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RESTRICTION OF THE GROWTH OF T7-LIKE PHAGES BY PLASMID PROPHAGE P1

T7-like phages represent an example of the saving utilization of the genome. Their DNA of about 40 kb contains genes solely of phage origin that are sufficient to perform the basic function of bacteriophage, its reproduction. They are lytic bacteriophages and hence do not waste coding capabilities of their genome neither for lysogeny establishment nor for transduction of bacterial genes. Considerable part of T7 phage genome though is responsible for the interaction with the host, peculiarly for overcoming the cell barriers [1]. Thus, the structure of the attachment apparatus that allows for adsorbtion to core of LPS is responsible for the expansion of host range of T7-like phages [1]. Some of them (eg FE44) are able to overcome intergenera and interspecies barriers what characterizes them as polyvalent [2]. The next step of interaction consists in phage avoidance of action of protective systems of bacterial cells, primarily the restriction-modification (RM) complexes. Interactions of T7 phage group members with RM systems of type I and II are relatively studied while the question of impact by the type III systems on their growth remains opened [3].

The purpose of our work was to develop a system allowing for studying on the gene level the interaction of bacteriophages with cells both of native and uncommon hosts; to explore the interaction of the active phages with prophage elements in these systems.

Materials and methods

The object of the study was a T7-like polyvalent phage FE44 [2] obtained on different host bacteria – *E. coli* C600 (FE44/C600) and *E. "horticola"* 450 (FE44/450). Its genome was recently sequenced, annotated and deposited to GenBank database under accession number KF700371. Other members of the group T3, T7 and BA14 were used as controls. *Podoviridae* phage E105 and *Siphoviridae* erwiniaphages 49, 59 [4] and 59 mod/P1, obtained by passaging on the lysogenic strain 450(P1) were used in experiments performed on phytopathogens. Phage P1Cm*c*1*ts*100 [5] carrying chloramphenicol resistance marker (*Tn*9) and temperature-sensitive repressor protein C1 was used for lyzogenization of the cells.

Three species of bacteria were used: laboratory strains of *E. coli (Eco)* C600, C1a, S/6, BE and 112(P1); the causative agent of fire blight disease *Erwinia amylovora (Eam)* strains K8 (ATCC 29850), L4, L6, L7, K4, K5; *Erwinia "horticola" (Eho)* 450, 60-1N, 60-3m, 43I, 43II, 120, 23a and artificially lysogenized strains 450(49) and 60 (59, E105) – the phytopathogenic bacteria causing black bacteriosis of apples and European beech [6].

For cells lysogenization a volume of 5 ml of concentrated phage P1Cm*c*1*ts*100 suspension near 10^8 PFU/ml was applied on the top layer of semisolid agar containing cells of the corresponding recipient: *Eam, Eho* and *Eco*. After drying the plates were overturned and incubated at 28 ˚C for 28–30 hours. Bacterial cells picked out from the area of application were carried into liquid LB medium and incubated for 8–10 h at the same temperature. When the early stationary phase of growth was reached the bacteria were propagated to individual colonies on selective LB-plates with chloramphenicol (Cm) in concentration of 14 μg/ml.

Bioinformatic research was performed using BLASTn and BLASTp common tools (http://blast.ncbi.nlm.nih.gov/Blast.cgi), COBALT (http://www.ncbi.nlm.nih.gov/tools/cobalt/) tool for multiple protein alignment and APE plasmid editor for virtual digestion (http://biologylabs.utah.edu/jorgensen/wayned/ape/).

Results and discussion

The *ocr* antirestriction function. T7-like phages are known to produce protein gp 0.3 (Ocr) to protect against type I RM systems. Ocr protein of T7 phage mimics the size and shape of curved DNA molecule while that of T3 possesses additional SAMase activity [1]. The role of gp 0.3 in T7-like phages interaction with type III RM system is a disputable issue: it can be argued that due to SAM-ase activity it counteracts type III restriction-modification complex which requires a cofactor AdoMet to function [3, 7].

FE44 phage turned out to be convenient to solve this issue since it is not able to express *ocr* function. When titrated on bacterial hosts carrying EcoB and EcoK restriction complexes (*E. coli* BE[EcoB], S/6[EcoB], J53[EcoK]) its efficiency of plating (EOP) decreased by value of 3–4 orders comparing to r^-m^- hosts (C600, C1a). In control experiments the EOP of T7 phage remained

constant or varied within one order of magnitude. Interestingly, bioinformatic analysis revealed the presence of gene 0.3 in FE44 genome. Predicted gp 0.3 protein features almost identical sequence to that of BA14 with single amino acid replacement of histidine in position 124 with proline found. The difference may seem insignificant however *ocr* expression is not observed. Thus, using FE44 the impact of type III RM enzyme on phage DNA excluding any interfering factors can be performed.

Construction of the bacterial strains systems and restriction exploration. Phage P1 is known to establish the type III RM-system EcoP1I in prophage state both in the cells of traditional host *E. coli* [5], and other enterobacteria *(Klebsiella, Pasteurella, Shigella)* [8], *P. atrosepticum* [9]. To study the interaction of EcoP1I with T7-like phages the systems formed by isogenic pairs comprising the parent and lysogen variant were constructed. Sensitive bacterial strains were treated with P1Cm*c*1*ts*100 and lysogens were selected as clones resistant to 14 μg/ml of chloramphenicol [5]. To verify the introduction of prophage DNA into the cell and its maintainance in it the electrophoretic analysis of extrachromosomal DNA extracted from the parent cells and CmR–strains of *Eho, Eam* and *Eco* was performed.

As shown in fig. 1, compared to the parent strains, resistant to Cm clones of *Eho* and *Eam* carry additional extrachromosomal circular DNA. These molecules of DNA coincide in size with that extracted from *E. coli* C600(P1), C1a(P1) and of control plasmid P1 of *E. coli* 112(P1) strain; hence, they appear to be a plasmid prophage P1. This shows that phage P1 can be maintained in the cells of uncommon host as a circular plasmid molecule of approximately 94.8 kb similar to its maintainance in native host *E. coli* [5].

To estimate the activity of RM-system in constructed bacterial cells the efficiency of plating (EOP) of phages on P1 lysogens was compared to that on parent strain. FE44 was the single phage able to grow on all used bacteria while T3 and T7 failed to infect most of the phytopathogenic strains (table 1). Therefore specific erwiniaphages E105, 59 and 49 were as the control in case of *Eho* system.

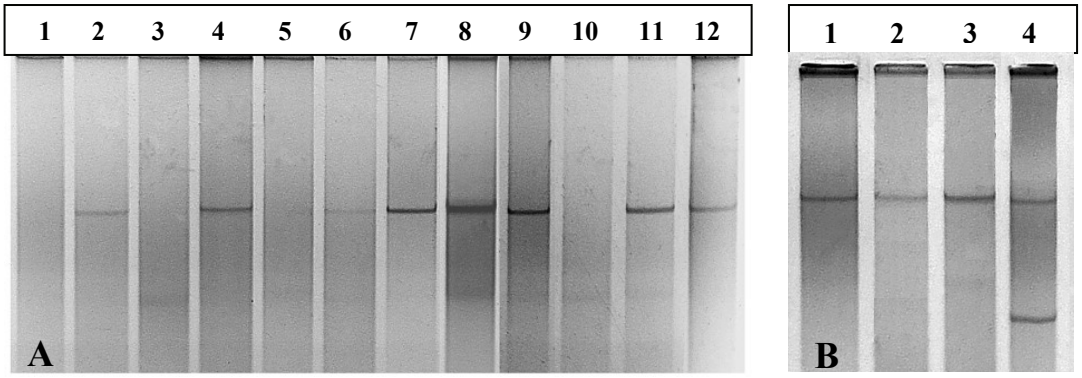

Fig. 1. Electrophoregram of extrachromosomal DNA extracted from parent and Cm^R– strains of *E. amylovora, E. "horticola"* and *E. coli*. **A.** *Eho*:1 – 450, 2 – 50 (P1), 3 – 60-3m, 4 – 60-3m (P1), 5 – 60-1N, 6 – 60-1N (P1), 8.–120 (P1); *Eco*: 7,9.– 12 (P1), 10.–C1a, 11.–C1a (P1), 12.– C600 (P1); **B.** 1 – *Eco* 112 (P1), 2 – *Eho* 120, 3 – 120 (P1), 4 – *Eam* L4 (P1)

Table 1. Sensitivity of *Erwinia* genus representatives to T7-like phages

Strain	*E. "horticola"*									*E. amylovora*		
Phage	60 - 3m	60 - 1n	60(59, E105)	450	450 (59)	43I I	43I	23a	120	L4	K8	K4
T7	–	–	–	–	–	–	–	–	–	–	$+^{N}$	–
T3	$+^{res}$	–	–	–	–	$+^{t}$	$+^{t}$	$+^{res}$	$+^{res}$	$+^{N}$	$+^{N}$	–
FE44	$+^{N}$	$+^{N}$	$+^{N}$	$+^{N}$	$+^{N}$	$+^{t}$	–	$+^{res}$	$+^{res}$	–	$+^{N}$	$+^{N}$

Note: "–" – here and in table 2 signifies insensibility to phage infection; "$+^{res}$" – indicates on the restriction of phage growth; "$+^{res}$" – stands for normal infection development.

Similar to other phages of T7 group interaction of phage FE44 with EcoP1I-carrying hosts results in the inevitable development of abortive infection (fig. 2). In system formed with *E. coli* C600 and C600(P1) and phage FE44/C600, as well as T3 and T7 phages their EOP decreased only by 1–2 orders of magnitude with each subsequent propagation. Still the form and size of plaques as well as the inability to restore normal phage reproduction in subsequent passaging indicated on the *abi*-infection. Interestingly, restriction of FE44 growth was similar to T3 while that of T7 was more stringent. This indicates that gp 0.3 is not involved in the interaction with type III RM system. DNA of T7-like phages is not modified by EcoP1I methylase, obviously for the reason of multiplicity of EcoP1I recognition sites on their DNA. Thus T7 DNA contains 126 and T3 has 154 recognition sequences while for FE44 this value constitutes 156.

Analogous abortive infection (EOP on lysogens about 10^{-3}) was discovered for phage E105 interaction with P1 RM-system in systems of *E. "horticola"* strain 60-1N and its lysogenic derivatives.

Another kind of interaction was determined in system formed by *E. coli* C1a and C1a(P1) strains and the used T7-like phages. The development of phage FE44/C600, T3 and T7 gave rise to abortive infection (*Abi*-phenotype) but the bacteriophages were completely eliminated after the first propagation on bacteria. Phage titers from 10^{9}–10^{10} PFU/ml on the parent strain decreased to zero on lysogenic. Similar results were obtained in *E. "horticola"* system in case of phage FE44/450 propagation on pairs 450, 450(P1) and 60-1N, 60-1N(P1). Such efficient restriction by P1 is likely associated not with the adsorption sites conversion in lysogens.

Again abortive was the phage T3 infection in lysogenic *E. amylovora* L4(P1) cells. This system was shown to be inefficient for studying the details of interaction between phage T3 and EcoP1I system due to the low EOP of phage both on lysogenic and parent strains.

In contrast to the mentioned cases phages 49 and 59 realize productive infection in *Eho* 450(P1) lysogens. EOP of phages decreased by 6 orders of magnitude after the first plating on P1 lysogens lawn. However plaque size and phage titers recovered in the following passages or when the phage 59 modP1 was used. Thus, DNA of 49 and 59 phages is efficiently modified by methyltransferase Mod of P1 RM-complex. Such features the phage lambda behavior in the system of *E. coli* P1 lysogens [5].

Conclusions

The constructed system of strains by P1 of *E. coli, E. "horticola"* and *E. amylovora* allow for the exploration of restriction-modification gene complex EcoP1I interaction with T7-like phages as well as with other polyvalent or specific phages.

The genes of EcoP1I RM system are fully expressed regardless the bacterial host lysogeinzed by phage P1. This proves the notion that RM-systems represent certain universal mobile genetic elements capable of functioning in any system and outspreading due to residing on phage DNA.

According to the level of restriction three types of phage-RM system interaction was discovered. Differences in phage responses to the presence of RM-system in the lysogenic host correlate with the number of recognition sequences on the DNA and the availability of adsorption sites while gp 0.3 Ocr protein was proved not to be involved in this interaction

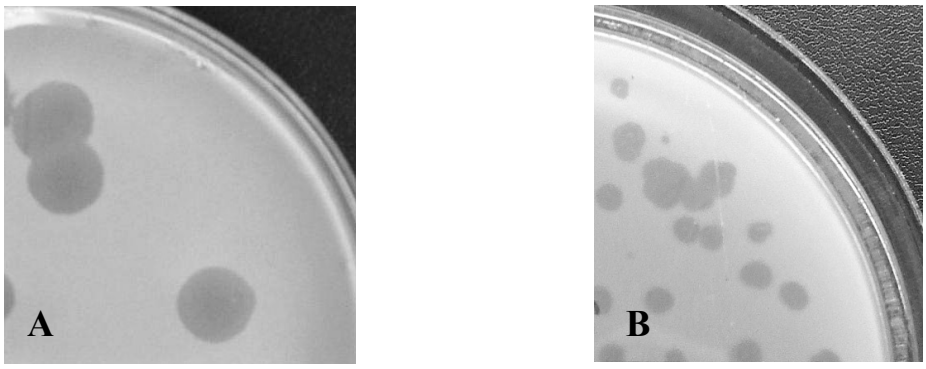

Fig. 2. Phage FE44 plaques formed on lawns of *E. coli* C600 (**A**) and C600 (P1) (**B**)

Table 2. Efficiency of plating of phages in the *E. coli* and *E. "horticola"* systems

Strain	Bacteriophage						
	T7	T3	FE44[a]	E105	59	59mod	49
Eco C600	1.0	1.0	1.0	–	–	–	–
C600(P1)	$5*10^{-2}$	0.18	0.16	–	–	–	–
C1a	1.0	1.0	1.0	–	–	–	–
C1a(P1)	0*	0*	0*	–	–	–	–
Eho 60-1N	–	–	1.0	1.0	x	x	x
Eho 60-1N	–	–	1.0	1.0	x	x	x
60-1N$(P1)_{-1}$	–	–	0*	$7\cdot10^{-3}$	x	x	x
60-1N$(P1)_{-2}$	–	–	0*	$8\cdot10^{-3}$	x	x	x
450	–	–	1.0	–	1.0	1.0	1.0
$450(P1)_{-2}$	–	–	0*	–	$4.3\cdot10^{-6}$	1.0	$9.5\cdot10^{-6}$

Note: "a" – On *Eco* strains the value is indicated for FE44/C600 phage and on *Eho* strains for FE44/450. "*0" – stands for the absence of individual plaques while zones of lysis are evident "x" – experiments not performed.

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RESTRICTION OF THE GROWTH OF T7-LIKE PHAGES BY PLASMID PROPHAGE P1

Aims. Considerable part of T7 phage genome is responsible for interaction with the bacterial host, primarily for the avoidance of action of protective systems of cells, the restriction-modification complexes. Interactions of T7-like phages with RM systems of type I and II are relatively studied while the question of impact by the type III systems on their growth remains unclear. Developing a relevant system would allow us to study the interaction of bacteriophages with host cells on the gene level including the interplay with prophage elements and RM-systems. ***Methods.*** Biological, genetics and molecular biology approaches combined with bioinformatic research were used. ***Results.*** The ability of P1 to infect and lysogenise *Erwinia amylovora* and *Erwinia "horticola"* cells as well as its maintainance as a single-copy plasmid in the cells of uncommon hosts was shown. A set of lysogenic strains was obtained. According to the level of restriction three types of phage-RM system interaction were discovered. Though polyvalent, phage FE44 undergoes abortive infection similar to other members of T7 phage group. ***Conclusions.*** The genes of restriction-modification complex EcoP1I are fully expressed regardless the bacterial host lysogeinzed by phage P1.

Differences in interaction with cells are likely associated with the number of enzyme recognition sequences and the adsorption sites availability while gp 0.3 Ocr protein is not involved in this interaction. The constructed systems allow for the exploration of EcoP1I interaction with polyvalent phages able to grow both on *E. coli* and on such phytopathogens as *E. "horticola"* and *E. amylovora*.
Key words: T7-like phages, Type III restriction-modification complexes, antirestriction, polyvalent bacteriophages, phytpathogens.