RESTRICTION OF PROPAGATION OF Escherichia coli O_{111} PHAGE BY ENTEROPATHOGENIC INTESTINAL BACTERIA

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Of 105 strains of enteropathogenic intestinal bacteria, 25 partially or completely restricted reproduction of the phage of Escherichia coli O_{111}. Adsorption of phage on cells of the limiting strains was understood. An increase in the multiplicity of infection, and also heating of the cultures to 50°C before phage infection, partially abolished the restrictive action of some strains. Streptomycin-resistant cells in a population of limiting strains has a much weaker restrictive action on phage propagation.

The effectiveness of seeding the same bacteriophage on different strains of bacterial hosts may differ essentially. As well as strains giving a high phage yield, other strains are found which limit its reproduction, manifested as low effectiveness of seeding or as the total absence of phage propagation. In some cases the process of limitation of phage reproduction is accompanied by modifications of the spectrum of lytic activity, which are controlled by the host and are phenotypic in character. The processes of limitation have been described for lambda [4], salmonella [9], staphylococcal [3,8], T-even [5,7], and T_1 [6] phages.

In the present investigation, the process of limitation of phage reproduction was studied in a system of enterophage O_{111} and its hosts – enteropathogenic types of intestinal bacteria.

EXPERIMENTAL METHOD

Enteropathogenic intestinal bacteria with the antigenic formula $O_{111}:B_4$ and $O_{26}:B_6$, isolated from infected children, were studied. A museum strain was used as the reference strain of host for the phage O_{111}. The ability of the bacteria to restrict propagation of phage O_{111} was determined from the effectiveness of seeding on the test strains. The number of negative colonies on a plate culture of the reference strain was taken as 100%. Adsorption of phage was studied by the usual method [1]. The lysogenicity of the test strains was determined by seeding the supernatant of 18-h broth cultures on indicator strains without ultraviolet induction. Colicinogenicity was determined on indicator strains φ and K_{12}.

EXPERIMENTAL RESULTS

To detect intestinal bacteria limiting reproduction of the phage of Escherichia coli O_{111}, 105 strains of enteropathogenic serotypes $O_{111}:B_4$ and $O_{26}:B_6$ were investigated. As a result of the experiments, the strains could be divided into three groups (Table 1).

Restriction of phage propagation by bacterial strains of all three groups was unconnected with adsorption because phage was adsorbed equally on strains completely restricting its reproduction and on those which did not restrict reproduction.

The results demonstrate that, just as with other phages, the reason for limitation in these experiments must be either a disturbance of the injection of phage DNA into cells of the limiting strains or destruction of the DNA after its penetration into these cells [2].

To examine this problem, 14 completely limited strains were infected with phage with a multiplicity of 10, i.e., a hundred times greater than in the previous experiments. The results showed that under these conditions reproduction of phage took place in the cells of 11 strains with a yield of $1 \cdot 10^1$-$1 \cdot 10^3$ particles/ml (effectiveness of seeding 0.0001-0.001%).

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TABLE 1. Effectiveness of Seeding and Adsorption of Phage O_{111} on Different Bacterial Hosts

Character of strain	Number of strains	Effectiveness of seeding (in %)	Phage titers	Adsorption in (%)
Nonrestrictive	80	100	10^9	87—90
Partially restrictive	9	0,0001—0,001	10^3—10^5	86—90
Totally restrictive	16	0	0	87—90

Note. Adsorption of phages was investigated on three nonrestrictive, five partially restrictive, and eight completely restrictive strains in relation to propagation of phage O_{111}. Multiplicity of infection in these experiments was 0.1.

TABLE 2. Reproduction of Phage E. coli O_{111} in Cells of Limiting Strains and Streptomycin-Resistant (Str^r) Clones Isolated from Them

Strain No.	Initial strain		Str^r strains	
	Phage titers	Multiplicity of infection	Phage titers	Multiplicity of infection
13	$8{,}0 \cdot 10^4$	0,1	$1{,}6 \cdot 10^6$	0,1
67	$6{,}0 \cdot 10^1$	10	$2{,}1 \cdot 10^8$	0,1
23	0	10	$3{,}0 \cdot 10^6$	0,1
62	0	10	$3{,}2 \cdot 10^5$	0,1
56	0	10	$2{,}0 \cdot 10^4$	0,1
34	$2{,}4 \cdot 10^5$	0,1	$7{,}6 \cdot 10^5$	0,1
45	$1{,}5 \cdot 10^4$	0,1	$1{,}4 \cdot 10^4$	0,1
68	0	10	0	10
81	0	10	0	10

Note. Adsorption of phage on all strains was 80-90%.

The possibility of overcoming the limiting properties of the strains by heating them or by inactivation of corresponding enzymes was next studied. The test strains grown in broth for 18 h were heated on a water bath for 10 min at 50°. After infection of 6 of the 13 heated strains, partial abolition of their limiting properties was observed. The phage titers were increased by from 4 to 100 times depending on the bacterial strain. However, the phage titer on none of the investigated heated strains reached values which were obtained by infection of unrestricted strains.

When the heated bacteria were cultivated for more than 3-4 h, they regained their limiting properties.

It can thus be postulated that heating the bacterial culture caused inactivation of a cell enzyme or enzymes responsible for destruction of the phage DNA. However, this inactivation was incomplete.

Besides the effect of temperature, that of streptomycin was studied, cells of two limiting strains of E. coli being grown in broth containing streptomycin in a concentration of 20 units/ml. This concentration of the antibiotic did not affect the development of these strains. After 18 h the bacterial cultures were infected with phage and plated out by the 2-layer method. The results showed that under these conditions the limiting properties of the test strains were unchanged.

Pursuing the same purpose, in the next series of experiments cells from nine limiting strains resistant to streptomycin in a concentration of 1000 units/ml were isolated. The percentage of such cells in a mixed population was 0.001-0.004. The original and the streptomycin-resistant strains were then infected with phage O_{111} in different multiplicities and plated out by the 2-layer method to determine the yield of phage particles (Table 2).

It will be clear from Table 2 that reproduction of phage took place in the cells of five Str^r clones, accompanied by an increase of titers by 2-8 orders by comparison with the original strain. Strain Nos. 67, 23, 66, and 56, completely limiting phage reproduction even when infected with high multiplicity, contained Str^r cells not limiting or only partially limiting the reproduction of phage O_{111}. However, the limiting properties of the bacteria could not be associated in every case with streptomycin resistant (Strain Nos. 68, 81). No definite conclusions can be drawn from these results, although they are indicative that the activity of the hypothetical limiting enzyme in streptomycin-resistant bacteria may be partially or completely inhibited.

Tests were also carried out on 14 limiting and 5 nonlimiting strains of E. coli for their content of prophages and colicins. The results of these experiments showed that all the strains were lysogenic and 14 of them were colicinogenic. During delysogenization, one of the test strains partially lost its limiting properties. Certain prophages may evidently play an essential role in the limitation of phage reproduction, a matter requiring further study. The ability of the cells to limit phage reproduction could not be associated with the presence of colicins in them.

LITERATURE CITED

1. M. Adams, Bacteriophages [Russian translation], Moscow (1961).
2. W. Arber and D. Dussoix, J. Molec. Biol., 5, 18 (1962).
3. E. Ashesnov and M. P. Jevons, J. Gen. Microbiol., 31, 97 (1963).
4. G. Bertani and J. J. Weigle, J. Bact., 65, 113 (1953).
5. S. Hattman, Virology, 24, 333 (1964).
6. A. Klein, Z. Vererbungsl., 96, 346 (1965).
7. S. E. Luria and M. L. Human, J. Bact., 64, 557 (1952).
8. D. J. Ralston and B. S. Baer, J. Gen. Microbiol., 36, 1 (1964).
9. H. Uetake, S. Toyama, and S. Hagiwara, Virology, 22, 202 (1964).