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CHARACTERISTICS OF ABORTIVE INFECTION IN LYSOGENIC SYSTEM OF *ERWINIA HORTICOLA*

The study provides evidence of the phage 49 abortive infection in the lysogenic culture of Erwinia horticola 450(59). ***Aim.*** *To study the peculiarities of abortive infection in lysogenic system of E. horticola under the propagation of phage 49.* ***Methods.*** *Erwiniophage 49 was obtained by propagation on E. horticola 450(59). The phage lysates were studied by using centrifugation in CsCl-gradients, electron microscopy and SDS-PAGE of the virion polypeptides.* ***Results.*** *The Abi-phenotype is manifested through the reduced efficiency of virus plating and the decrease of amount of phage progeny. The phage lysates contain an excess of capsid structures, incomplete virions and polytails. The polypeptide profile of these capsid structures corresponds with the one of the native phage particles.* ***Conclusions.*** *The obtained data suggest that the current phage-bacterial system develops the Abi-phenotype affecting the phage morphogenesis.*

Key words: abortive infection, bacteriophage 49, Erwinia horticola, capsid, polytails, Abi-phenotype.

As a component of phage-host "arms-race", the abortive infections (*Abi*) interrupt the productive phage development targeting translation, replication, genome packaging and assembly of mature virions [1, 12]. The *Abi*-mediated "suicide" of infected cells leads to the release of few or no progeny particles and prevents the spreading of infectious virus within the bacterial population [10, 12].

Although many Abi-systems (*Abis*) have been found in various bacteria, they are the most well-studied for lactic acid bacteria [3]. A highly effective *Abis*, designated ToxIN, has been described for the phytopathogenic *Pectobacterium carotovorum* subsp. *atrosepticum* [4]. An *Abis* inherents for the pectolytic bacterium *P. carotovorum* subsp. *carotovorum* was detected in the course of infection with bacteriophage ZF40. It is characterized by the overproduction of separate phage capsids [9].

Recently, a study of polyvalent T7-like bacteriophages development in the presence of prophage elements has demonstrated that phage FE44 is restricted when single or double lysogens of non-pectolytic phytopathogenic bacterium *E. horticola* are infected [3]. Phage infection led to the formation of aberrant capsids in phage progeny that point out at the abnormal virion assembly of T7-like phages as a result of an abortive infection.

Despite its abundance in the nature, *Abis* remain insufficiently studied in comparison with the lysogenic or lytic development of bacteriophages. Thus,

the aim of this research was to study the characteristics of abortive infection in the lysogenic system of *E. horticola* 450(59*)* under the propagation of phage 49.

Materials and methods. The object of the study was the phage 49 of *E. horticola*. The strain *E. horticola* 450 lysogenized by erwiniophage 59 was used as an indicator culture.

For lysogen construction, phage was applied on the bacterial lawn of the parental strain *E. horticola* 450. Then, cells from the zones of secondary growth were cultivated in the broth (LB). After serial cloning, bacteria were analyzed for the lysogenic induction ability and resistance to lysogenisation by homoimmune phage [3].

The phage lysates were obtained by the confluent lysis. The phage particles were concentrated and purified by differential centrifugation (the SW28 rotor, Spinco L7-70, 24,000 g, 3 h, 10°C). The profound purification and density estimation of the phage particles were carried out by centrifugation in the preformed cesium chloride gradients with using the SW55 rotor at 35,000 rpm, 4 h, at 10°C [13].

Micrographs of the phage particles, contrasted with 2% uranyl acetate solution, were taken with the electron microscope JEOL1400.

SDS-PAGE-electrophoresis of the structural polypeptides of the phage virions was performed with the standard method by Laemmli [7]. Electrophoresis data was processed with the help of the computer program Total Lab (version 2.01).

Statistical data analysis was done in Microsoft Excel and STATISTICA [9].

Results. In the earlier studies [11] it was shown that phage 49 and 59 are heteroimmune representatives of the polylysogenic system of *E. horticola.* This allows us to explore the lytic and lysogenic process under the lysogenic state of the host formed by one of them. For this purpose, a monolysogenic strain *E. horticola* 450(59) was constructed.

The obtained results have shown that phage 49 is a subject of considerable restriction by *E. horticola* 450(59). In this case, the efficiency of plating (EOP) decreases to 88% in comparison with the reference EOP on the parental strain *E. horticola* 450. In addition, it should be noted that the further plating on the lysogenic culture resulted in the formation of unusual and much smaller negative colonies.

Preparative obtaining of the phage 49 particles on the lysogenic strain *E. horticola* 450(59) has shown the reduction of phage progeny release. In comparison with the control experiments with the parental non-lysogenic culture *E. horticola* 450, the phage 49 titer decreased 1-2 fold and was estimated at 10^{10} PFU/ml.

The CsCl gradient centrifugation of the obtained phage lysates has indicated that sedimentation profiles (fig. 1A) are characterized by the prevalence of the band with a low buoyant density (1.35 g/cm^3).

Based on the electron microscopy data it was determined that the band with low density in CsCl-gradient contains incomplete virions and separate phage components with an excess of capsid structures (fig. 1B).

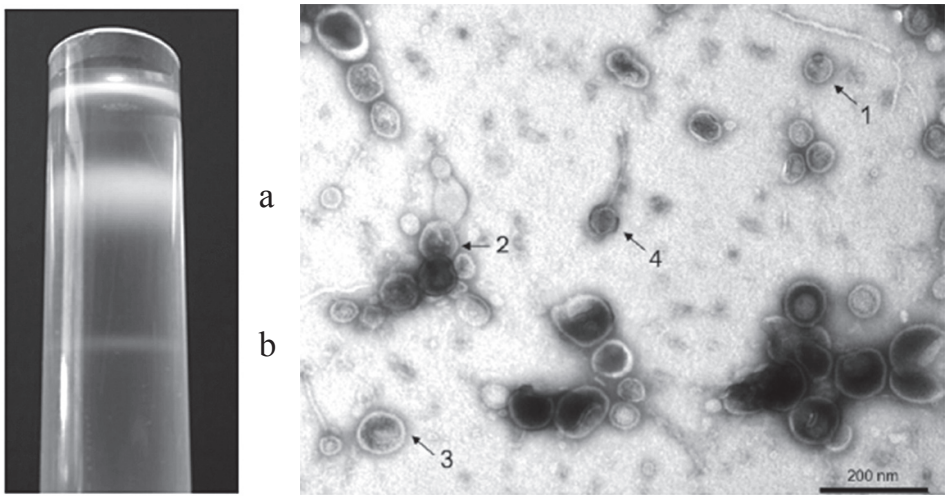

Fig. 1. A. The CsCl-gradient profile of phage 49 obtained on lysogenic strain *E. horticola* 450(59). a – the band with buoyant density 1.35 g/cm^3; b – the band of native phage particles (1.5 g/cm^3). B. Electron-microscopic image of the materials from the CsCl-band with the low buoyant density. 1, 2, 3 – the capsid structures of I, II, II classes, respectively; 4 – incomplete phage particles.

The histogram on the fig. 2 indicates that there are several classes of capsid structures with sizes 55-60 nm (I class), 65-70 nm (II class) and 91-96 nm (III class). All of the mentioned capsid classes were characterized by the similar structure with an explicit coat. The electron microscopy data suggest that capsid structures of the I class dominate in the lysates. Their diameters are similar to the head size of native phage particles (53 nm).

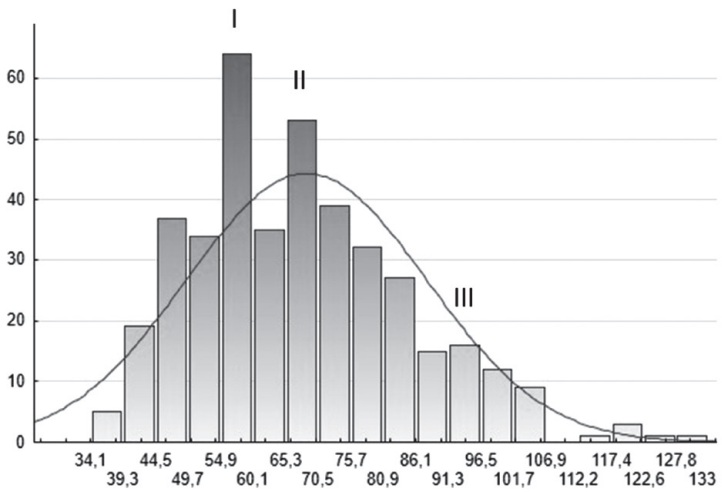

Fig. 2. Size distribution of capsid structures from the lysates of phage 49. I, II, III – the basic statistics classes of capsid structures.

In addition to incomplete virion particles and capsid structures, the occasional separate phage tails occur on the electron micrographs. Apart from that, some unusual entities were identified. Their structure is similar to phage tails but are characterized by a much greater length (fig. 3).

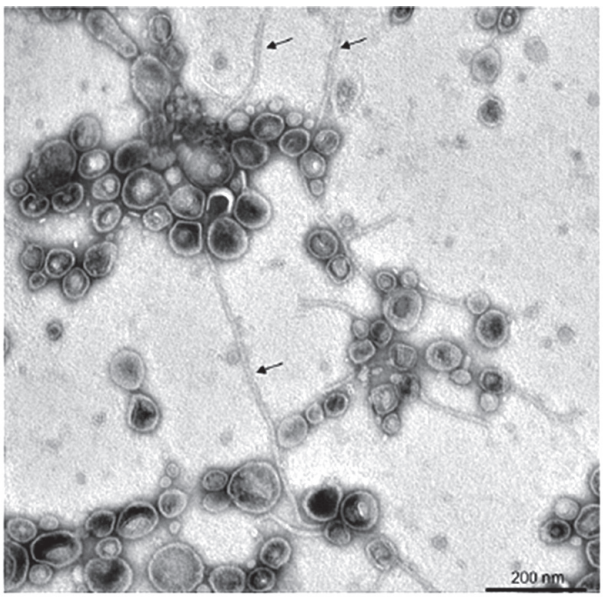

Fig. 3. Polytails (pointed arrows) in the lysates of bacteriophage 49

The width and length of the tail within the intact infective phage 49 virions equal 7.7 ± 0.3 nm and 153.2 ± 4.2 nm, respectively. The width of the phage tail-like particles found in the lysates was not reliably different from the one in the native phage (8.1 ± 0.5 nm). However, their length was several times greater (200-700 nm).

Further research aimed to prove that the detected capsid structures are the intermediate products of virion formation. Using gel filtration chromatography on a column with Toyopearl HW-75, the fractions enriched by capsid structures of different classes were obtained. This was confirmed by the electron microscopy data of the samples. After that, we performed a comparative analysis of polypeptide profiles of capsid structures from different fractions and native phage particles.

According to the electrophoregram presented on fig. 4, the peptide profile patterns are similar. But the profiles of fractions with capsid structures contain an additional protein band with molecular weight of 36 kDa, which does not present among the structural proteins of native phage particles.

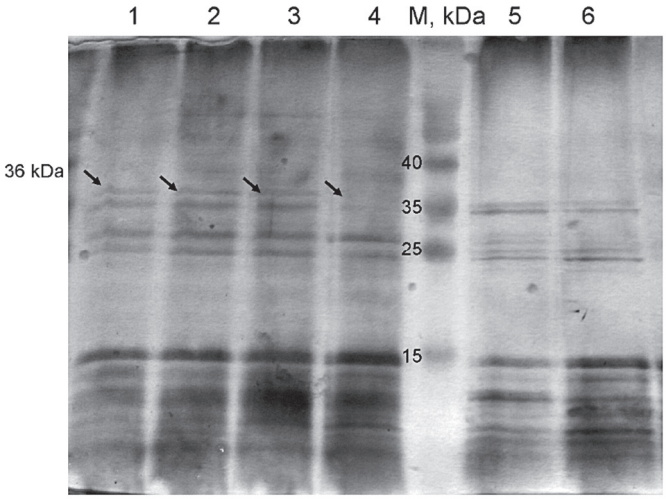

Fig. 4. Protein profiles of phage 49. 1-4 – fractions enriched by the capsid structures; M – molecular weight marker; 5-6 – native page particles. Arrows point at the additional protein band with molecular weight of 36 kDa

Discussion. Bacteria are well recognized to impact natural ecosystems, industrial processes and human health. Recently, bacteriophages have also gained relevance as they modulate the processes through cell lysis, horizontal gene transfer and metabolic reprogramming during infections [10]. For better understanding of the roles of phages in nature, an understanding of their possible interactions with their hosts is necessary [2].

Most of the *in vitro* studies are based on the idea of phage development in according to the lytic or lysogenic life cycle. At the same time, numerous data indicate that phages have diversity of possible life cycles depending on the interaction with their physical environment and bacterial host. Such widespread phenomena as pseudolysogeny, chronic and abortive infection are difficult to study due to the absence of adequate models and methods [2, 5].

In fact, the arms-race between phage and bacteria is predicted to have an impact on global nutrient cycling, on global climate, on the evolution of the biosphere, and also on the evolution of virulence in pathogenic hosts. Faced with the rapid evolution and turnover of phage particles, bacteria have evolved various phage resistance mechanisms (the restriction-modification and CRISPR/Cas systems, abortive infections, etc.) [6, 10].

This paper is devoted to the study of the interaction between the phage 49 and the amilovora-like bacterium *E. horticola* 450(59). It was found that the endogenous genetic elements, such as integrated heteroimmune prophages 59, interfere with the exogenous phage 49. The phage exclusion is expressed in the EOP reduction and the excess of aberrant capsid structures in lysates. The identity level of the polypeptide profiles of native virions and fractions with enriched capsid structures indicates that the observed capsid structures are relevant to the bacteriophage assembly. This assumption is supported by the fact that the capsid structures of dominated class are similar in size to the

capsid of native particles. Additionally, numerous polytails in the lysates refer to a significant disruption of virion morphogenesis.

The obtained results suggest that the abortive infection occurs in the case of phage 49 reproduction on the lysogenic heteroimmune culture *E.horticola* 450(59). Although the development of the *Abi* phenotype is associated with the integration of a prophage element in the genome of the host bacteria, the mechanism and genetic determinants of this phenomenon are unknown and require further study.

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ОСОБЕННОСТИ АБОРТИВНОЙ ИНФЕКЦИИ В ЛИЗОГЕННОЙ СИСТЕМЕ *ERWINIA HORTICOLA*

Резюме

Показано, что при репродукции фага 49 на лизогенной культуре *Erwinia horticola* 450(59) инфекция имеет абортивный характер. **Цель работы.** Изучить свойства абортивной инфекции в лизогенной системе *E. horticola* под действием бактериофага 49. **Методы.** Для получения эрвиниофага 49 использовали лизогенный штамм *E. horticola* 450(59). Для исследования свойств фаговых частиц применяли центрифугирование в градиентах CsCl, электронную микроскопию и SDS-ПААГ-электрофорез вирионных полипептидов. **Результаты.** Абортивная инфекция проявлялась в снижении эффективности посева вируса, а также сокращении количества жизнеспособного потомства при препаративном получении фаговых частиц. С помощью електронной микроскопии установлено, что фаговые лизаты содержат избыток капсидных структур, не полностью сформированные вирионы и полихвосты. Полипептидный профиль капсидных структур совпадает с таковим нативных фаговых частиц. **Выводы.** Полученные результаты свидетельствуют о том, что в исследуемой системе проявляется *Abi* фенотип на уровне морфогенеза и морфопоэза фагового потомства.

Ключевые слова: абортивная инфекция, эрвиниофаг 49, *Erwinia horticola*, капсидные структури, полихвосты, *Abi* фенотип.

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ОСОБЛИВОСТІ АБОРТИВНОЇ ІНФЕКЦІЇ В ЛІЗОГЕННІЙ СИСТЕМІ *ERWINIA HORTICOLA*

Резюме

Показано абортивний характер протікання інфекції при репродукції ервініофага 49 на лізогенній культурі *Erwinia horticola* 450(59). **Мета роботи.** Дослідити властивості абортивної інфекції в лізогенній системі *E. horticola* під впливом бактеріофага 49. **Методи.** Для отримання ервініофага 49 використовували лізогенний штам *E. horticola* 450(59). Для дослідження властивостей фагових часток застосовували центрифугування в градієнтах CsCl, електронну мікроскопію і SDS-ПААГ-електрофорез віріонних поліпептидів. **Результати.** Абортивний характер інфекції проявлявся в зниженні ефективності висіву віруса та зменшенні виходу життєздатного фагового потомства при препаративному отриманні фагових часток. За допомогою електронної мікроскопії встановлено, що лізати містять надлишок капсидних структур, не повністю сформовані віріони та поліхвости. Поліпептидний профіль капсидних структур співпадає з таким у нативних фагових часток. **Висновки.** Отримані результати свідчать про те, що в дослідженій системі *Abi* фенотип проявляється на рівні морфогенезу та морфопоезу фагового потомства.

Ключові слова: абортивна фагова інфекція, ервініофаг 49, *Erwinia horticola*, капсидні структури, поліхвости, *Abi* фенотип.

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