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Phage Multiplication on Two Hosts. Isolation and Activity of Variants of Staphylococcus Phage P_1.* (19575)

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By simultaneously titrating phage on two susceptible hosts, the authors have been able to detect and isolate variants from a stock staphylococcus phage P_1. The dual host assay system has provided a sensitive means of studying certain unusual activities of one of the phage variants.

Experimental procedures and results. I. *Isolation of phage variants from stock P_1.* When aliquots of stock P_1, prepared on host strain Staphylococcus K_1 in tryptose-phosphate both, were titrated by plaque count(1) on two hosts—strains K_1 and WF 145‡—a slightly but consistently higher titration value was found with the latter. This difference could not be ascribed to rates of adsorption, latent periods, or burst sizes of the phage for the two hosts. Stock phage P_1 was then produced on strain 145 in trypticase soy broth. At intervals aliquot samples were titrated on the two hosts. The ratio of titer per ml on 145 to titer per ml on strain K_1 (hereafter designated $145/K_1$) was found to increase to approximately 4 during the growth period of the phage. Plaque isolates were made from samples exhibiting this high ratio. Virus from plaques on strain 145 was suspended in saline and aliquots plated on the two hosts. Four isolates of 32 exhibited distinct differences in titration ratio $145/K_1$, and, in 2 cases, formed small sized plaques on K_1 cells. Growth in broth on 145 cells resulted in characteristic curves of phage titratable on the two hosts. Two variants, No. 15 and 16, showed very similar curves and titration ratios $145/K_1$ = ca 5. Since they also formed small plaques on K_1 cells, they were considered identical isolates. One variant, No. 5, exhibited a growth curve much like that of stock phage and proved to have a titration ratio of 1. The lysate of the fourth, phage 14, exhibited a ratio $145/K_1$ = ca 40. This variant was selected for detailed investigation to determine the factors involved in producing this high ratio.

II. *Observations on phage 14.* A. *Attempted strain separation by plaque isolation.* The high plaque ratio $145/K_1$ of P_{14} lysates of 145 cells pointed to a possible mixture of virus strains, one of which produces no plaques on K_1 assay cells. A series of 10 successive serial passages of P_{14} on host K_1 was made by plating saline suspensions of virus from individual plaques. On the tenth transfer the saline suspension of virus assayed on the 2 hosts in a ratio $145/K_1$ = 1.3. A similar

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TABLE I. Effect of Host Passage on Titration Ratio $145/K_1$ of Phage Variants.

Variant No.	Plaque formation on K_1 cells	Avg value $R145/K_1$ of virus suspended from plaques, after		
		10 passages by plaque transfer on		1st transfer from K_1 to Host 145
		Host K_1	Host 145	
5	Indistinguishable from Stock P_1	1	1	
14	"	1.3	40	40
15	Tiny	1	7	

series of 10 passages was carried out on strain 145. (Hereafter, P_{14} which has been carried on K_1 cells will be referred to as P_{14} (K_1), and that passaged on strain 145 as $P_{14}(145)$. After 10 serial passages, virus from plaques on strain 145 assayed in a high ratio, suggesting that a separation of at least 2 strains had been made. However, when virus which had been carried on K_1 cells was transferred back to host 145, it produced plaques whose contents assayed again in a high ratio. This return to a high ratio occurred in the first passage and showed that no separation had been achieved. Two other variants, No. 5 and 15, were also passaged on each host. One of them, phage 5, maintained a ratio of one independent of the host used for passage, indicating that the shift in ratio occurring with P_{14} is a property peculiar to this variant. Representative data are summarized in Table I.

B. *Titration ratios of P_{14} in adsorption and multiplication studies.* 1) *Production in broth on 2 hosts.* P_{14} infections of log phase 145 cells in Broth III (3% tryptose phosphate, 3% trypticase soy, and 0.5% yeast extract) invariably produced a clear lysate with a high ratio $145/K_1$, whereas on K_1 cells, the phage in the lysate assayed in a ratio approaching one. This occurred regardless of which host the phage had previously been passaged on. The ratios are characteristic for assays of free phage and correspond to similar assays of saline suspensions of virus obtained from plaques in the same medium.

2) *Adsorption on two hosts.* Phage 14 was adsorbed on both 145 and K_1 cells in broth III for 20 minutes at 37°C, in a ratio of phage/cells = ½. At the end of this period, samples were centrifuged and resuspended to volume in broth III, to collect infected cells; and were filtered through super cell pads, to obtain the unadsorbed free phage. Dual assays were run on 1) total phage, 2) infected cells, and 3) free phage. As summarized in Table II, when $P_{14}(K_1)$ was added to K_1 cells or to 145 cells, 98-99% of the phage was absorbed in 20 minutes. In both cases, the residual free phage assayed in a ratio $145/K_1 = 1$. The infected cells (both K_1 and 145) also assayed in a ratio of 1, and the absolute titer corresponded to the value of the total free phage on each host. When $P_{14}(145)$ was added to K_1 or to 145 cells, about 99% of the phage was adsorbed in 20 minutes. In both cases ($P_{14}(145)$ plus K_1 cells and $P_{14}(145)$ plus 145 cells), the residual free phage assayed in the high ratio characteristic of dual assays of the total free phage. The infected K_1 and 145 cells showed assay ratios $145/K_1 = 1$ when aliquots of each were titrated on the two hosts. However, the absolute titer per ml of the infected K_1 cells was reduced to the titer exhibited by total free $P_{14}(145)$ on host K_1, whereas the titer of the infected 145 cells corresponded to the total free phage as measured on 145 cells. Since 99% of the phage had been adsorbed onto the K_1 cells, and since the titer of infected cells was very low, these results show that a high percentage of adsorbed particles produced no plaques on host K_1. Furthermore, this same percentage of "infected" K_1 cells release no phage capable of forming plaques on host 145. On the other hand, when the same quantity of phage 14(145) is

adsorbed on strain 145, most or all of these infected cells are capable of forming plaques in both K_1 and 145 assay suspensions. Thus, the high ratio 145/K_1 of free phage 14(145) is not the result of great differences in the amount of adsorption onto the two hosts, but is apparently related to the release of phage from "infected" cells. 3) *Latent periods and Burst sizes of P_{14} on the two hosts.* The latent period of P_{14}(145) added to either host K_1 or host 145 log phase cells was found to be around 30 minutes at 37°C. This value was independent of the strain used to assay the rise in titer. Tests on 14(K_1) show a similar latent period on the two hosts.

Log phase K_1 cells infected with 14(145) released about 60 particles per infected cell when aliquots were assayed on strain K_1, and 75 particles per infected cell when assayed on strain 145. Similar results were obtained with P_{14}(K_1) infections of K_1 cells. This showed that phage released in the first burst from infected K_1 cells reproduces the assay ratio of ca 1.3 characteristic of broth lysates of strain K_1. A log phase infected Staph. 145 cell was found to release by burst only 2 to 3 particles capable of forming plaques on host K_1 as compared with 80 particles capable of producing plaques on host 145. Thus, analyses of phage produced in the first burst showed an assay ratio 145/K_1 of ca 40. Again the burst size was independent of the host used to passage the virus P_{14}. *Viz.* Table II.

C. *Tests for inhibitors.* The fact that most of the phage particles produced by host 145 adsorb onto K_1 cells but do not form plaques suggested the possibility of an inhibitor for K_1 lysis associated with phage 14 and strain 145. 1) Assays of filtered phage from P_{14} multiplying on host 145 showed a consistently high ratio 145/K_1, indicating that free phage early in production is no different than that released in the last bursts, and that the high ratio does not result from an accumulation of an inactivator. 2) Filtrates from log phase normal K_1 or 145 cells produced no inhibitory effect on bursts of phage from strain 145. 3) P_{14}(K_1) was mixed with P_{14}(145) and stored at 4°C overnight. The total count was equal to the sum of controls mixed similarly with

TABLE II. Summary of Titration Ratios in Adsorption and Burst Size Studies.

Phage and passage on cells	Phage assay on cells	Plaque suspension after 10 passages/ml*	Total phage in filtered broth lysate per ml†	Adsorbed on K_1 cells 20 min 37°C			Adsorbed on WF145 cells 20 min 37°C			
				Residual free phage per ml‡	% adsorbed	Infected cells per ml§	Burst size per infected cell‖	Residual free phage per ml	% adsorbed	Infected cells per ml	Burst size per infected cell
14(K_1)	K_1	1.1×10^6	1×10^8	1.1×10^6	98.9	5.6×10^7	100	1.6×10^6	98.4	4.4×10^7	2
	145	1.4×10^6	1.4×10^8	1.3×10^6	99.1	5.8×10^7	100	2.5×10^6	98.2	5.6×10^7	60
	R145	1.3	1.4	1.2	—	1	1	1.5	—	1.2	30
	K_1										
14 (145)	K_1	5×10^4	2×10^6	1.1×10^4	99.4	1.3×10^6	60	2.1×10^4	99	3.6×10^7	2
	145	2.2×10^6	6.8×10^7	4.7×10^5	99.3	1.5×10^8	75	6.8×10^5	99	5.6×10^7	83
	R145	40	34	42	—	1.2	1.3	33	—	1.5	42
	K_1										
Stock P_1(K_1)	K_1	—	6.9×10^7	5.2×10^5	99.2	6.2×10^7	80	5.1×10^5	99.3	6.6×10^7	58
	145	—	9.4×10^7	6.2×10^5	99.3	6×10^7	85	6.5×10^5	99.3	7.2×10^7	62
	R145	—	1.4	1.2	—	1	1.1	1.3	—	1.1	1.1
	K_1										

* Ten ml saline suspension of virus from plaque after 10th passage. † Log phase cells infected with phage in Broth III, incubated 37°C until lysis. Filtered through super cell. Diluted in Broth III. ‡ Adsorption mixture P/B=$1\times10^8/2\times10^8$ per ml. Shaken 20 min 37°C, chilled ½ min, filtered through super cell. § Adsorption mixture P/B=$1\times10^8/2\times10^8$ per ml. Shaken 20 min 37°C, chilled ½ min, centrifuged in cold. Cells resuspended to volume. Some cells are lost in supernate. Recovery is about 50 to 75% of total cells. ‖ Unit infection of log phase cells P/B=$3\times10^8/1\times10^8$. Adsorbed at 37°C, free phage removed by centrifugation or determined by filtration of chilled sample through super cell. Infected cells diluted to *ca.* 10000/ml to prevent readsorption of phage. Average values reported.

broth. The mixture was additive for assay on K_1 and on 145 cells. The results were the same with phage mixed in dilute or in concentrated suspension. 4) Supernates from P_{14} (K_1) and P_{14}(145) were crossed after high speed centrifugation. Phage resuspended in these supernates was equal in titer to controls which had been centrifuged and resuspended in their own supernate. The above tests indicated that there was no interaction of phage lots passaged on different hosts. 5) Heat inactivation of P_{14}. Lots of $P_{14}(K_1)$ and P_{14}(145) were heated to 60°C. Dual titrations of samples removed at intervals revealed that activity for the K_1 cells decreased more rapidly than that for the 145 strain. Finally samples were obtained which had no phage active on K_1 but which exhibited residual activity for 145 cells. When fresh phage was diluted with these heated samples and exposed to 59°C, its activity decreased at rates similar to a control containing fresh phage diluted in broth, indicating that neither had anything heat stable been released nor had an inactivator been destroyed during heat treatment.

Examination of the last survivors of heat inactivation by plaque isolation from growth on 145 cells showed that all 47 isolated produced plaques both on K_1 and 145 cells. This occurred regardless of previous host passage. The ratio 145/K_1 obtained by plating aliquots of plaque suspensions again approached 40.

Summary. Analysis of phage activity by titration on two hosts has revealed the presence of variants hitherto undetected in stock phage P_1. One isolate, Phage 14, exhibits great changes in titration ratio on passage through the two hosts K_1 and WF 145. Produced on K_1 cells, the ratio of free phage assayed on two hosts is 1.3, whereas made on 145 cells, the free phage titrates in a ratio 145/K_1 of ca 40. This occurs regardless of the host employed in previous passage and is reproduced in the very first burst from infected cells. Attempts to isolate different strains from this phage by usual plaque isolation technics were not successful. The high ratio obtained with free phage made on 145 cells could not be explained on the basis of differences in adsorption onto or latent periods in the two hosts. The difference was traced to the production of a large number of particles from host 145 which adsorbed on K_1 but formed no plaques. No evidence was found for an inhibitor of K_1 activity associated with phage 14 production on 145 cells. Heat inactivation destroyed phage activity for K_1 cells much more rapidly than for 145 cells. There was no interaction of heat killed and active phage on exposure to 59°C.

Evidence has been accumulated which indicates that the phage P_{14} is altered on passage through host 145. The fact that phage particles surviving a heat treatment which destroyed all activity for K_1 cells produce on strain 145 a mixture of two phages (one active on K_1 and one—or both—active on strain 145) points to an unusual host effect on virus reproduction.

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Soluble Specific Leptospiral Complement-Fixing Antigens. (19576)

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Leptospiral complement-fixing antigens have been prepared from suspensions of the organisms by various methods in several laboratories (1-4). Carlinfanti(5) reported a "lipoidal" antigen extracted from washed, dried leptospirae. These antigens are characterized by relatively broad spectra of reactivity with antibodies against heterologous leptospiral strains.

This paper reports the demonstration of