FREQUENCY OF PHAGE-RESISTANT MUTATIONS AND EFFECT OF BACTERIOPHAGE ON THE FORMATION OF RESISTANT FORMS OF Vibrio cholerae

A. G. Somova

UDC 576.851.315.095.57.095.38:576.858.9

It is a well known fact that phage-resistant mutants or secondary cultures of Vibrio cholerae may be formed by the action of certain types of homologous bacteriophages on a bacterial population. According to the author's findings the number of phage-resistant colonies depends on the properties of the strains of V. cholerae and the lytic activity of the type and series of bacteriophages [2].

As evidence that the mutation process is independent of the action of bacteriophage, the fluctuation test of Luria and Delbrück and the results of the investigations of J. and E. Lederberg [3, 4], demonstrating the pre-existence of phage-resistant cells in a population of bacteria before contact with bacteriophage, are usually mentioned.

The object of the present investigation was to determine the frequency (the probability) of mutations from phage-sensitivity to phage-resistance and to establish the role of bacteriophage in the process of formation of phage-resistant forms of V. cholerae.

EXPERIMENTAL METHOD

Sixty typical strains of V. cholerae, stored for different periods from the time of isolation, and of different virulence, were investigated. The behavior of the V. cholerae cells to bacteriophages of types C and D, and Mukerjee type 1, contained in commercial batches of polyvalent cholera bacteriophage, and to a polyvalent bacteriophage was studied. The primary selection of bacteriophage-sensitive strains was carried out by seeding 0.1 ml of a 24 h broth culture of the test strain on to the surface of three agar plates on which 0.1 ml bacteriophage had been first applied, and thoroughly dried. The appearance of phage-resistant colonies on one or more plates indicated high resistance to bacteriophage. These combinations of bacteriophage and strain of V. cholerae were not subsequently used in the experiments. Combinations of bacteriophage and strain with which growth of phage-resistant colonies was not obtained on 3 plates also often failed to yield mutant colonies in the subsequent investigated in a series of 25 parallel cultures. The frequency of mutations from phage-sensitivity to phage-resistance was determined by the first method of Luria and Delbrück as modified by Newcombe [5], in accordance with the formula:

$$a = -(\ln 2)\cdot\left(\frac{\ln \cdot P_0}{N}\right),$$

where a is the frequency of mutations per cycle of division of one bacterial cell, P_0 the proportion of cultures not having phage-resistant colonies to the number of cultures investigated, and N the mean number of bacteria in the culture at the end of growth on the agar plate without phage. The cell composition of the initial sensitive and phage-resistant mutants of V. cholerae was studied by means of the impression method [3].

EXPERIMENTAL RESULTS

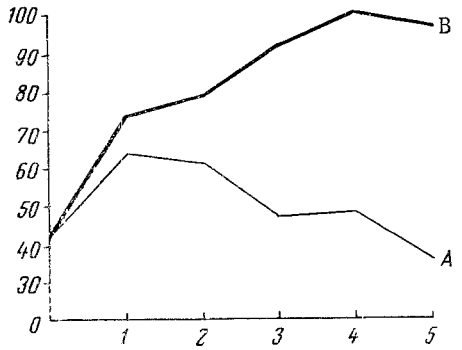
The mean results of experiments with three repetitions to determine the sensitivity of 60 strains of V. cholerae to type-specific and polyvalent cholera bacteriophages are given in Table 1.

It is clear from Table 2 that the frequency of mutations of phage-resistance in V. cholerae to type-specific and polyvalent bacteriophages is low, of the order of tenths and hundredths of the number of original cells on the plates without bacteriophage. Sensitivity to bacteriophage did not determine capacity of mutation of phage-resistance, but was evidently responsible for certain other properties of individual strains and bacteriophages.

Rostov-on-Don Plague Research Institute (Presented by Active Member of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 11, pp. 99-103, November, 1966. Original article submitted March 29, 1965.

TABLE 1. Primary Selection of Strains of V. cholerae Sensitive to Type-Specific and Polyvalent Bacteriophages

Type of phage	Number of phage-resistant colonies		
	0	1—50	Over 50
	Number of strains		
Polyvalent RPChI, batch 10. .	54	6	0
Mukerjee type 1 .	46	11	3
C	38	14	8
D	16	18	26



Mean results of fluctuations of sensitivity to type D bacteriophage in a population of V. cholerae strain No. 1601 during passage without bacteriophage and in its presence (investigation by the impression method after J. and E. Lederberg). Along the axis of ordinates—percentage of phage-resistant colonies; along the axis of abscissas—subcultures. Passages in broth: A) without phage; B) with a sublytic dose of bacteriophage.

For instance, it was impossible to find phage-resistant mutants of strains numbers 1082 and 1601 with a high degree of sensitivity to type C bacteriophage, or strain No. 1766 to Mukerjee type 1 bacteriophage. These same strains gave no secondary growth with polyvalent bacteriophage. Consequently, the frequency of mutations from phage-sensitivity to phage-resistance could be detected by means of the first method of Luria and Delbrück in V. cholerae cells, with the investigation of 25 parallel cultures, in a limited number of strains only.

The method of Luria and Delbrück is regarded as an example of the statistical approach to the solution of a biological problem [1]. While noting the validity of use of the Luria and Delbrück fluctuation test in the system bacteriophage—V. cholerae, it must be stated that the detection of phage-resistant mutants in parallel cultures is carried out by means of bacteriophage. However, the supporters of the mutation theory, despite the obvious fact that bacteriophage plays a principal role in the process of formation of phage-resistant forms, deny such an influence.

To discover the role of bacteriophage in the process of formation of phage-resistant forms of V. cholerae, strains of V. cholerae sensitive to type D bacteriophage were investigated by the impression method. Between 760 and 1840 colonies of each strain were studied. As Table 3 shows, all the strains were of heterogenous cell composition relative to type D bacteriophage. Besides sensitive cells, these strains contained from 7 to 84.7% of phage-resistant cells.

Phage-resistant D-mutants obtained from these strains of V. cholerae by seeding on agar plates soaked with bacteriophages of type D and C and Mukerjee type 1 were investigated in the same way. It must be pointed out that before the cell composition was studied the phage-resistant mutants were kept in a lyophilized state for between 8 months and 1 year 2 months. During investigation of between 770 and 1560 colonies of phage-resistant D-mutants by the impression method it was found that they showed a fairly homogeneous cell composition of the V. cholerae cells resistant to type D bacteriophage. The exceptions were three mutants of strains Nos. 1606-D, 1664-D, and 1668-D, in which 90.9-81.4% of phage-resistant cells respectively were found.

The cell composition of the population of the original strains and the phage-resistant mutants to type D phage relative to the other types of bacteriophage (C and Mukerjee type 1) was heterogeneous and no regular changes were observed in the mutants.

The final stage of the investigation was to study the changes in the composition of the population by the impression method in subcultures of V. cholerae, strain No. 1601 during passage in Martin's broth without bacteriophage, and in a parallel series in the presence of sublytic concentrations of type D bacteriophage. The mean results of an experiment with three repetitions after appropriate analysis are shown in the figure. Passages of subcultures of strain No. 1601 in broth without bacteriophage were accompanied by the usual fluctuations in the composition of cells sensitive and resistant to bacteriophage D typical of this process (42.2% of phage-resistant cells in the original culture and 36% in the 5th subculture. Passages in the presence of bacteriophage were accompanied by a regular decrease in the sensitivity of the population to the homologous type of bacteriophage (42.2% of phage-resistant cells in the original culture, 100% in the 4th subculture, 97.1% in the 5th subculture).

The existence of a definite heterogeneity of this particular population in relation to sensitivity to bacteriophage is firmly established, and under the influence of bacteriophage selection of phage-resistant cells takes place.

TABLE 2. Frequency of Mutations of V. cholerae from Phage-Sensitivity to Phage-Resistance to Type-Specific and Polyvalent Bacteriophages Calculated by the First Method of Luria and Delbrück as Modified by Newcombe

Type of bacteriophage	Strain no. of V. cholerae	Area of parallel cultures												
		1	2	3	4	5	6	7	8	9	10	11	12	13
		No. of phage-resistant colonies												
C	1128	71	0	44	0	118	47	8	0	0	0	4	16	0
C	1408	0	0	0	0	0	34	17	84	71	69	118	160	4
C	1766	0	0	0	44	5	6	11	21	7	4	3	102	90
D	1082	9	15	24	0	92	46	22	18	16	20	27	42	32
D	1766	16	21	0	72	52	0	0	16	10	6	28	14	27
Mukerjee type 1	1082	6	0	15	0	0	0	38	0	0	0	0	0	0
"	1128	0	0	49	0	0	0	0	4	0	0	0	71	0
"	1408	26	0	184	201	0	164	112	78	69	27	57	48	37
Polyvalent, RPChI, batch 10	1128	112	0	0	88	0	0	0	0	0	0	42	0	36
	1408	45	0	24	16	33	0	78	112	0	200	40	0	71

Type of bacteriophage	Strain no. of V. cholerae	Area of parallel cultures												$N \cdot 10^8$	P_0	$a \cdot 10^{-8}$
		14	15	16	17	18	19	20	21	22	23	24	25			
		No. of phage-resistant colonies														
C	1128	36	29	19	15	10	0	8	82	111	186	14	6	1,33	0,28	0,66
C	1408	9	44	26	30	17	11	51	3	2	3	15	3	2,1	0,2	0,52
C	1766	84	27	3	16	19	25	17	42	54	22	18	16	1,8	0,12	0,06
D	1082	12	0	5	1	31	19	0	0	102	17	0	0	1,7	0,24	0,58
D	1766	46	0	0	12	29	4	102	48	32	25	40	6	1,3	0,2	0,75
Mukerjee type 1	1082	0	14	0	0	25	0	0	0	0	0	0	0	1,46	0,8	0,1
"	1128	16	22	9	0	0	0	0	3	0	0	0	0	2,7	0,7	0,08
"	1408	24	14	82	60	27	51	0	17	20	24	0	21	1,6	0,16	0,73
Polyvalent, RPChI, batch 10	1128	0	110	0	144	115	39	81	17	0	29	15	0	1,47	0,52	0,3
	1408	6	0	0	0	3	15	21	19	0	0	25	34	1,6	0,36	0,44

TABLE 3. Cell Composition of Population of Original Sensitive Strains and of Phage-Resistant Mutants of V. cholerae, Relative to Type D Bacteriophage

No. of original phage-sensitive strain	No. of colonies investigated		No. of phage-resistant mutants	No. of colonies investigated	
	total	phage-resistant (in %)		total	phage-resistant (in %)
438	760	30,2	1—438-D	870	100
1395	920	18,4	4—1395-D	1210	100
1399	880	42	7—1399-D	820	100
1601	1230	42,2	11—1601-D	1165	100
1668	1510	23,8	14—1668-D	1120	96,4
1082	1112	15,3	17—1082-D	1450	100
1600	811	17,2	20—1600-D	856	100
1599	980	12,2	21—1599-D	910	100
1606	822	24,3	27—1606-D	770	90,9
1666	793	21,5	43—1666-D	845	100
1128	1840	23,9	49—1128-D	962	100
1391	1540	12,3	53—1391-D	1270	100
1382	1190	26	56—1382-D	1064	100
1403	777	15,5	58—1403-D	1423	100
1604	850	7	62—1604-D	1243	100
1664	870	27,5	67—1664-D	1560	81,4
1126	990	21,1	88—1126-D	946	100
1758	1440	84,7	154—1758-D	1031	100

LITERATURE CITED

1. S. E. Bresler, Introduction to Molecular Biology [in Russian], Moscow—Leningrad (1963), p. 300.
2. A. G. Somova, Byull. éksp. Biol., No. 1, 75 (1965).
3. J. Lederberg and E. M. Lederberg, J. Bact., Vol. 63 (1952), p. 309.
4. S. E. Luria and M. Delbrück, Genetics, Vol. 28 (1943), p. 491.
5. H. B. Newcombe, Genetics, Vol. 33 (1948), p. 447.