g) was flooded with water (2 l) and boiled for 3–4 h. The obtained suspension was filtered and sterilized by autoclaving.

Phage lysates were obtained by the confluent lysis. Phage particles were concentrated and purified by differential centrifugation (the SW28 rotor, Spinco L7-70, 24000 g, 3 hours, 10 °C).

The profound purification and density estimation of the native phage particles were carried out by centrifugation in the preformed cesium chloride gradients. Such gradients were made by layering three concentrations of CsCl (1.4 g/cm^3, 1.5 g/cm^3 and 1.6 g/cm^3, 1.5 ml each) and phage suspension (0.5 ml) into the 5 ml centrifuge tubes. They were centrifuged in the SW55 rotor at 34000 g for 4 hours at 10 °C. At the end of run, the tube content was photographed and collected for further investigation. The virus titer was determined for the separated fractions. The absorbance was determined by a NanoDrop ND 1000 (Thermo Scientific, USA). Densities of the phage particles were calculated using the refraction index determined with refractometer URL (model-1).

The phage sample was adsorbed to carbon-coated Formvar grids and stained with 2 % uranyl acetate. Electron micrographs were taken using the microscope JEOL JEM-1400 at the instrumental magnification 20,000–40,000x. The maximum diameter of a capsid was measured as the distance between the opposite vertices. The distance between portal vertice of the head and the end of baseplate was calculated as the tail length.

The phage DNA was extracted by the SDS-pronase method [15]. The restriction analysis was carried out using the endonucleases *Sma*I and *Sal*I. The enzymatic hydrolysis was performed according [15]. Electrophoretic separa-

tion of the restriction products was done in 0.8–1.0 % agarose gels. 1.0 % agarose gels were used for separation of the phage virions and genome DNA.

The SDS-PAGE of the structural viral polypeptides was performed according to the Laemmli protocol [7]. After an electrophoretical run the gels were stained with Coomassie brilliant blue G-250. PageRuler Prestained Protein Ladder (Fermentas, USA) was used as a molecular weight standard. Molecular weight analysis was calculated using the computer program Total Lab (version 2.01).

The spontaneous induction frequency was calculated using the equation f=P/B, where P is the phage titer in the cultural liquid, B – the titer of lysogenic bacterial cells. Lysogenization frequency was determined as the ratio between the number of lysogenized cells and the total cell number in the cultural medium [6].

Statistical data analysis was done in Microsoft Excel and STATISTICA [12].

Results. In the earlier studies [4] it was shown that phytopatogenic bacteria are characterized by the presence of structural heterogeneity of virions within their populations.

Similar results were obtained in curent investigation for temperate erwiniophage 59 in the case of its adaptation to reproduction in cells of the auxotrophic mutant *E. horticola* 450 His3. The pool of phage particles produced two equimolar bands in CsCl gradient (Fig. 1A). Both bands contained viable bacteriophages with a titer of $1–2\times10^{11}$ PFU/ml.

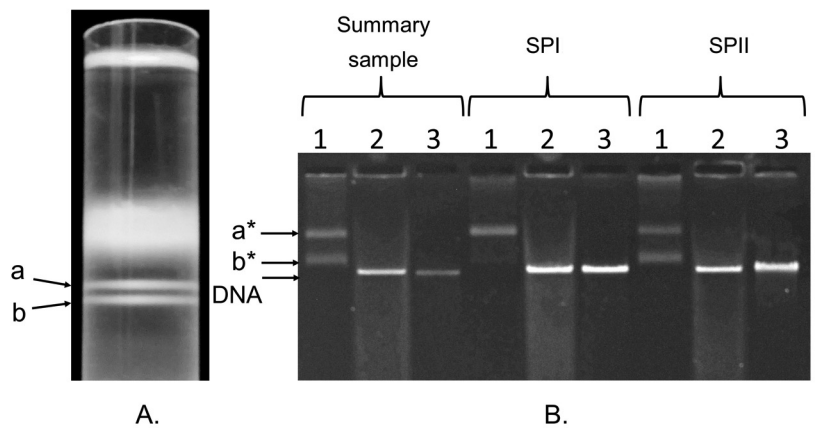


Fig. 1. The CsCl-gradient profile of the phage 59 obtained on the auxotrophic mutant *E. horticola* 450 His3 (A); a, b – bands with the buoyant density of 1.45 and 1.47 g/cm^3 respectively. B – electrophoregram of the native virions and genome DNA of phage 59/450 His3. SPI, SPII – phage particles of different subpopulations; 1 – native particles; 2 – phage particles treated with 0.8 % SDS; 3 – phage particles treated with pronase B; a*, b* – virions with different electrophoretic mobility.

It was established that the upper band of virus material has a density of 1.45 g/cm^3 while for the lower band of the CsCl-gradient this value was estimated at 1.47 g/cm^3. It should be mentioned, that this band's density is 0.03 g/cm^3 lower than it was mentioned in earlier works [3]. The obtained data indicate the presence of a considerable populational diversity that effects density of phage particles. For simplicity of notation, we assume to designate the virus-containing material of bands with the lower and higher density as subpopulation I (SPI) and subpopulation II (SPII) respectively.

The electrophoretic separation of the summary sample and the analysis of SPI and SPII contents showed that the general pool of phage particles is characterized by two groups of virions (Fig. 1B). The SPI was homogeneous according to the electrophoretic mobility and coincided with the top band of the summary sample. The overwhelming majority of SP II corresponds to the lower band of the summary pattern. The electrophoretic mobility of DNA molecules extracted with SDS and pronase B was the same in all cases.

Identical restriction patterns were obtained using *Sal*I restriction enzyme for both populations (Fig. 2). DNA fragments have the same size and relative content. This leads to a conclusion that the phage particles of subpopulation I and subpopulation II carry genomes of identical size. Therefore, we assume that the different electrophoretic mobility of phage particles is resulted by the change in virion structure.

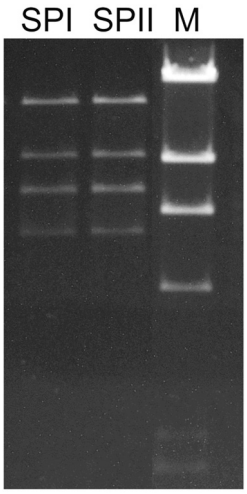


Fig. 2. ***Sal*I restriction profiles of DNA isolated from phage 59/450 His3 subpopulation I (SPI) and subpopulation II (SPII). M – *Hind*III-fragments of λ DNA.**

The study of plaque morphology inferred that SPI and SPII produce morphologically identical plaques on the lawn of sensitive bacteria. Phage plaques were characterized by turbid centers, clear halos and uniform sizes.

The fact of different lysogenization frequency of *Eho* 450 His3 by SPI and SPII phage has been established. This index differs by about 2 orders and equals 3.3 and 1.7 % for SPI and SPII phage particles respectively. Furthermore, at least two stable lysogenic types of the host-microorganism were detected. The first lysogen formed small zones of bacterial lysis on the lawn of sensitive culture while the second lysogenic colony was characterized by big lysis zone of the indicator strain. Subpopulation I and II produced both lysogenic types, but the percentage of the second type lysogen was several orders of magnitude smaller (Table 1).

The isolated lysogens differed by the frequency of bacteriophage spontaneous induction. The spontaneous induction frequency of the first type lysogen after 20 h of incubation reached 2×10^{-2}. For the second type lysogen this index differed by two-orders of magnitude (3×10^{-4}). Similar feature was earlier established for bacteriophage ZF40 forming several lysogenic states after lysogenisation of the same sensitive host [6].

Table 1.

Lysogenization of *E. horticola* 450 His3 by two variants of phage 59/450 His3

Populations of phage 59	Lysogenization frequency, %	
	The first type lysogen	The second type lysogen
Subpopulation I	2.0	0.4
Subpopulation II	1.7	0.03

Lysogenic strains obtained using *Eho* 450 His3 on the base of SPI and SPII phages were shown to be resistant to homoimmune superinfection and cross-infection. It should also be noted that when SPII phage suspension was applied to the lawn of lysogenic *E. "horticola"* 450(59), the spots of lysis and plaques were formed with a frequency of 1×10^{-8}. It is not excluded that the spots of lysis may be resulted by cooperative killing from the extrinsic side. The nature of separate plaques is not clear; the emergence of vir-mutants or the phage-phage induction phenomenon seems to be possible in these conditions [17].

The average values of the maximum capsid diameter and the tail length were established for SPI and SPII virions by means of electron microscopy (Table 2). The linear sizes were determined for 71, 159 та 110 phage particles of the summary sample, SPI and SPII respectively.

Table 2.

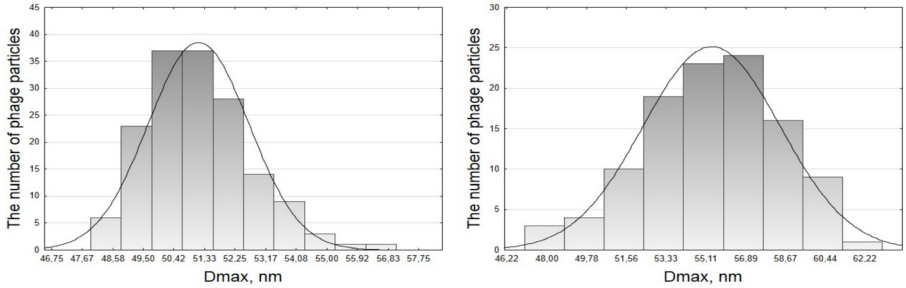
The linear parameters of phage particles 59/450 His3

Structural components of virions	Average size, nm		
	Summary sample	Subpopulation I	Subpopulation II
Maximum diameter of capsids	54.04 ± 0.66	51.16 ± 0.23	55.36 ± 0.57
Tail length	ND	147.73 ± 2.12	149.25 ± 1.75

ND – not determined

The maximum diameter of the phage capsids equaled 51.16 ± 0.23 nm for SPI and 55.36 ± 0.57 nm for SPII capsids, which is 8.2 % lager than the one for SPI capsids. As demonstrated on Fig. 3, the SPI and SPII capsid size distributions are close to normal distribution. In contrast, the distribution of capsids by size in the summary sample indicated the presence of several classes of data. It might be considered that the general phage pool contains particles with different structural and morphological organization of the virions.

Since the average values overlap within the margin of error it is impossible to assume that the tail size difference between SPI and SPII phage is accurate. Moreover, the SPII tail size data does not demonstrate the normal distribution. For this reason, we assume that the tail lengths vary due to the flexibility of a proximal part of the tail and different orientation of particles on the surface of the supporting grid.



Subpopulation I Subpopulation II

Fig. 3. Capsid size distribution of the phage 59/450 His3 different variants.

In an attempt to eliminate randomly obtained results, we have repeatedly propagated the viral contents of the summary preparation and SPI and SPII using strain *Eho* 450 His3. As seen from Fig. 4, all three lysates contain both types of phage particles with the corresponding electrophoretic mobility. The varying intensity of fluorescence of the EtBr-nucleic acid complexes in bands points at differences in the quantitative ratio between two types of virions within the general phage pool.

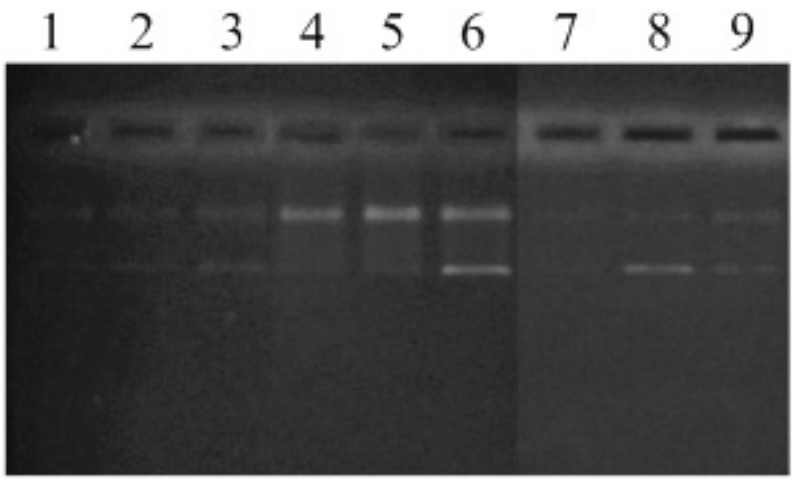


Fig. 4. Electrophoregram of phage 59 CsCl-fractions. The viral particles of the summary sample (1–3), the subpopulation I (4–6) and the subpopulation II (7–9) were propagated on *E. horticola* 450 His3

Next, the host influence on the adaptation process and the phage morphogenetic development were investigated. The adaptation was performed by means of 4–5 serial clonings through a single plaque on an appropriate strain. As the previously obtained result indicates that both SPII and SPPI phage particles produce heterogeneous progeny, the viral material of the summary sample was chosen for the initial passage. Gradient profiles of the recloned phages indicate that all the studied strains produce heterogeneous phage pools characterized by quantitative redistribution of the viral material between SPI and SPII depending on the strain and the number of reclonings (Fig. 5). Thus, strains *E. horticola* 450, 450(P1)$^-$ and 450(49) produce bands of equal intensity in the CsCl-gradient. The lower band is dominant in 59/450 His3 and 59/450(49) preparations while for 59/60 it is the upper one. Different band intensity points at the strain specificity in regard to the virion density correlation. It is worthy

to note that the virus sample recloned and obtained using strain *Eho* 450 have a slightly visible top band. Such redistribution of virion classes within the population may be an evidence of the tendency to homogeneity with subsequent passages when grown on an adequate host. Thus, the result of the current investigation stage is the detection of 5 strains *E. horticola* able to produce the heterogeneous populations of phage 59.

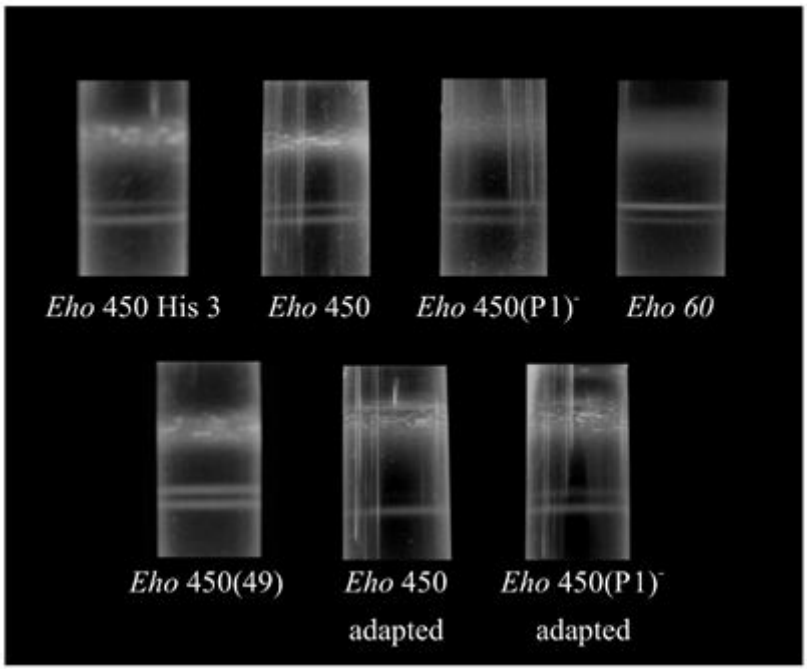


Fig. 5. The CsCl-gradient profiles of phage 59 obtained on and adapted to different strains of *E. horticola* (noted in the text). "adapted" – see text.

Based on the electrophoretic data it was determined that SPI and SPII phage particles are characterized by identical polypeptide profiles (Fig. 6). The phage virions include at least 8 structural polypeptides with molecular weights ranging from 11 to 126 kDa. Three of them (p4, p5, p7) are the major ones.

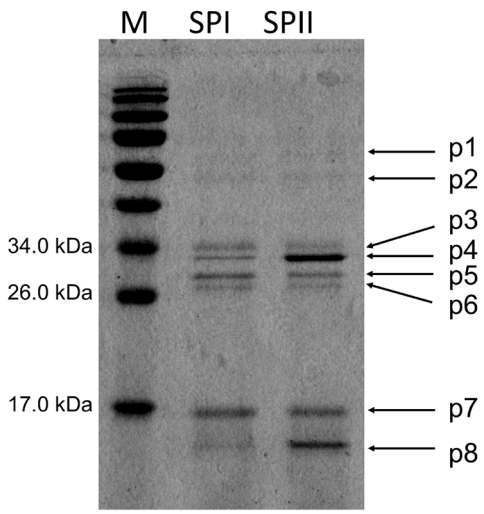


Fig. 6. Polypeptide profiles of two variants of bacteriophage 59/450. M – molecular weight marker; SPI – subpopulation I; SPII – subpopulation II.

The obtained data and their comparison with literature sources make it impossible to determine the function of any of the identified polypeptides. However, the difference in percentage relation between p4 and p7 probably is related to the distinction in capsid structures; thus, SPI and SPII phage particles have different protein-DNA relation. This may possibly explain the electrophoretic mobility variation of the virions within a particular population.

The phage 59 DNA packing into mature heads is performed according to the headful mechanism [15]. Such DNA is characterized by the presence of circular permutation. We attempted to determine the influence of the indicated structural changes on the packaging and permutation of DNA.

The *Sma*I endonuclease fragments of SPI and SPII DNA had the same electrophoretic mobility (Fig. 7). The *Sma*I digests of the phage 59 DNA contains six basic fragments, the set of heterogeneous fragments ("a") and one submolar fragment ("b"). These results correspond to the literature data [15]. Consequently, the indicated change in the capsid structure doesn't affect the phage DNA packaging system and permutation.

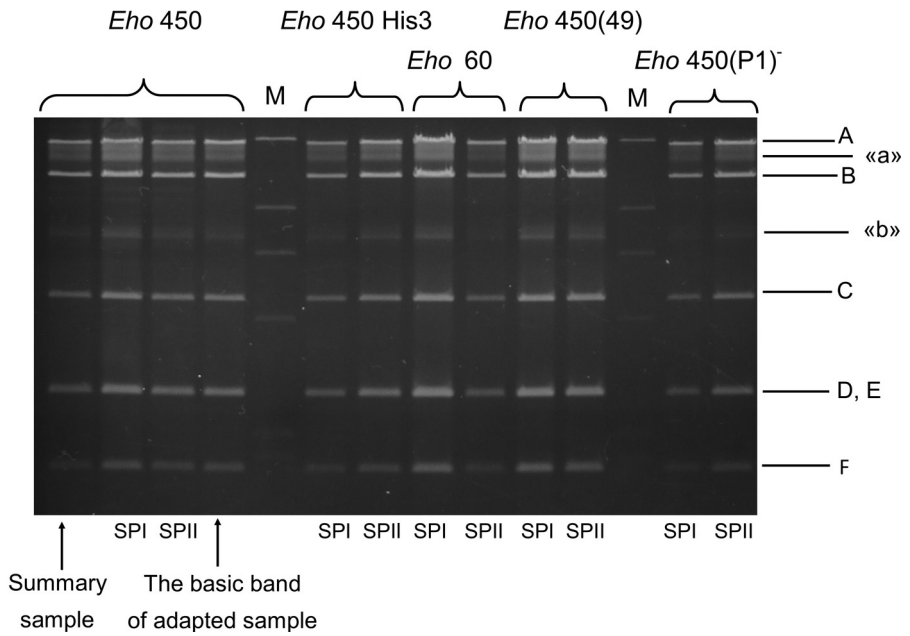


Fig. 7. The *Sma*I restriction profiles of the genomic DNA obtained from different variants of phage 59. M – *Hind*III-fragments of λ DNA; SPI, SPII – subpopulation I and subpopulation II respectively; A-F – normal restriction fragments of DNA; "a" – a set of heterogeneous fragments; "b" – a submolar fragment.

Discussion. It is well known that a capsid is the most labile component of a virion while the tail with its appendages stays stable as a rule. The formation of aberrant capsids in particular bacteriophages occurs with low frequency. Therefore, in general, a subpopulation of normal virions considerably dominates in the pool of phage particles supporting the viability of population at a proper level.

Usually, the capsid size of bacteriophage morphological variants is expressed by discrete values and significantly differs from the size of a normal capsid. For example, the population of coliphage P1 consists of several separate subpopulations heaving head diameters of 86 nm (P1B), 65 nm (P1S) and 47 nm (P1M) [18]. All three types are composed of the same protein subunits

but have different triangulation numbers – 16, 9 and 4 respectively. However, only P1B particles package complete genome and are able to realize a productive infection. The phenomenon of morphological heterogeneity takes place independently from the host strain and demonstrates the functional peculiarities at the level of expression of several structural phage genes [8].

The process of formation of a heterogeneous population in the P2–P4 phage system is drastically different from the one mentioned above but their results are similar. The satellite phage P4 head volume is three times smaller than the head volume of its helper and contains a smaller genome [10]. Morphological variants have been also observed for coliphage T4. Besides normal phage with elongated polyhedral heads (A2-morphotype) and short-headed isometric forms (petit), the lysates of T4 also contain morphological variants with abnormal (multiprolate) heads. The last ones are able to package complete genome but they fail to productively infect sensitive bacterial cells.

This manuscript provides evidence that morphological heterogeneity of the temperate erwiniophage 59 significantly differs from such "classical" coliphages as T4, P1 and the phage system P2–P4. Although the equimolar subpopulations of the phage are represented by two discrete sets of virions with varying capsids, the difference between their average diameters is insignificant and equaled 5 nm. Nevertheless, the density indexes and the virion electrophoretic mobility in agarose gels proved to be significantly distinguished for the two morphological types. The mentioned biophysical parameters characterize the change in a protein/DNA ratio of the virions as well as the alteration in the value of their surface charges. If one considers that the subpopulation II is close to the original phage 59 [3], the traditional host for which is the strain *Eho* 450, then it may be assumed that the variation in the mentioned parameters concerns the subpopulation I.

As shown in Fig. 5, in addition to the strain *Eho* 450 His3 on which the phage was first isolated, the phenomenon of morphological heterogeneity spreads to two isogenic strains *Eho* 450(49) i *Eho* 450(P1)$^-$, to the original strain *Eho* 450 and to the related bacterium *Eho* 60. The quantity correlations between the two subpopulations obtained on various hosts are generally distinguished, but systematic passages of a summary population on the initial strain *Eho* 450 leads to a considerable domination of the subpopulation II, namely the authentic phage 59. Therefore, this fact counts in favor of the idea that morphological heterogeneity of a phage population depends on the host cells and is formed due to the fine phage morphogenesis mechanisms in an infected cell [4].

Since the emergence of the subpopulation I on the background of the initial phage 59 is relegated to the capsid, the phage genome and its virion polypeptide patterns were characterized (Fig. 6). It was identified that both morphological variants have the same genome sizes. Furthermore, the subpopulations I and II detected on all of the studied strains contain DNA with identical *Sma*I-restriction patterns. Hence we presume, that its DNA packaging according the headfull packaging mechanism remains constant [17]. In contrast, the virion polypeptide content of all phage variants has undergone changes in comparison to the ones of the subpopulation II. As indicated on the electrophoregram (Fig. 6) there is significant difference between the two phage types in the relative content of some polypeptides (probably the major ones) at the while their

molecular weights remain the same. Therefore, the values of buoyant density and the data of electron microscopy together with SDS-PAAG definitively confirm the change in the physical and chemical properties of the capsid under the formation of an addition subpopulation within the phage 59 standart pool. Actually, as shown in Table 2, the changes do not affect the tail size, its linear length probably remain constant. We cannot be sure, however, that there are no structural changes in baseplate of the SPI-particles. The pleiotropic effect related to the populational heterogeneity and variations of such key indicators of lysogeny as the frequency of lysogenization and spontaneous induction counts in favor of such changes (Table 1). On the other hand, the previous findings indicate that the related phages 59 and 49 except the baseplate are assembled with the same proteins [17]. It should be noted that the interconnection between the structure of a virion and a manifestation of lysogeny was first observed in this paper.

It has been shown recently that, under particular conditions, vir-mutants of phage ZF40 *Pectobacterium carotovorum* subsp. *carotovorum* are able to produce two types of capsids with diameters of 60.3 and 65.0 nm [4]. This difference is close to the one in case of the phage 59 subpopulations. Hence, the described phenomenon of the population heterogeneity may have analogies for other bacteriophages of phytopathogenic bacteria.

The reasons for the occurrence of an additional phage particle set within the phage 59 population remain unclear. It may be supposed that the morphological heterogeneity may be caused by changes in the phage genes as well as by the conditional changes of the infection process. One should not also exclude that the new subpopulation appears as a reaction of the pseudolysogenic system *E. horticola* to the infection by a homologous phage 59. Thus, the system including *E. horticola* – temperate phage 59 should be considered not only from the physiological side but also in relation to genome organization of both the bacteriophage and its host.

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М.А. Златогурська [1], Ф.І. Товкач [2]

[1] Одеський національний університет ім. І.І. Мечникова,
вул. Дворянська 2, Одеса, 65082, Україна
[2] Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України,
вул. Академіка Заболотного, 154, Київ, 03143, Україна

МОРФОЛОГІЧНА ГЕТЕРОГЕННІСТЬ ПОМІРНОГО ЕРВІНІОФАГА 59

Р е з ю м е

Дана стаття присвячена феномену морфологічної гетерогенності популяції часток помірного ервініофага 59, яка не має аналогів серед інших вірусів бактерій. **Мета роботи.** Дослідження основних характеристик гетерогенної популяції фага 59 аміловороподібної бактерії *Erwinia "horticola"* (*Eho*), отриманої в умовах інфікування ним клітин різних ізогенних бактерій-хазяїв. **Методи**. Для отримання ервініофага 59 використовували його традиційного хазяїна *Eho* 450, три ізогенні штами, а також близькоспоріднену бактерію *E. "horticola"* 60. Для дослідження фізико-хімічних властивостей часток застосовували центрифугування в градієнтах CsCl, електрофо-

рез часток в агарозних гелях, електронну мікроскопію, рестрикційний аналіз ДНК і SDS-ПААГ-електрофорез віріонних поліпептидів. **Результати**. Показано, що при розмноженні на *Eho* 450 His3 та його ізогенних штамах, а також на *Eho* 60, пул помірного фага 59 є гетерогенною популяцією, яка складається з двох типів часток. Ці типи являються дискретними і відрізняються за плавучою густиною на 0,02 г/см^3, що дозволяє ізолювати окремі субпопуляції для детального дослідження. Субпопуляцію з більшою густиною часток ідентифікували як аутентичний бактеріофаг 59 (субпопуляція II з діаметром капсиду 55,36 нм). Діаметр капсиду часток субпопуляції I складає 51,16 нм. Встановлено, що обидва типи часток не відрізняються за розміром ДНК та мають однакові рестрикційні узори. За характером патерна *Sma*I-рестрикції можна зробити висновок щодо незмінності упаковки фагового генома в капсид, що здійснюється згідно headfull- механізму. Однак субпопуляція I відрізняється від вихідної відносним вмістом деяких віріонних поліпептидів при сталості їх молекулярних мас. Цікавим є те, що субпопуляції I і II мають різні показники частоти лізогенізації та лізогенної індукції. **Висновки**. Вперше виявлено незвичайний тип морфологічної гетерогенності часток помірного ервініофага 59, яка проявляється в наявності двох еквімолярних субпопуляцій з різними фізико-хімічними параметрами часток. Гетерогенність популяції, хоч і стосується фагового капсиду, суттєво відрізняється від такої у коліфагів T4 і P1, а також фагової системи P2–P4.

К л ю ч о в і с л о в а: помірний ервініофаг 59, морфологічна гетерогенність фагової популяції, фізико-хімічні властивості часток, рестрикційний аналіз ДНК, лізогенія.

М.А. Златогурская [1], Ф.И. Товкач [2]

[1] Одесский национальный университет им. И.И. Мечникова, ул. Дворянская, 2, Одесса, 65082, Украина
[2] Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины, ул. Академика Заболотного, 154, Киев, 03143, Украина

МОРФОЛОГИЧЕСКАЯ ГЕТЕРОГЕННОСТЬ УМЕРЕННОГО ЭРВИНИОФАГА 59

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Данная статья посвящена феномену морфологической гетерогенности популяции частиц умеренного эрвиниофага 59, которая не имеет аналогов среди других вирусов бактерий. **Цель работы.** Изучение основных характеристик гетерогенной популяции фага 59 амиловороподобной бактерии *Erwinia "horticola"* (*Eho*), полученной в условиях инфицирования им клеток различных изогенных бактерий-хозяев. **Методы.** Для получения эрвиниофага 59 использовался его традиционный хозяин *Eho* 450, три изогенных штамма, а также близкородственная бактерия *E. "horticola"* 60. Для исследования физико-химических свойств частиц применяли центрифугирование в градиентах CsCl, электрофорез частиц в агарозных гелях, электронную микроскопию, рестрикционный анализ ДНК и SDS-ПААГ-электрофорез вирионных полипептидов. **Результаты**. Показано, что при размножении на *Eho* 450 His3 и его изогенных штаммах, а также на *Eho* 60 пул умеренного фага 59 является гетерогенной популяцией, которая состоит из двух типов частиц. Эти типы являются дискретными и различаются по плавучей плотности на 0,02 г/см^3, что позволяет изолировать отдельные субпопуляции для детального изучения. Субпопуляцию с большей плотно-

стью частиц идентифицировали как аутентичный бактериофаг 59 (субпопуляция II с диаметром капсида 55,36 нм). Диаметр капсида частиц субпопуляции I составляет 51,16 нм. Установлено, что оба типа частиц не отличаются по размеру ДНК и имеют одинаковые рестрикционные узоры. По характеру паттерна *Sma*I–рестрикции можно заключить о неизменности упаковки фагового генома в капсид, которая происходит по headfull-механизму. Однако субпопуляция I отличается от исходной по относительному содержанию некоторых вирионных полипептидов при постоянстве их молекулярных масс. Интересным является то, что субпопуляции I и II имеют разные показатели частоты лизогенизации и лизогенной индукции. **Выводы.** Впервые обнаружен необычный тип морфологической гетерогенности частиц умеренного эрвиниофага 59, которая проявляется в наличии двух эквимолярных субпопуляций с различными физико-химическими параметрами частиц. Гетерогенность популяции, хотя и затрагивает фаговый капсид, существенно отличается от таковой у колифагов T4 и P1, а также фаговой системы P2–P4.

К л ю ч е в ы е с л о в а: умеренный эрвиниофаг 59, морфологическая гетерогенность фаговой популяции, физико-химические свойства частиц, рестрикционный анализ ДНК, лизогения.

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