UDK578.81

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CHARACTERISTICS OF DEFECTIVE PHAGE PARTICLES OF *PECTOBACTERIUM CAROTOVORUM* ZM1

S u m m a r y

It is shown for the first time that the expression products of defective prophages are typical of defective lysogenic systems of phytopathogenic Pectobacterium carotovorum. It is established that virus-like particles (VLP) such as phage capsids are packing bacterial DNA which size is determined by pulse field gel electrophoresis separation. Based on data about capsid structures which are formed by the virulent mutant ZF40/421, there is made a suggestion about the forming mechanism of defective virions of P. carotovorum.

K e y w o r d s: Pectobacterium carotovorum, defective lysogeny, phage, virus-like particles (VLP)

Determination of the relationship between viable and defective viruses in the environment is an important goal of modern virology. Formation of defective virions during viral infections is associated with attenuation of virus population and the "extinction" of infections. From a theoretical point of view the assembly of bacteriophage particles and especially the capsid folding are included in the category of the most urgent problems of biology.

The presence of defective phages is due to many reasons. Firstly, they may be the result of incomplete expression of prophages, which have retained the structural genome region [1].

Secondly, the defects may often be caused by abortive phage infections which result in accumulation of incomplete virus particles [2].

Thirdly, there are original phage systems in nature, such as the P2-P4. Development of the defective satellite phage P4 is possible only if it uses the products of many genes of the helper phage P2 [3].

Although the defective phages are unable to produce a complete infection, they may play an important role in horizontal gene transfer by transduction in water and soil ecosystems. In addition, their presence is indicative for defective lysogenic systems and abortive infection.

Pectobacterium carotovorum subsp. *carotovorum* (Pcc) is a representative of the bacterial consortium associated with different plants. The study of this phytopathogenic bacterium cannot exclude the participation of temperate phages and lysogenic state in its ecology.

As it was previously shown, the induction of lysogenic strains of *Pcc* of different origin is characterized by synthesis of individual components of phage particles – capsids, base plates and tails, which are not gathered into a whole infectious virion [1]. These tail-like particles are macromolecular carotovoricins (MCTV) able to kill closely related strains of *P. carotovorum*. Recently it was shown for the first time that the capsid structures of defective temperate erwinia phages are filled with the DNA molecules [4]. The origin of this DNA is unknown. On the other hand, it has not been established, whether the phage capsid-like particles are able to perform generalized transduction between the strains of *P. carotovorum*. This work is aimed at solving these problems.

Materials and Methods

The strain of *P. carotovorum* subsp. *carotovorum* ZM1 was used as the producer of virus-like particles (VLP). Lysogenic induction of the cells was performed with mitomycin C (1μg in 1 ml of actively growing cells) and nalidixic acid (20 μg/ml). To determine the relative killer activity (A_{rel}) the method of lysis zones was used. The value was expressed as the ratio of diameter of the individual zones to the diameter of maximum area in the series of studies [5]. Concentrated preparations of DNA containing phage capsid-like particles (DNA-VLP) were obtained by differential ultracentrifugation and stored in the buffer STMG (200 mM NaCl; 10 mM Tris–HCl, pH 7.4; 10 mM $MgCl_2$; 100 μg/ml gelatine).

The native DNA-VLP and restriction products were separated in agarose gels. The virulent mutant ZF40/421 lysing the specific lysogenic strain Pcc 62A-d1($ZF40_{c10}$), was used as a model of complete phage infection [6]. The phage was propagated by the confluent lysis method on the

minimal medium A with lactose. For the simultaneous concentration and purification of the phage particles the fibrous cellulose (type DEAE 23SS, capacity 0.74, Serva) in 0.01 M sodium phosphate buffer, pH 7.0, was used. After layering the lysate, the column was eluted with 0.15 M NaCl and then fractionated by 0.25 and 0.4 M NaCl.

The content of each fraction was analyzed for the presence of VLP by electrophoresis in agarose gel. After that the titer of the phage was determined for the peak fractions ZF40\421-f16 and ZF40\421-f17 and also the presence of the capsids and complete phage virions was shown by the electron microscopy.

Results and Discussion

We have previously shown that the strain Pcc ZM1 as the result of lysogenic induction produces DNA-containing particles that are close to the capsids of phage ZF40 by their mobility in agarose gels [4].

It was also established that macromolecular carotovoricins (MCTV) of this strain cause the phage-phage induction and lead to the change from pseudolysogenic state of phage ZF40 to lytic or lysogenic development [7]. Bacteriocins of this strain formed negative lysis spots on the lawns of the indicator culture Pcc 66A [1] and the size of these spots correlates with the killer activity. There is no much information about the bacteriocins with such properties in *P. carotovorum*, therefore their study might lead to obtaining new information on defective lysogeny and bacteriocinogeneity in *P. carotovorum*.

In the process we used two spontaneous mutants of Pcc ZM1 – Pcc ZM1/40i1 and Pcc ZM1/40i6 which give an increased yield of MCTV. Fig. 1.a shows the dynamics of changes in the negative zones of lysis on the indicator strain Pcc 66A, depending on the age of cultures Pcc J2/S2 [5] and Pcc ZM1/40i1, that were grown in the minimal A medium with lactose. A more detailed analysis of the killer activity (A_{rel}) showed that in the case of bacterial mutant Pcc ZM1/40i1 the yield of MCTV has an undulating character, regardless of which inductor was used (Fig. 1.b). As it is evident by the presented results there are three "waves" of yield of the MCTV in the strain Pcc ZM1/40i1.

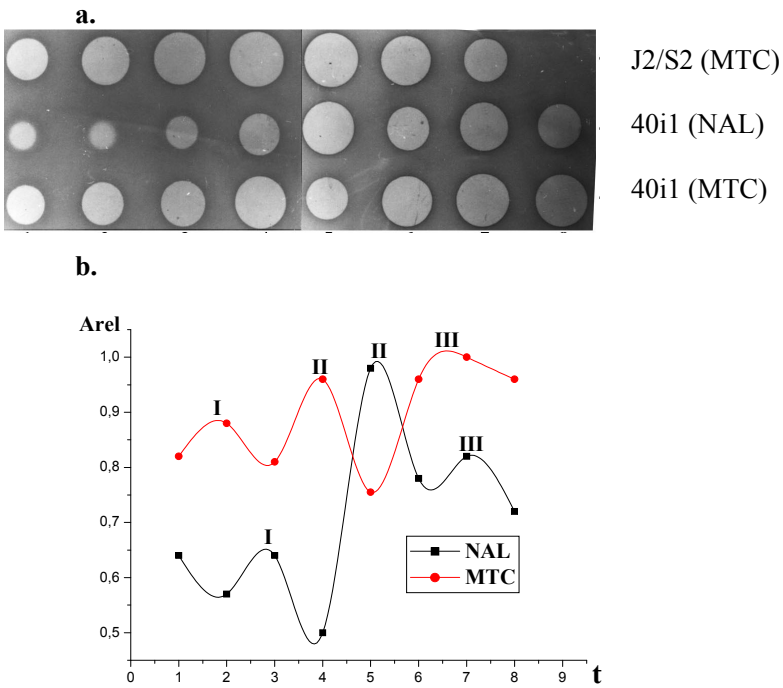

Fig. 1. a. Lysis zones of MCTV *P. carotovorum* J2/S2 and ZM1/40i1, that were obtained on minimal medium A plates with lactose. 1 – 8 – growth period in hours.

b. Wave-like yield of MCTV *P. carotovorum* ZM1/40i1 that was obtained by mitomycin C (MTC) and nalidixic acid (NAL) induction. I, II, III – maximum yield of carotovoricins. A_{rel} – comparative activity of MCTV, t – time in hours.

The first wave is typical of the initial log phase of growth – about 10^8 cells/ml (Fig. 1.b, I). The second wave is indicative for new pool of bacteriocins. They appear in the middle (mitomycin C) or in the late logarithmic (nalidixic acid) growth phases of the culture (Fig. 1.b, II). The third wave of the yield is observed in the initial stationary period, when the cell number reaches to 10^9 or more per ml (Fig. 1.b, III). As it can be seen from the graph (Fig. 1.b), "wave" yields for the two inductors are in antiphase, i.e. when there is a maximum output of MCTV in mitomycin-treated cells, the cells affected by nalidixic acid give a minimum of bacteriocins. Obviously, this difference reflects the different mechanisms of action of mitomycin C and nalidixic acid on the SOS-system activation which ultimately leads to the destabilization of defective lysogeny of *P. carotovorum* and the induction of defective prophages.

When the cells of Pcc J2/S2 are exposed to both inductors the wave yield of MCTV is not detected. Nevertheless, it is typical of thymine auxotrophic mutants of this strain [5]. Although the overall yield of carotovoricins and, consequently, the efficiency is higher for the induction of mitomycin C (Fig. 1.b), the nalidixic acid was used for obtaining the DNA-VLP from the strains Pcc ZM1. This choice is based on the fact that under the influence of the nalidixic acid on the lysogenic erwinia cells there is a partial reorientation of the synthesis, leading to increased production of structures such as the base plates and the phage capsids, in comparison to the phage tail-like particles [1]. This fact, together with the wave yield of MCTV (Fig. 1.b) is likely to confirm the position of defective lysogeny of the plural nature in phytopathogenic erwinia and its gradual depression of lysogenic induction [5].

Electrophoretic analysis and electron microscopy of preparations concentrated by the method described earlier [7] showed that nalidixic lysates of both bacterial mutants Pcc ZM1/40i1 and Pcc ZM1/40i6, although in small numbers, do contain DNA-VLP (Fig. 2, 3). In order to determine the origin of DNA packed in these particles the restriction analysis using endonucleases *Hind*III, *BamH*I, *Eco*RI and *Sal*I was carried out. It is evident from the data presented in Fig. 4 that the hydrolysis of DNA of VLP Pcc ZM1/40i1 does not lead to the formation of discrete fragments. This fact is the fairly convincing evidence that bacterial DNA is packed in VLP. However, we cannot completely exclude the possibility that this DNA can occur from multiple prophages. This is possible provided that the phage capsids formed after induction are of the same size and presented in the preparations obtained in equimolar amounts.

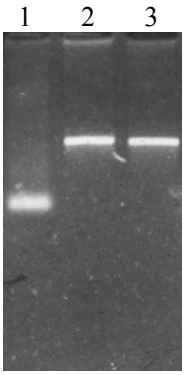

Fig. 2. Electrophoregram of native VLP Pcc ZM1/40i1 (1) and DNA of VLP Pcc ZM1/40i1 (2) and VLP Pcc ZM1/40i6 (2).

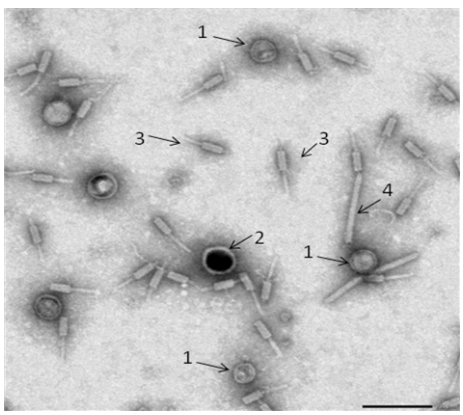

Fig. 3. Electron microscopy of phage particles of *P. carotovorum* ZM1/40i1. 1 – capsids which packed DNA molecules, 2 – an empty capsid, 3 – contracted tail-like particles, 4 – relaxed tail-like particles. Bar – 200 nm.

The approaches for genetic markers transduction using Pcc ZM1 and its DNA-VLP were not developed in our laboratory. In our independent studies an attempt to transfer the plasmid pKM101 (marker of ampicillin resistance) by VLP-particles of Pcc 62A into the cells of *Escherichia coli* C600 was made [unpublished data]. Since in these cases the positive result was not obtained, we

hypothesized that the DNA-VLP is not capable to the infectious process, and indeed is defective in contrast to the active colispecific MCTV of this strain.

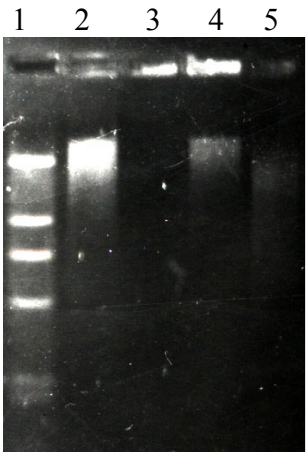

Fig. 4. Restriction digestion of DNA of VLP Pcc ZM1/40i1 by endonucleases HindIII (2), EcoRI (3), SmaI (4) and HpaI (5). 1 – HindIIIλ fragments.

For the further explanation of the underlying process of formation of defective phage particles, we used virulent mutants ZF40/421 and $c_{5/5}$ [6]. It was shown that, as well as the mutant $c_{5/5}$, ZF40/421 has defects in the morphogenesis. Its main capsid accumulates in excess in phage lysates and has mobility less than that of the normal capsid of the wild-type phage. Mutations that lead to the occurrence of mutants ZF40/421 and $c_{5/5}$ are pleotropic. In addition to the formation of abnormal phage capsids the phage ZF40/421 could lead to abortive infection that has as a result a yield of complete phage capsids of two different types. The phage mutant ZF40/421, unlike phage ZF40 wild-type, is unable to generalized transduction of plasmid pKM101, whereas the mutant $c_{5/5}$ is able to transfer this plasmid with a much lower frequency than other clear-mutants of the phage ZF40. In addition, the restriction patterns of phages ZF40/421 and $c_{5/5}$ have significant differences from those of phage ZF40 wild type.

Separation of phage particles by the ion-exchange chromatography with DEAE-cellulose column showed that the phage population of ZF40/421 consists of two components. The first component contains viable phage particles and capsids, which were eluted from the column with 0.25 M NaCl, the second one – virions and capsids that can be eluted at higher ionic strength – 0.4 M NaCl. The two-component nature of the phage population of ZF40/421 was determined for the first time in this work however, the reasons for this heterogeneity require additional research. As for the capsids structures, in both cases, their numbers prevail over the numbers of native virions. Electron microscopy and electrophoresis showed that the great majority of capsids were filled with DNA (Fig. 2 and 3). In addition, the capsids of ZF40/421 were similar in electrophoretic mobility to DNA-VLP Pcc ZM1/40i1.

Thus, *P. carotovorum* may have one of the common mechanisms of formation of defective virions, which, as it was previously assumed [1], is associated with the assembling of complete phage particles at the level of attachment of the phage tail to the head.

Pulse field gel electrophoresis of DNA from VLP Pcc ZM1/40i1 showed that they really pack the molecules of the same length (Fig. 5, 1). According to the preliminary assessments the size of this DNA is about 45 kb that matches with phage ZF40/421 DNA (Fig. 5, lane 5), as well as with possible size of the ZF40/421-f16 and ZF40/421-f17 virion DNA (Fig. 5, lane 7 and 6, respectively). Thus, the detection of these two subpopulations of the phage particles with different affinity to the DEAE-cellulose but with virion DNA of similar size also is an evidence in favor of the heterogeneity of phage ZF40/421 population. A more profound study of defective lysogeny of *P.carotovorum* at the level of sequence of the prophage genomes is necessary to determine exact mechanisms of the emerging of the prophage defects and formation of heterogeneity of the prophage populations.

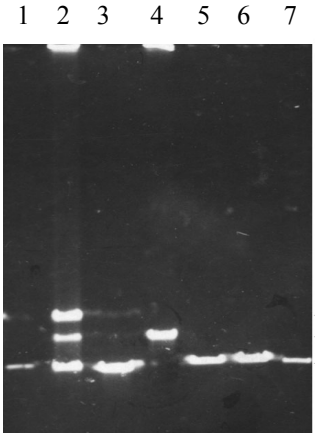

Fig. 5. Pulse field gel electrophoresis separation of the phage DNA in 1% agarose. Lines at the bottom of the gel conform the DNA of VLP Pcc ZM1/40i1 (1), phage ZF40/421 (5) and phages ZF40/421-f17 (6) and ZF40/421-f16 (7) Size and separation markers: mixture of T7, T5 and T4 DNA with molecular size are 40, 122 and 168 kb from bottom to top respectively (2), T7 DNA (3) and T5 DNA (4).

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ХАРАКТЕРИСТИКА ДЕФЕКТНЫХ ФАГОВЫХ ЧАСТИЦ *PECTOBACTERIUM CAROTOVORUM* ZM1

Р е з ю м е

Впервые показано, что продукты экспрессии дефектных профагов характерны для дефектно-лизогенных систем фитопатогена *Pectobacterium carotovorum*. Установлено, что вирусные частицы типа фаговых капсидов упаковывают бактериальную ДНК, размер которой определен при помощи пульс-форетического разделения. На основе данных о капсидных структурах, которые образует вирулентный мутант ZF40/421, сделано предположение относительно механизма формирования дефектных вирионов у *P. carotovorum*.

К л ю ч е в ы е с л о в а: *Pectobacterium carotovorum,* дефектная лизогения, фаг, вирусоподобные частицы (VLP)

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ХАРАКТЕРИСТИКА ДЕФЕКТНИХ ФАГОВИХ ЧАСТОК *PECTOBACTERIUM CAROTOVORUM* ZM1

Р е з ю м е

Вперше показано, що продукти експресії дефектних профагів характерні для дефектно-лізогенних систем фітопатогена *Pectobacterium carotovorum*. Встановлено, що вірусні частки типу фагових капсидів упаковують бактеріальну ДНК, розмір якої визначений за допомогою пульс-форетичного розділення. На основі даних про капсидні структури, які утворює вірулентний мутант ZF40/421, зроблено припущення щодо механізму формування дефектних віріонів у *P. carotovorum*.

К л ю ч о в і с л о в а: *Pectobacterium carotovorum,* дефектна лізогенія, фаг, вірусоподібні частки (VLP). .

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Отримано 08.01.2011