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BIOLOGICAL CHARACTERISTICS OF PLAGUE BACTERIOPHAGES

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BIOLOGICAL CHARACTERISTICS OF PLAGUE BACTERIOPHAGES

[Following is the translation of an article by M. A. Shashayev, Central Asia Scientific Antiplague Research Institute (Alma-Ata), published in the Russian-language periodical Zhurnal Mikrobiologii Epidemiologii i Immunobiologii (Journal of Microbiology Epidemiology and Immunobiology), #12, 1964, pages 32-35. It was submitted to the editors on June 14, 1963. Translation performed by Sp/4 Richard M. Koplen.]

Certain biological properties of plague bacteriophages were studied by Yershov and Bikova (1962, 1963) and Shashayev (1963). Results are presented in the present report of a study of the morphology of negative colonies, serological properties, sorption, latent period, productivity, and sensitivity to X-rays of 13 plague phages.

The phages which were subjected to study were isolated in 1920--1956 from various sources and places: 9 phages -- in natural foci of plague in the Soviet Union, 3--in the Mongolian Peoples Republic and 1--in South Vietnam (table 1).

Each phage of an individual negative colony multiplied on a virulent strain of plague microbe No. 319. Seedings were made on Hottinger's broth with a pH of 7.3--7.4 and 54--58 mg. % residual amino nitrogen. The morphology of negative colonies was studied by the two-layer method on Hottinger's meat peptone agar with a pH of 7.1. Twenty-two hours after incubation at 28°C on plague microbe strain No. 257, the phages formed large negative colonies of a regular round form with a transparent center and a zone of incomplete lysis. The negative colonies were 10--11 mm. in diameter.

Serological properties of phages were studied in cross-neutralization reactions with homologous and heterologous antiphage sera according to Adams, with the calculation of the speed constant of phage neutralization varying from 1 to 34.6 min.⁻¹ (table 2). It is known that the value of the constant during the cross neutralization reaction serves as evidence of the serological relationship between phages. Proceeding from the value of the constants, which were obtained in our tests, we attributed all 13 phages studied to one serological group.

Sorbition was studied by the method of numerical determination of infected bacteria (Adams, 1961), and the reproduction and productivity of a phage -- in tests of a single cycle of phage multiplication by the method of Ellis and Delbryuk (Cited by Adams, 1961). The tests were conducted at 28° with a broth culture of plague microbe No. 257, containing $5 \cdot 10^7$ microbial cells in 1 ml., and with a corresponding phage, containing $5 \cdot 10^7$ phage particles in 1 ml., with a multiplicity of infection equal to 0.1.

For determination of the sensitivity to X-rays, extracellular plague phages were exposed to the RVM-7 apparatus with a 0.09 mm. aluminum filter, at 50 kv., 10 ma., with focal length equal to 75 mm. and at a power of 11,000 roentgens per minute. The plague phages with a concentration which contained 10^7 phage particles in 1 ml. were exposed to 220,000 roentgens. The data received was expressed in inactivating doses, that is in the doses necessary for reducing the titer of the phage population by a specific number of times ("e"). The strain of plague microbe No. 257 served as the indicator strain.

The investigations showed (table 3) that on P. pestis No. 257 in the course of 5 minutes, 21.8 -- 57.5% of the phage was sorbed, and in the course of 12 minutes -- 27 -- 83.4%. The duration of the minimum latent period was 22 -- 28 min. The average yield on one infected bacterial cell was 51 -- 148 phage particles.

Based on the rate of sorbition and the yield of phage particles on one infected bacterial cell, it is possible to divide the plague phages into two groups. The sorbition ability and productivity of phages of Pokrovskoy, Osolinkera, Marinoy, Ivanova, No. 67, 1497, 1048, 210, 2938, which were isolated in plague foci of the Soviet Union, and d'Erellya (South Vietnam) was somewhat higher than foci of Berlina, Mikhalevoy, and No 57 phages, which were isolated in the Mongolian Peoples Republic.

Based on sensitivity of plague phages to X-rays, it is also possible to divide them into two groups: Phages No. 210, 2938, 1497, 67, Marinoy, Osolinkera, and d'Erellya proved to be more stable, and phages of Pokrovskoy, Berlina, Mikhalevoy, Ivanova, No 57 and 1048 -- less stable.

Literature

- a. Yershov, F. I., Bykova, Z. A., Zh. Mikrobiol., 1963, No. 4, page 131.
- b. Idem, Zh. Mikrobiol., 1962, No. 4, page 121.
- c. Shashayev, M. A., In the book: Materials of the Scientific Conference Concerning the Natural Focalness and Prophylaxis of Plague, Alma-Ata, 1963, page 254.
- d. Adams M., Bacteriophages, Moscow, 1961, pages 406.

Certain Record Data of Plague Phages

Phage	When and by whom isolated	Place and source of isolation
D'Erellya	1920, D'Erellya	Province of Nambo (Cochin China), vicinity of Fan-Tiet; from rat feces.
Pokrovskoy	1929, M. P. Pokrovskoy	Salskiy Orrug, Tsimlyanskiy Rayon (from the organs of a dead suslik).
Berlina	1932, A. L. Berlina	Mongolian People's Republic (Central Aimak), from the bone marrow of a dead tarbagan.
Osolinkera	1936, B. Ye. Osolinkera	Kalmytskaya ASSR, Dolbanskiy Rayon, from the organs of a suslik.
Mar'inoy	1938, Yu. N. Mar'inoy	Rostovskaya Oblast, Zimovnik village, from suslik No 5026.
Mikhalevoy	1944, V. L. Mikhalevoy	Mongolian People's Republic, Dzak-Samon, from the organs of a dead Mongolian mouse hare.
Ivanova	1945, I. Kh. Ivanova	Guryevskaya Oblast, Makatskiy Rayon, from a strain of plague microbe.
No 67	1950	Zabaykal, from a strain of plague microbe.
No 1497	1955, T. I. Filimonova	Kzyl-Ordinskaya Oblast, from a strain of plague microbe isolated from a great gerbil.
No 57	1956, R. V. Kovaleva	Mongolian People's Republic, Bayan-Khongorskiy Aimak, Bayan-Bulak Soman, from the spleen of a biotest animal, infected with the organs of a tarbagan.
No 1048	1956, T. I. Filimonova	Kzyl-Ordinskaya Oblast, from plague microbe strain No 1048, isolated from a Ceratophyllus laeviceps flea, taken from the wool of a red-tailed gerbil.
No 2938	1956, T. I. Filimonova	Kzyl-Ordinskaya Oblast, from plague microbe strain No 2938, isolated from a red-tailed gerbil.
No 210	1956, T. I. Filimonova	Kzyl-Ordinskaya Oblast, from a plague microbe strain No 210, isolated from the organs of a great gerbil.

Table 2

Rate constants (in min.^{-1}) in the cross neutralization reaction of phages with antiphage sera

Phage	Antiphage serum												
	d'Erel'lya	Pokrovskoy	Berlina	Osolin'kera	Mar' inoy	Mikhail' evoy	Ivan ova	No 67	No 1497	No 57	No 1048	No 2938	No 210
D'Erel'lya	17.7	6.4	20.7	9.9	13.8	4.6	4.1	8.5	3.7	3.5	3.7	12.7	11.9
Pokrovskoy.....	10.4	7.8	21.8	5.7	9.7	5.0	5.0	8.7	2.6	5.1	7.4	13.0	13.1
Berlina.....	11.8	4.3	34.6	9.6	10.6	6.3	4.2	8.3	4.8	4.7	7.3	16.9	16.6
Osolin'kera.....	16.4	7.6	22.7	14.5	9.7	4.8	5.2	8.4	4.2	5.9	5.1	13.2	12.8
Mar' inoy.....	16.5	7.7	26.3	3.3	16.3	5.8	5.5	8.2	3.1	3.4	7.1	14.0	16.4
Mikhalevov.....	11.3	4.8	16.1	5.7	11.5	12.0	2.7	7.3	4.2	3.9	4.6	16.7	9.9
Ivanova.....	11.0	5.0	14.1	5.0	11.0	5.4	13.3	8.6	3.6	3.5	3.2	9.3	12.2
No 67.....	11.1	4.1	12.7	8.5	10.0	3.7	9.4	16.4	3.1	2.3	2.7	1.0	12.7
No 1497.....	13.3	5.0	22.2	12.5	11.0	4.8	7.8	8.3	14.1	3.5	5.0	15.8	17.2
No 57.....	9.4	5.4	18.0	10.8	4.8	4.6	9.7	7.1	4.6	9.3	4.0	18.0	15.0
No 1048.....	10.8	6.2	15.6	10.8	12.3	5.6	3.4	8.1	3.2	4	12.6	13.6	15.1
No 2938.....	10.8	6.5	30.2	3.2	10.0	4.9	3.2	5.3	3.9	3.6	6.5	24.5	14.6
No 210	10.8	5.9	14.0	5.7	11.4	6.3	9.8	5.4	4.6	3.6	9.8	16.8	18.0

Results of determining sorbtion, reproduction, productivity and sensitivity to x-rays of plague phages.

Phage	Sorbition of phages (in %) after contact over a period of		Duration of minimum latent period (in min.)	Average number of phage particles on one cell	Sensitivity of phages to the action of x- rays (in inactivating doses)
	5 min.	12 min.			
D' Erellya	53.1	83.4	25--26	117	0.29
Pokrovskoy	46.2	58.7	24--25	147	0.16
Berlina	30.2	45.5	26--27	54	0.18
Osolinkera	57.5	81.4	26--28	138	0.37
Mar' inoy	38.2	56.9	24--26	138	0.28
Mikhalevoy	28.4	40.4	26--28	61	0.2
Ivanova	48.5	73.2	24--26	135	0.2
No 67	42	51.8	27--28	124	0.32
No 1497	39.6	50.4	22--24	124	0.29
No 57	21.8	27	27--28	51	0.17
No 1048	40.4	47.7	27--28	125	0.18
No 2938	48.8	75.3	26--28	101	0.37
No 210	39.2	62	26--27	130	0.26