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PHYTOPATHOGENIC BACTERIA PHENOTYPE CONVERSION AS A RESULT OF THEIR LYSOGENISATION BY COLIPHAGE P1

A set of lysogenic strains of phytopathogenic bacteria Erwinia "horticola" and Erwinia amylovora associated with woody plants was obtained using bacteriophage P1Cmc1ts100. The phenotype conversion from Cm^S to Cm^R was shown to be connected with introducing of authentic prophage DNA of 94.8 kb as a single-copy plasmid into the cells. Prophage state is unstable; P1 plasmid is spontaneously lost with high frequency by the cells. In lysogenic cells the prophage genes of type III restriction-modification complex EcoP1I are actively expressed. The system formed by E. "horticola" 450 and 60 as well as their lysogenic derivatives and specific bacteriophages provides an opportunity to divide the latter into three groups according to the level of restriction in the course of their interaction with the enzyme EcoP1I. The difference in phage responses to the endonuclease presence in a lysogenized host presumably correlates with the number of enzyme recognition sequences and the adsorption sites availability. After the prophage plasmid DNA curing the characteristic value of phage sensitivity of cells is changed.

The lysogenic strains obtained in this work allow for the exploration of EcoP1I restriction-modification gene complex interaction with polyvalent phages able to grow not only on E. coli, but also on such phytopathogens as E. "horticola" and E. amylovora.

Key words: *lysogenic conversion, type III restriction-modification system, polyvalent bacteriophages, phytopathogens, Erwinia amylovora.*

Having a wide host range the bacteriophage P1 and its derivatives P1Cm*c*1*ts*100 and P1Km-*c*1ts100 are common genetic tools in molecular biology studies both of the *Enterobacteriaceae* genera representatives and many other Gram-negative bacteria [13]. Establishment of lysogeny and conversion of lysogenized cells by P1 are described not only in *Escherichia coli* [16, 17], but also in such heterologous systems as: *Klebsiella, Pasteurella, Shigella* [10, 18, 20], as well as some pathogenic bacteria *Pectobacterium carotovorum* and *Dickeya dadantii* [1, 2].

Lysogenic by P1 *E. coli* cells synthesize defective structural components of LPS and as a consequence their phage sensitivity spectrum alters [17], naturally resistant to tellurite *Klebsiella pneumoniae* cells lose this property after being lysogenized [19]. Furthermore, P1Cm*c*1*ts*100 in the prophage state establishes in a host cell the type III restriction-modification system EcoP1I, which efficiently restraints the growth of the majority of phages in these cells [16].

As lysogenic systems formed by different bacteria and phages are unique and can vary substantially [7] the purpose of this paper was to investigate the peculiarities of P1 lysogeny in *Erwinia amylovora* and *Erwinia "horticola"* bacteria associated with trees.

Matherials and methods. The following strains of pathogenic bacteria were used: fire blight disease pathogen *E. amylovora (Eam)* K8 (ATCC 29850), L4, L6, L7, K4, K5; amylovora-like bacteria causing forest beech black bacteriosis *E. "horticola" (Eho)* 450, 60-1N, 60-3m, 43I, 43II, 120, 23a [6] and artificially lysogenized strains of *E. "horticola"* 450(49) and 60(59, E105) obtained in previous studies [5]. Laboratory strains of *E. coli (Eco)* C600, C1a and BL21 were used as control.

Genetic tool P1Cm*c*1*ts*100 [16] carrying chloramphenicol resistance marker (Tn*9*) and temperature-sensitive repressor protein C1 was used for lysogenization of cells. Other biological experiments were performed using *Podoviridae* phages: phage FE44 (GenBank accession no. KF700371), [4] obtained on different host bacteria – *E. coli* C600 (FE44/C600) and *E."horticola"* 450 (FE44/450); T3 and T7, kindly provided by Dr. I.J. Molineux; phage E105 and *Siphoviridae* phages 49, 59 [5] and 59 mod/P1, obtained by passaging on the lysogenic strain 450(P1).

Phage P1Cm*c*1*ts*100 with the concentration of about $1–2 \cdot 10^8$ PFU/ml was obtained by thermoinduction of *E. coli* strains C600(P1) and 112(P1). The lysogenic cells were grown in LB medium till saturation ($2 \cdot 10^9$cells/ml), then diluted 10–20 times in fresh LB-medium and grown for 3 h at 30˚C, after that the temperature was drastically raised to 42˚C. The cells were incubated for about 3 hours

at this temperature; after cells lysed chloroform was added to suspension (1/50 part of the initial volume of suspension). The lysates were stored at 4˚C.

Lysogenization of bacteria was performed as described in [2].

Extrachromosomal DNA was extracted using [9] and analyzed by horizontal electrophoresis in 0.7% agarose gel in buffer E for 4–8 hours. P1 plasmid of 94.8 kb isolated from *E. coli* strain 112(P1) was used as a standard [11].

Results.

Phage P1Cm*c*1*ts*100 interaction with cells of phytopathogens. Sensitivity of *E. amylovora* and *E. "horticola"* strains to phage P1 infection was determined by the formation of specific lysis spots that indicated the lysis from outside. Four out of six used strains of *Eam* (L4, K4, K8 and L6) and 80% of *Eho* strains (except for the strains 23a and 43II) appeared to be susceptible to the P1. Two strains of *E. amylovora*, L4 and K8 and 4 strains of *E. "horticola",* 450, 60-1N, 60-3m and 120 were selected for further research.

As P1Cm*c*1*ts*100 carries transposon Tn*9*(Cm^R) the phenotype of the lysogenized cell changes from Cm^S to Cm^R due to the functioning of chloramphenicol acetyltransferase expressed from a *cat* gene that is placed within transposon inverted repeats [3, 16]. Therefore, among the cells that survived the phage P1 lysis the potential lysogens were selected as clones resistant to 14 μg/ml of chloramphenicol.

Six clones of *E. amylovora* L4 strain with altered Cm^R phenotype were selected. Despite P1 phage formed lysis zones on the lawn of *Eam* K8 obtaining of any Cm^R-clone was impossible for unknown reason. However, chloramphenicol-resistant clones of four $P1^S$ strains of *E. "horticola"* as well as of two control *E. coli* strains (C1a and C600) were selected under the same conditions.

Obtaining of chloramphenicol resistance by pathogens after their interaction with phage P1Cm*c*1*ts*100 can be provided by following factors: *cat* gene transduction, its transposition as a part of Tn*9* into chromosome or extrachromosomal DNA of the recipient strain, and, finally, introduction of the authentic prophage DNA into the cell. The last explanation is the most plausible whereas the establishment and maintenance of plasmid P1 in phytopathogenic bacteria *P. carotovorum* was shown with assurance [2] .

To verify this hypothesis electrophoretic analysis of extrachromosomal DNA extracted from the parent cells and Cm^R–strains of *Eho, Eam* and *Eco* was performed. As shown in Fig. 1A, compared to the parent strains (lanes 1, 3, 5), resistant to chloramphenicol clones of *Eho* carry additional extrachromosomal circular DNA (lanes 2, 4, 6). These molecules of DNA coincide in size with that extracted from *E.coli* C600(P1) and C1a(P1) strains (lanes 11 and 12, Fig. 1A). Accordingly their electrophoretic mobility corresponds to that of control plasmid P1 of *E. coli* 112(P1) strain; hence, they appear to be a plasmid prophage P1. In addition, prophage DNA coexists with resident plasmids of about 20 and 100 kb in *Eam* L4 and *Eho* 120 cells respectively (lanes 3 and 4, Fig. 1B). Thus, prophage P1 is maintained as a single-copy plasmid of approximately 94.8 kb in cells of phytopathogens *E. amylovora* and *E. "horticola"* similar to its maintainance in strains of native host *E. coli* [16].

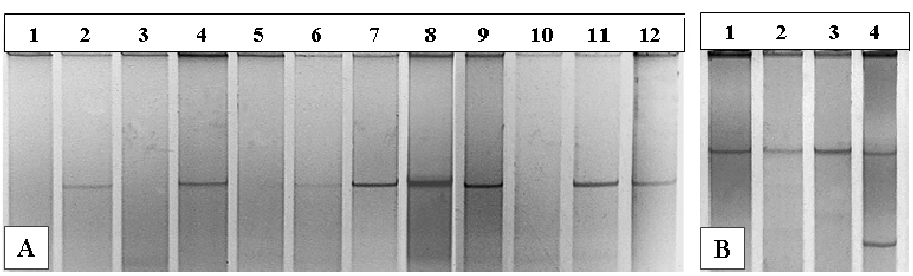

Fig.1. Electrophoregram of extrachromosomal DNA extracted from parent and Cm^R–strains of *E. amylovora, E. "horticola"* and *E. coli*.

A. *Eho*:1.–450, 2.–450(P1), 3.–60-3m, 4.–60-3m(P1), 5.–60-1N, 6.–60-1N(P1), 8.–120(P1); *Eco*: 7,9.–112(P1), 10.–C1a, 11.–C1a(P1), 12.–C600(P1);

B.1.–*Eco* 112(P1), 2.–*Eho* 120, 3.–120(P1), 4.–*Eam* L4(P1)

To inspect the stability of Cm^R-phenotype inheritance coupled with the plasmid P1 maintenance a series of experiments for *Eho* strains spontaneous loss of chloramphenicol resistance was carried out. *E. coli* cells lose the P1 plasmid with low frequency (10^{-4} – 10^{-5} cells per generation) since the majority of cured cells are killed by toxin-antitoxin (TA) system PhD/Doc [11].

E. "horticola" strains spontaneously loose Cm^R marker with a sufficiently high frequency. It constituted 5.5% for strain 60-1N(P1); for strains 450(P1) and 60-3m its values were about the same – 2.1 and 2.4%, respectively. In the case of strain 120(P1) only 2 of the 204 tested clones reverted to Cm^S phenotype (0.98%). Under the same conditions of spontaneous curing none of the tested *E. coli* lysogens lost resistance to chloramphenicol. Consequently lysogenic system formed by *E. "horticola"* and phage P1 is less stable than the system of phage P1–*E. coli*.

Next, by means of electrophoretic analysis *Eho* strains 450, 60-1N and 120 which lost resistance to chloramphenicol (all tested clones) were shown to be devoid of plasmid. Interestingly, strain 60-3m cells though become chloramphenicol-sensitive, yet carry the extrachromosomal DNA. Moreover the two clones of this strain differ (lane 11, Fig. 2A, and lanes 2, 3, Fig. 2B): 60-3m(P1)$^-$-1 and 60-3m(P1)$^-$-2 carry a plasmid which has slightly smaller size than P1, but the lane that corresponds the clone 2 also features additional band of circular extrachromosomal DNA.

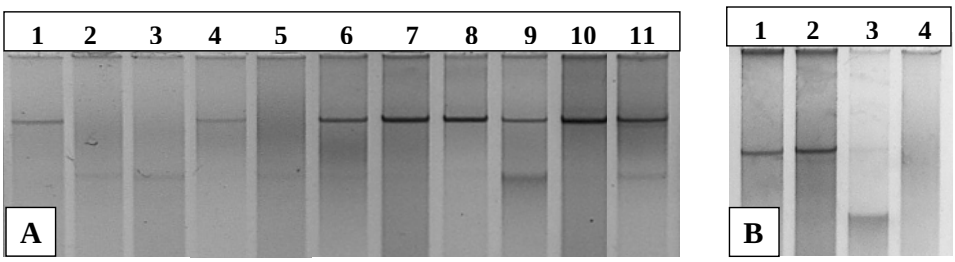

Fig.2. Electrophoregram of extrachromosomal DNA extracted from parent, lysogenic and cured strains of *E. amylovora* and *E. "horticola".*

A. *Eho*:1.–60-1N(P1), 2,3.–60-1N(P1)$^-$, 4.–450(P1), 5.–450(P1)$^-$, 7.–120(P1), 8, 9.–120(P1)$^-$, 10.–60-3m(P1), 11.–60-3m(P1)$^-$-2, 6.–*Eco* 112(P1);

B. *Eho:* 1.–60-3m(P1), 2.–60-3m(P1)$^-$-1, 3.–60-3m(P1)$^-$-2, 4.–60-3m.

Thus, *E. "horticola"* cells' loss of chloramphenicol resistance correlates with the loss of plasmid prophage. By analogy with [3] the loss of the marker Cm^R by 60-3m(P1) cells can be suggested to occur due to deletion of the Tn*9* left terminal repeat along with the acetyltransferase gene. The loss of significant part of plasmid DNA in conjuction with transposon is likely to occur in the clone 60-3m(P1)$^-$-2.

EcoP1I restriction-modification system functioning in cells of phytopathogenic bacteria. Phage P1 is able to establish the type III RM-system EcoP1I in prophage state not only in the cells of traditional host *E. coli* [14], but also of other enterobacteria (*Klebsiella, Pasteurella, Shigella*) [10, 18, 20] and *P. atrosepticum* in particular [2]. To estimate the activity of RM-system in *E. "horticola"* and *E. amylovora* cells the efficiency of plating (EOP) of phages on P1 lysogens was compared to that on parent strains and cured strains obtained in the previous study. FE44 phage which carries 156 EcoP1I recognition sequences on DNA and erwiniaphages E105, 49 and 59 with unknown number of such sites were used [5].

The phage P1 gene complex EcoP1I was shown to be successfully expressed and to function both in the cells of *E. "horticola"* and *E. amylovora*. Three types of enzyme EcoP1I interactions with the phages were determined (Table 1).

In systems formed by *Eho* pairs 450, 450(P1) and 60-1N, 60-1N(P1) the development of phage FE44/450 gave rise to abortive infection (*Abi*-phenotype). Phage titer from 10^8 PFU/ml on the parent strain decreased to zero on lysogenic; even though more concentrated phage suspensions of FE44/450 had been applied on the lawn of *Eho* 450(P1) plaque formation was not observed. Such effective restriction by P1 is likely associated not only with RM-system functioning but also with the adsorption sites conversion in lysogens. A similar kind of interaction was shown in control experiments for system formed by *E.coli* C1a and C1a(P1) strains and the T7-like phages.

Table 1

Efficiency of plating of phages in the system formed by *E. "horticola"* and its lysogenic derivatives

Strain	Bacteriophage				
	FE44/450	E105	59	59mod	49
60-1N	1,0	1,0	x	x	x
60-1N(P1)-1	0*	$7 \cdot 10^{-3}$	x	x	x
60-1N(P1)-2	0*	$8 \cdot 10^{-3}$	x	x	x
450	1,0	–	1,0	1,0	1,0
450(P1)-2	0	–	$4{,}3 \cdot 10^{-6}$	1,0	$9{,}5 \cdot 10^{-6}$

Note: "*0" – here and in table 2 stands for the absence of individual plaques while zones of lysis are evident; "–" – here and in table 2 signifies insensibility to phage infection, "x" – experiments were not performed.

The plating of phage E105 on strains *Eho* 450, 60-1N and their lysogenic derivatives also resulted in the development of *Abi*-infection. In this case the phage titer decreased only by two 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 abortive character of infection (Fig. 3). Analogous abortive infection (EOP on lysogens about 10^{-2}) was discovered for phage FE44 interaction with P1 RM-system in *E.coli* C600 cells.

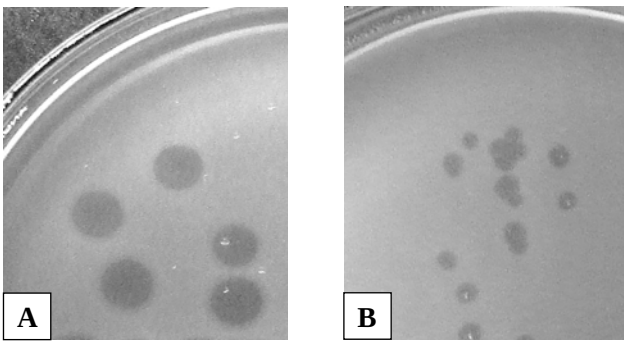

Fig.3. Phage E105 plaques formed on lawns of *E. "horticola"* 60-1N (A) and 60-1N(P1) (B).

In contrast to the two mentioned viruses, phages 49 and 59 conduct normal 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 and next time when such DNA enters the P1-lysogenic cell the EcoP1I enzyme recognizes it as self. Such interaction is the traditional type of RM-system interaction with the phage DNA and also characterizes the phage lambda behavior in the system of *E. coli* P1lysogens [14].

The phage infection in lysogenic *E. amylovora* L4(P1) cells also appeared to be abortive. 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.

Therefore genes coding for the two enzymes of restriction-modification system, endonuclease (Res) as well as methyltransferase (Mod) are actively expressed in *E. "horticola"* and *E. amylovora* cells. Differences in phage response to the presence of RM-system in the lysogenic host correlate with the number of recognition sequences on their DNA and the availability of adsorption sites.

Changes in *E. "horticola"* sensitivity to phages after P1 plasmid loss. Since *Eho* 60-1N(P1) and 450(P1) were able to lose the plasmid prophage P1 with high frequency several isogenic $P1^-$-clones were tested for sensitivity to phages FE44, E105, 49 and 59. As it can be seen from the results presented in Table 2 all cured bacteria regained sensitivity and significantly changed the efficiency of plating value regarding the indicated phages. Thus the phage FE44/450 EOP on the cured variants of 60-1N and 450 increased by 2–3 times. A slight increase of this value was also observed for phage 49 which was titrated on P1-cured variants of both strains. At the same time, the phage 59 is characterized by both slight increase or decrease of EOP depending on certain isogenic clone of

60-1N(P1⁻) used. Bacteria 450(P1⁻) albeit slightly but in general increased the titer of this phage. Finally, in contrast to phages FE44, 49 and 59 the phage E105 when titrated on P1⁻ derivatives of 60-1N strain lost EOP by approximately 50% compared to that on the parent strain.

Table 2

Efficiency of plating of phages FE44, E105, 49 and 59 on strains *E. "horticola"* 60-1N, 450 and their derivatives

Strains	Bacteriophages			
	FE44/450	E105	59	49
60-1N	1,00	1,00	0*	1,00
60-1N(P1⁻)-1	2,70	0,37	1,50	0,85
60-1N(P1⁻)-2	2,00	0,57	1,20	1,25
60-1N(P1⁻)-3	2,40	0,57	1,50	1,95
60-1N(P1⁻)-4	2,20	0,63	0,50	1,30
60-1N(P1⁻)-5	2,90	0,40	0,90	1,25
450	1,00	–	1,00	1,00
450(P1⁻)-1	2,25	–	1,40	1,20
450(P1⁻)-2	3,00	–	1,10	1,60

Thus the data convincingly confirm the alteration of such phenotypic feature as sensitivity to phages of cured *E. "horticola"* 60-1N and 450 clones.

The functioning of the immunity module and module of morphological and structural organization. The defense of the host cell from homoimmune phage superinfection is accomplished by activity of the phage repressor protein C1 [11]. The ability of P1 prophage to express the repressor in lysogenic *Eho* cells was estimated by development of resistance to P1 reinfection. When phage suspension was applied to the lawn of lysogenic *E. "horticola"*(P1) the typical spots of lysis from outside were formed; the lysogens remained sensitive to phage P1. Thus, the immune system is working inappropriately or is not expressed in the cells of phytopathogenic erwinia.

We also were not able to recover phage progeny after induction of *E. "horticola"* 450, 60-3m and 120 lysogens. At the same time due to the thermoinduction of *E. coli* strains C600(P1) and C1a(P1) viable P1Cm*c*1*ts*100 phage particles were obtained in concentration of about 10^8 PFU/ml. Obviously phage P1 fails to express the structural module genes in the heterologous system, *E. "horticola"*.

Discusssion. A comparison of the phage-host systems is essential for understanding the mechanisms of virus adaptation to variations of the environmental conditions with supreme usage of its own genetic potential. Upon that the polyvalent bacteriophages able to expand the bacterial host range overcoming species and genera barriers are of particular interest. On the other hand the study of the bacteriophages adaptive potential is impossible without the introduction of a defined set of sensitive bacteria and especially isogenic pairs that would permit to investigate the adaptive response of phage as response to a certain signal.

Phytopathogenic bacteria sensitive to three different types of phages as well as their isogenic partners lysogenized by P1 were used in this paper. In our view this approach can simplify the investigations greatly as they are reduced to a phage-prophage interaction regardless of other conditions. Furthermore, lysogenic conversion with the participation of prophage P1 in case of uncommon under normal conditions pathogenic bacterial hosts could expand our understanding of heterologous phage-bacterial systems.

P1 prophage was for the first time shown to be maintained as an extrachromosomal plasmid DNA in phytopathogenic bacteria *E. amylovora* and *E. "horticola"*. The size of the DNA in the cells of all lysogenic strains is 94.8 kb. Thus, the process of phytopathogens lysogenization by phage P1 does not significantly differ from that of its related host, *E. coli.*

It was also shown that the plasmid prophage P1 is stably maintained in the presence of selective pressure and is able to coexist with resident plasmids of *Eho* and *Eam* cells. Even despite the strain *Eho* 120 plasmid has the similar size as P1 they do not displace each other from the cell. Coexistence of plasmid P1 and cryptic plasmid with size of 18.5 kb was previously shown in cells of

P. carotovorum [2]. Obviously, in this case and in the case of *Eho* 120 and *Eam* L4 plasmids belong to different incompatibility groups with respect to P1.

The high frequency of curing of the studied *E. "horticola"* and *E. amylovora* strains in the absence of selective pressure indicates a discordance of the plasmid separation and segregation with those of bacterial cells and lack of toxin-antitoxin PhD/Doc system expression. Bacteria occupying a common or similar to *E. coli* ecological niche including *Klebsiella, Shigella* are cured from prophage P1with low frequency [18, 20]. We noticed that the strain *Eho* 120 cells lost Cm^R marker with the lowest frequency, and obviously the systems of resident plasmid maintaining also stabilize the prophage DNA. Interestingly, in *Eho* 60-3m cells plasmid replicon is not removed while the parallel loss of resistance to Cm occurs. The latter is most likely caused by deletion in the region of Tn*9* [3].

The most demonstrational was the cell lysogenic conversion by phage P1 due to establishment the type III restriction-modification system within them. The sensitivity to phages of lysogens was shown to significantly vary depending on the phage and host strain. It is known that in lysogenic cells of *E. coli* EcoP1I system cleaves and modifies DNA of phage lambda [14], T-even phages [15] and representatives of T7 phage group [12]. Using the system formed by strain *Eho* 450, its lysogenic derivatives and various phages the latter can be divided into three groups according to the rate of restriction of their development. Differences in the interaction can be explained by variant numbers of recognition sites on phage DNAs (phage FE44 has 156). Therefore DNA of phage E105 can be assumed to contain fewer EcoP1I recognition sites than that of phage FE44 because the restriction of E105 in the same system of strains is less strict than of phage FE44.

Different levels of phages growth restriction most likely depend not only on the interaction of their DNA with intracellular EcoP1I enzyme, but also largely on the molecular structure of the host cell surface and adsorption sites accessibility. It is possible that phages E105 and FE44 exploit cellular receptors located in different loci of LPS molecule. Obviously, in the case of phages 49 and 59 their DNAs contain a small number of EcoP1I sites as in the case of phage λ, which has 49 recognition sites. Therefore methyltransferase manages to modify their DNA before it will be destroyed by restriction endonuclease.

Expression of the RM-system genes was confirmed in *K. aerogenes* [8]. Decrease in efficiency of plating of phages T7, H and φIV on lysogens of *Pasteurella pseudotuberculosis* by 6-8 orders of magnitude also indicates on successful expression of this module [10]. EcoP1I functioning has been also shown in cells of *S. dysenteriae* that were able to restrain the phage T1 development [20]. Obviously, both methyltransferase and restriction endonuclease of RM gene complex are fully expressed and function regardless the species or genera of bacteria lysogenized by phage P1.

An important phenomenon established in the course of work was the alteration of *E. "horticola"* strains sensitivity to specific phages 49, 59 and E105 after loss of the plasmid P1. It is known that after lysogenization by P1 *E. coli* cells lose their ability to adsorb P1*vir* and λ*vir* due to the changes in surface structures. Lysogenic cells switch to the synthesis of heptose-deficient LPS molecules [17]. Conversion of the surface structures regarding phage MS2 was shown for *Pasteurella* [10]. However after curing from P1 surface structures of *E. coli* completely reverse to the original form, so such conversion can explain the changes in phage EOP on lysogens but not on cured variants.

In our case it can be assumed that phage P1 selects cells with mutant forms of LPS from all primary population of *Eho* and *Eam* strains since only such variants are able to adsorb the phage. Tomas and Kay reached the same conclusion when explored the phage P1 lysogenic conversion of *Klebsiella.* They showed that its P1 lysogens as in the case of *E.coli* possess a reduced LPS molecule. But in contrast to *Escherichia* cured *Klebsiella* cells LPS form did not revert and remained reduced [18]. Presumably the tail fibers lability provided by Cin-invertase of *cix-cin* recombination system [11] allows phage to infect different parts of the strain population. It is possible that as a result of plasmid DNA interaction with host chromosome the properties of cell irreversibly change and the complete restoration of the original phenotype after plasmid loss is impossible. It was previously shown that susceptible to P1 cells were coincidently mutant in some cellular functions: for example, $P1^S$-cells of *S. typhymurium* carried *galE* mutations; among *K. aerogenes* cells susceptible to infection were *gal-bio* deletion mutants [8]. This phenomenon, however, along with the possibility of phage P1 *c*1gene expression in lysogens remains unclear.

Since we were unable to induce phage recovery from *Eho* and *Eam* cells apparently structural module is not expressed in these cells. Phage progeny also failed to obtain from lysogenic *P.carotovorum* cells [8], although this possibility was described for some bacteria (*Proteus, Enterobacter*) [13].

Thus among all functions encoded by prophage plasmid P1 the only ones effectively expressed in the system of *E. amylovora* and *E. "horticola"* cells are the genes of mobile genetic elements – trasposoneTn*9* (*cat*-gene) and RM-system EcoP1I. Expression of all other genes required for the functioning of prophage and phage progeny production under thermoinduction conditions is either insufficient or absent.

According to conventional conception the phage DNA is constructed from discrete modules. Obviously, their rates of autonomy differ and the obtained results indicate that most independent are the restriction-modification system along with transposon DNA. 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.

The obtained lysogenic strains allow for the exploration of restriction-modification gene complex EcoP1I interaction with polyvalent phages able to grow not only on *E. coli*, but also on such phytopathogens as *E. "horticola"* and *E. amylovora*.

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КОНВЕРСІЯ ФЕНОТИПУ ФІТОПАТОГЕННИХ БАКТЕРІЙ ВНАСЛІДОК ЇХ ЛІЗОГЕНІЗАЦІЇ КОЛІФАГОМ P1

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За допомогою бактеріофага P1Cm*c*1*ts*100 отримано набір лізогенних штамів фітопатогенних бактерій *Erwinia "horticola"* і *Erwinia amylovora,* асоційованих з деревними рослинами. Показано, що фагова конверсія фенотипу клітин з Cm^S на Cm^R пов'язана з привнесенням в клітини аутентичної профагової ДНК розміром 94,8 кб у вигляді малокопійної плазміди. Профаговий стан є нестабільним; плазміда P1 спонтанно втрачається клітинами з високою частотою. В лізогенізованих клітинах активно експресуються гени профагового комплексу рестрикції-модифікації III типу EcoP1I. Система, утворена *E. "horticola"* 450 і 60 та їх лізогенними похідними і специфічними бактеріофагами дозволяє поділяти останні на три групи за ступенем їх обмеження при взаємодії з ферментом EcoP1I. Різна відповідь фагів на присутність ендонуклеази в лізогенізованому хазяїні, ймовірно, корелює з кількістю сайтів розпізнавання для ферменту та доступністю сайтів адсорбції. Після виліковування від плазмідної профагової ДНК клітини змінюють показник фагочутливості.

Створені в даній роботі лізогенні штами дозволяють досліджувати взаємодію генного комплексу рестрикції-модифікації EcoP1I та полівалентних фагів, які здатні розвиватись не тільки на *E. coli*, але і на таких фітопатогенах, як *E. "horticola"* і *E. amylovora*.

Ключові слова: лізогенна конверсія, система рестрикції-модифікації III типу, полівалентні бактеріофаги, фітопатогени, *Erwinia amylovora.*

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КОНВЕРСИЯ ФЕНОТИПА ФИТОПАТОГЕННЫХ БАКТЕРИЙ ВСЛЕДСТВИЕ ИХ ЛИЗОГЕНИЗАЦИИ КОЛИФАГОМ P1

Р е з ю м е

С помощью бактериофага P1Cm*c*1*ts*100 получен набор лизогенных штаммов фитопатогенных бактерий *Erwinia "horticola"* и *Erwinia amylovora,* ассоциированных с древесными растениями. Показано, что фаговая конверсия фенотипа клеток от Cm^S к Cm^R связана с привнесением в клетки аутентичной профаговой ДНК размером 94,8 кб в виде низкокопийной плазмиды. Профаговое состояние является нестабильным; клетки спонтанно теряют плазмиду P1 с высокой частотой. В лизогенизированных клетках активно экспрессируются гены профагового комплекса рестрикции-модификации III типа EcoP1I. Сис-

тема, образованная штаммами *E. "horticola"* 450, 60, их лизогенными производными и специфическими батериофагами позволяет разделять последние на три группы по степени их ограничения при взаимодействии с ферментом EcoP1I. Разный ответ фагов на присутствие эндонуклеазы в лизогенизированном хозяине, скорее всего, коррелирует с количеством сайтов распознавания для фермента и доступностью сайтов адсорбции. После излечения от плазмидной профаговой ДНК клетки изменяют показатель фагочувствительности.

Созданные в данной работе лизогенные штаммы позволяют исследовать взаимодействие генного комплекса рестрикции-модификации EcoP1I и поливалентных фагов, способных развиваться не только на *E. coli*, но и на таких фитопатогенах как *E. "horticola"* и *E. amylovora*.

Ключевые слова: лизогенная конверсия, система рестрикции-модификации III типа, поливалентные бактериофаги, фитопатогены, *Erwinia amylovora.*

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