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Articles and Statements

Complementation and Recombination Tests between Phage T4brii-1272 Mutant and Related Wild-Type Zonne Phages

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Abstract

The investigation is concerned with the detection of the rII region in the phages, related to T-even, by means of some complementation and recombination tests with deletion mutant of phage T4BrII-1272. The restoration of the damaged function and the recombinants were detected by planting the progeny of reference and mutant strains of bacteria.

The analysis of the results obtained shows the ability of five phages out of the eight to restore the damaged function in deletion mutant of phage T4BrII-1272 (Zonne 2, Zonne 3 and Zonne 4 phages were not complement). The formation of recombinants was noted only with DDVI and Zonne 7 phages. The percentage of recombinants was 0.004 and 0.2 respectively.

Keywords: bacteriophages, recombinant phages, cistron, complementation, deletion mutant, phage T4BrII-1272, incubation efficiency, lysogenic, serological properties.

1. Introduction

To establish a genetic relationship between T-even phages, recombination analysis of population was successfully applied, which was generated by infecting common host cells (Hartl, Jones, 1998; Bohmer et al., 2010; Weaver, 2011; Kurtboke, 2012). It is noteworthy that recombinant phages may possess both parental properties, but it is not always possible to introduce the genes of one of the phages into the given genome (Laszlo et al., 2013). With the recombination, defined a degree of nonhomologous genes of T6 type phage groups was identified. They were separated from their natural habitats because they could not breed.

While working with a set of bacteriophages, it is difficult to prove, whether there are commonly identified properties for all bacterial infections in nature, or these qualities only characterize a definite group of phages.

The analysis of Benzer's complementation test (Benzer, 1957) with rII regions of the T4 phage helped to identify 2 cistrons (A and B) of this virus. In addition, the wild-type phage could restore both cistron's damaged functions.

Studies have shown (Chanishvili i dr., 1975) that both T4 phage and DDVI phage have rII-region (region/locus), which is also composed of 2 cistrons. A relatively simple method to identify the existence of rII regions in phages related to T–even phages can be a complemeting test of wild-type phages with T4BrII-1272 deletion mutant, which does not have both cistrons.

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The goal of the research was to detect rII regions with the complementation method in T-even phage-related wild-type zonne phages and with recombination testing of deletion mutant of phage T4BrII-1272.

2. Materials and methods

Bacteriophages: T2 and T4BrII-1272 Mutant phages, DDVI phage, Zonne 2 and Zonne 7 phages. Bacteria: *E. coli* B and *Sh. sonnei* 1188 strains were used to determine titers of phages, for crossbreeding and the analysis of the total number of progeny: E. coli B – with high titers in the complementation tests. Mutant clones – B / 2, B / 4, B / VI, II88 / 2, II88 / 4, II88 / VI, II88 / Zonne 2 – Zonne 7, K / 2, K / 4, K / VI, K // Zonne 2 – Zonne 7 – We used to analyze the progeny and calculate the share of the recombinants.

The quantitative complementation method is adopted from Jazikov et al. (Zhazykov i dr., 1970; Metody obshchei bakteriologii, 1984; Toth et al., 2013). The crossbreeding of phages was conducted in the following way: we added liquid culture of host bacterium to a mixture of two related phages. It was equally susceptible to both phages. The number of viable bacteria was determined with the photoelectric colorimeter. The infection rate equaled to 6 for every parental phage. After completing the time required for adsorption, we removed remained phage with a completely neutral dose of anti-phage serum, then we mixed the infected suspension with new broth and left at 30°C until the end of the lysis. The corresponding mixtures of lysate were incubated in etalon and mutant strains, allowing us to determine the production of parental and recombinant types of phages.

3. Results

We used the incubation method with indicator strains to determine the interdependence of different genotypes and populations (Harley, 2017). Table 1 presents the determining data of the effectiveness of bacterial viruses in etalon and mutant clones.

The presented material shows that all the clones of the phages are reproduced on *Sh. sonnei* 1188 and *E. coli* K-12 (λ) strains, the effectiveness of the incubation efficiency is insignificant (0,9-1,0). The exception is the T4BrII-1272 deletion mutant whose characteristic is only *E. coli* K-12 (λ phage lysogenic) adsorption properties on the strain without releasing the mature progeny. The efficiency of incubating bacteria in other sterile and mutant clones is 0,7-1,0. Phage Zonnes 2, 3, 4 are not reproduced on *E. coli* B (etalon and mutant) strains. Phage Zonne 5 is characterized by low efficiency in strains *E. coli* B and equals to 0,001.

Table 1. Comparative efficiency of phage incubation

Strains	Phages								
	T2	T4BrII-1272	DDVI	Zonne					
				2	3	4	5	6	7
E. coli B	1	1	1	-	-	-	0,001	1	1
E. coli K-12 (λ)	1	0	0,9	1	1	0,9	1	1	1
Sh. sonnei 1188	1	1	0,97	1	1	1	1	1	1
B/2	0	0	1	-	-	-	-	-	-
B/4	0	0	1	-	-	-	-	0,9	1
B/VI	0	0,9	0	-	-	-	-	-	-
1188/2	0	0	1	-	-	-	-	-	-
1188/4	0	0	1	0,8	0,95	-	-	0,9	0,9
1188/VI	-	1	0	-	-	-	-	-	-
1188/Zonne 2	-	1	-	0	0	0	0	-	-
1188/ Zonne 3	-	0,75	-	0	0	0	0	-	-

1188/ Zonne 4	-	1	-	0	0	0	0	-	-
1188/ Zonne 5	-	1	-	0	0	0	0	-	-
1188/ Zonne 6	-	0,75	-	-	-	-	-	0	-
1188/ Zonne 7	-	1	-	-	-	-	-	-	0
K/2	0	0	-	-	-	-	-	-	-
K/4	0	0	0	-	-	-	-	-	-
K/VI	-	0	0	-	-	-	-	-	-
K/ Zonne 2	-	0	-	0	0	0	0	-	-
K/ Zonne 3	-	0	-	0	0	0	0	-	-
K/ Zonne 4	-	0	-	0	0	0	0	-	-
K/ Zonne 5	-	0	-	0	0	0	0	-	-
K/ Zonne 6	-	0	-	-	-	-	-	0	-
K/ Zonne 7	-	0	-	-	-	-	-	-	0

Comparative analysis of the obtained results enables us to conclude that the strain *E. coli* B can not be used as bacteria-host for all phages. On the basis of the above mentioned we have used *Sh. sonnei* 1188 clone. The results of the test are presented in Table 2.

Table 2. Quantitative complementation between phages

Phage experimental solution	**The phage production calculated on single infected bacteria**	
	Wild type	**T4BrII-1272**
T2 and T4BrII-1272	40	54
DDVI და T4BrII-1272	100	52
Zonne 2 და T4BrII-1272	10	0
Zonne 3 და T4BrII-1272	17	0
Zonne 4 და T4BrII-1272	18	0
Zonne 5 და T4BrII-1272	46	11
Zonne 6 და T4BrII-1272	86,9	129
Zonne 7 და T4BrII-1272	60,6	75

Simultaneously infecting wild type and T4BrII-1272 deletion mutant phage of bacterial cells confirms that positive complementation was only in the following cases - T2 and T4BrII-1272 (control), DDVI and T4BrII-1272, Zonne 5 and T4BrII-1272, Zonne 6 and T4BrII- 1272, Zonne 7 and T4BrII-1272. This circumstance proves that the function of the rII region in T4 phage can be compensated by the region function of some wild-type phages. Complementation was not observed in the following cases – Zonne 2 and T4BrII-1272, Zonne 3 and T4BrII-1272, Zonne 4 and T4BrII-1272. It is also detected that some phages multiplied with deletion mutants (produce 10-18 particles in a cell).

A high range of phage mixture cross-breeding was conducted in Sh. sonnei 1188 cells by infecting; one of which was the T4BrII-1272 mutant, and the second- wild-type, and then analyzed the reproduction. We considered the particles as recombinants if they gave transparent negative colonies in reference to the wild type phages in *E. coli* K-12 sustainable mutant strain, as the genome of the recombinant phage should contain the locus which is responsible for rapid lysis – in a wild type of phage, and a locus responsible for the lysis spectrum – T4BrII-1272 phage. The results of the test are summarized in Table 3 with a mean value of 3-4 tests.

Table 3. Recombination between deletion Mutant T4BrII-1272 and wild type of zonne phage

Cross-breeded phages	Recombination, %
T2 and T4BrII-1272	0,12
DDVI and T4BrII-1272	0,004
Zonne 2 and T4BrII-1272	-
Zonne 3 and T4BrII-1272	-
Zonne 4 და T4BrII-1272	-
Zonne 5 და T4BrII-1272	-
Zonne 6 და T4BrII-1272	-
Zonne 7 და T4BrII-1272	0,2

The recombination between the phages was observed only in the following cases: T2 and T4BrII-1272 (control), DDVI and T4BrII-1272, Zonne 7 and T4BrII-1272. The percentage of the recombination rate was 0.004-0,12. Obtained recombinants were selected and incubated in a host strain and re-examined on the activity of lytic spectrum.

In other cases, crossbreeding was not observed. The low percentage of recombination between T2 and T4BrII-1272 phages may be attributed to characteristics of Sh. sonnei 1188 strain which was used for the tests, as the recombinant percentage of T2 and T4BrII-1272 increased up to 1,4 by the hybridization of these viruses in E. coli B strain.

Three types of reactions were revealed by quantitative complementing experimental analysis between T4BrII-1272 deletion mutant and morphologically identical and serologically relevant phage types:

1. T4BrII-1272 Function restoration in both phages during simultaneous reproduction (T2 and T4BrII-1272, DDVI and T4BrII-1272, Zonne 6 and T4BrII-1272, Zonne 7 and T4BrII-1272)

2. Function restoration of T4BrII-1272 during partial inhibition of its reproduction (Zonne 5 and T4BrII-1272);

3. No complementation (Zonne 2 and T4BrII-1272, Zonne 3 and T4BrII-1272, Zonne 4 and T4BrII-1272).

The system of phage incubation developed by us, after the compound infection of the cells, in the phage-resistant wild type *E. coli* K-12 (λ) mutant strains, in which the wild clone under the study in normal conditions is not adsorbed, and the deletion mutant is not producible, enabled us the possibility to discern quite rare recombinants, that bear rII region from the wild type phage, while h locus – from T4B phage. Taking into account the final solutions, we could propose even isolation of recombinant units. The use of this system is justified by the fact that none of the phages under the study had corresponding h locus of T4 phage, whereby resistant mutants were simultaneously exposed to lysis.

As the results of the studies have shown, only in case of cross-breeding of the following phages T2 and T4BrII-1272, DDVI and T4BrII-1272, Zonne 7 and T4BrII-1272, recombinants were produced, whose stability was established in the next generations. The frequency of appearance of recombinant, as well as in the case of T-even crossbreeding, depends on the binding quality of genes. The greater the distance between the genes, the greater the likelihood of cross-binding between them and the higher the share of recombinant generations.

4. Conclusion

The study of the correlation between the restoration of function and recombination between viruses used in the tests shows that when the phage can restore the damaged function of the rII region, not in all cases cross-breeding is possible. It is not always possible to establish complementation, recombination, and relationship between biological signs (capsid's structure, serological properties, etc.).

Thus, three types of reactions identified between selected 8 phages and the genomes of T4BrII-1272 deletion mutant indicate the existence of different degrees of relations between these bacterial viruses that were developed due to evolution.

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