Propagation of Staphylococcal Typing Phages on a Common Host, *Staphylococcus aureus* K₁, and Host-Controlled Changes in Their Lytic Range

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SUMMARY

A broad range of typing phages belonging to lytic groups I, II, III, IV and those classified as miscellaneous produced plaques on *Staphylococcus aureus* strain K₁. Two variant forms of this strain were compared, K_{1N} and K_{1H}. Phages belonging to lytic group II, of both A and B serology, had low efficiencies of plating (EOP) for the K_{1N} variant but had high EOP values for the K_{1H} variant. In general, of phages from other lytic groups, those of B serology tended to plate with low EOP values on both variants, whereas those of A serology plated with high EOP values. Propagation of several of the phages on both K₁ hosts resulted in host-modifications of the phage progeny. Similar host-induced modifications were produced by propagation of the phages on ps 73. The host-modified phages showed striking losses in plating efficiency for their usual propagating hosts. The significance of these findings with respect to genetic classification of the phages and hosts is discussed.

INTRODUCTION

This investigation has given information on the relative efficiencies of plating (EOP) of a series of staphylococcus phages for a common host, *Staphylococcus aureus* K_{1N}. This host exhibits a broad susceptibility to members of all groups of typing phages. These phages are classified as members of groups I, II, III and IV, or are placed in a category designated miscellaneous (Blair & Williams, 1961). By comparative titration of these phages on their common propagating strain and on the K_{1N} strain, we have detected interesting patterns of behaviour which seem to be related in some cases to their lytic group and in others to their antigenic structure.

This study is part of a series designed to outline the nature and extent of host modifications of phages brought about by various staphylococci. In previous work we have shown that two forms of the K₁ strain released the host-induced restriction of the polyvalent K₁₄ phage (also designated P₁₄) which occurs during replication on staphylococci typing with group II phages (Ralston & Krueger, 1952, 1954; Ralston & Baer, 1964*a*). Evidence is presented in this paper to indicate that propagation of the typing phages on these cultures of strain K₁ results in marked host-modified changes in phage host range so as to mask the original high efficiency of plating for their usual propagating host strain, thus altering the lytic group of the phages.

In future publications it will be shown that the K₁ strain is only one of a series of staphylococci which can profoundly host-modify the lytic specificity of phages.

MATERIALS

Bacterial strains and phages. *Staphylococcus aureus* K₁ cultures were maintained on Difco-tryptose phosphate agar. This strain was isolated from a case of multiple furunculosis in 1929 by Dr A. P. Krueger. When originally tested it was a golden pigmented, slightly haemolytic, coagulase-positive strain; it fermented mannitol, reduced nitrate, and liquefied gelatin. It has been subcultured frequently on laboratory media, and at present is non-pigmented, mannitol positive, coagulase negative, produces no detectable amounts of hyaluronidase, haemolysin, or extra-cellular deoxyribonuclease, and has lost its pathogenic potential for rabbits. This strain has been the propagating host for the well-known polyvalent phage K, now designated phage K₁ in this laboratory, and for the host-alterable variant phage K₁₄—also referred to as the P₁ and P₁₄ phages in former publications (Ralston & Krueger, 1952). Two forms of the K₁ strain were used in these studies: K_{1N} and K_{1H}. The host-restricted phage K₁₄ plates with a low efficiency of plating (EOP) on strain K_{1N} but with a high EOP on strain K_{1H}.

Phages and bacterial hosts of the typing series were obtained through the courtesy of Dr J. E. Blair (N.Y. Hospital for Joint Diseases, New York City, U.S.A.) and from the late Dr A. Browne (Calif. State Dept. of Public Health, Berkeley, Calif., U.S.A.), who originally obtained his set from Dr Blair. Stock bacterial cultures were maintained on trypticase soy (hereafter TS) agar (Baltimore Biological Laboratories, Baltimore, Md., U.S.A.), and were stored at 4°. Twenty-four hr cultures were used in all experiments. The stock suspensions of phage were stored at 4°.

Phage assay. Phage titres were determined by plaque count by a typical Gratia technique. All dilutions were made in TS broth. Suitable dilutions of phage were mixed with 5×10^7 cocci/ml. in 0.3% (w/v) TS agar supplemented with 400 µg. calciumion/ml. One ml. amounts of the phage + cocci assay mixtures were spread over a basal layer of 2.5% (w/v) TS agar. The plates were incubated at 28°. Freshly poured basal agar was essential for obtaining plaque counts.

RESULTS

Efficiencies of plating of individual typing phages for Staphylococcus aureus K₁ cultures as compared with their respective propagating strains

The individual phages tested in this series represented members of the major lytic groups as defined by Blair & Williams (1961). These include phages of A, B, F and L serology. The phage K₁₄ (not a typing phage) is of D serology. It was used in its host-restricted form after modification by propagation on host PS 51. The phages were assayed by plaque count on host K_{1N} and on their usual propagating hosts (H). A comparison was made of the relative plaque-forming ability of each phage stock for the two hosts by calculating an efficiency of plating: $EOP\ K_{1N}/H = \text{titre/ml. on strain } K_{1N} \text{ divided by the titre/ml. on the usual propagating host for a given phage (H)}$. Table 1 shows that 23 of 28 phages could produce plaques on the K_{1N} strain; and these showed patterns of EOP K_{1N}/H values as follows: (1) In general, serological type B phages from all lytic groups had low EOP K_{1N}/H values, i.e. they formed many fewer plaques on strain K_{1N} than on their respective

Table 1. Differences in plaque-forming abilities of typing phages of known serological type as determined by comparative assays on their propagating hosts, on *Staphylococcus aureus* K_{1N} and *Staphylococcus aureus* K_{1H}

Stock phages, prepared on their respective hosts, were diluted in TS broth and assayed on their propagating host, on strain K_{1N} and K_{1H}. The EOP values were calculated from the resultant titres. Titres for the assays on K_{1H}, which provided data for the calculations of EOP K_{1N}/K_{1H}, are not included in the table.

Typing phage group	Phage	Serotype of phage	Titre/ml.		EOP* K _{1N} /H	EOP K _{1N} /K _{1H}
			<i>S. aureus</i> K _{1N}	Propagating host (H)		
I	29	B	7.3×10^2	7.3×10^7	0.00001	n.t.†
	44A	B	3.1×10^2	6.2×10^6	0.00005	n.t.
	52	B	1.4×10^4	1.0×10^7	0.001	1.0
	52A	B	$< 1.0 \times 10^2$ (R)‡	1.7×10^4	—	0 (R)
	79	B	3.1×10^6	3.6×10^6	0.9	1.0
	80	B	7.4×10^4	5.0×10^6	0.01	2.5
II	3A	A	4.0×10^5	1.0×10^7	0.04	0.001
	3B	A	1.5×10^8	3.5×10^9	0.04	0.001
	3C	A	3.6×10^5	2.0×10^7	0.02	0.002
	71	B	2.9×10^2	4.1×10^4	0.007	0.005
	55	B	$< 1.0 \times 10^2$ (R)	2.0×10^8	—	0 (R)
	89	B	$< 1.0 \times 10^2$ (R)	5.0×10^7	—	0 (R)
	523	B	$< 1.0 \times 10^2$ (R)	1.0×10^7	—	0 (R)
III	6	A	2.4×10^9	3.9×10^8	6.3	1.0
	7	A	1.8×10^7	8.8×10^8	2.4	1.0
	42B	A	4.4×10^9	7.4×10^9	0.60	1.0
	42E	A	2.4×10^9	1.2×10^9	2.0	0.67
	47	A	2.6×10^4	3.0×10^8	0.008	5.0
	54	A	1.0×10^8	4.7×10^8	5.0–20.0§	1.0
	70	A	5.0×10^9	1.0×10^9	5.0	1.0
	73	A	6.0×10^6	6.0×10^6	1.0	1.0
	75	A	1.7×10^9	2.0×10^9	0.8–1.6	1.0
	53	B	4.0×10^5	5.0×10^7	0.008	1.0
	83	B	5.0×10^5	5.0×10^7	0.01	1.0
	77	F	1.5×10^4	1.5×10^8	0.0001	1.0
IV	42D	B	1.6×10^6	4.0×10^6	0.3–3.0	1.0
Misc.	81	A	6.0×10^6	1.2×10^7	0.5–8.0	1.0
	187	L	$< 1.0 \times 10^2$ (R)	2.5×10^7	—	0 (R)
Non-typing phages	K ₁₄ (Produced on group II hosts)	D	2.0×10^7	1.0×10^9	0.02	0.02
	K ₁₄ (Produced on non-group II hosts)	D	1.0×10^9	1.0×10^9	1.0	1.0

* EOP designates efficiency of plating, obtained from the ratio: titre/ml. on host K_{1N} ÷ titre/ml. on host (H), etc.

† Designates no test carried out.

‡ R designates resistant to all concentrations of phage tested. In this test 10^{-2} dilution was the highest concentration plated. In other tests higher concentrations of phage failed to form any plaques. No EOP was calculated because the phages were considered to be incapable of forming plaques on K_{1N} (designated R). In the case of phage 187, failure to form plaques was found to be due to failure to adsorb; in the case of phage 55, adsorption was so slow as to be almost insignificant. Failures of the other phages have not been analysed further.

§ A range of variation in values is included. All calculations are to the first significant decimal place.

propagating hosts. In addition, of five phages which failed to form any plaques on strain K_{1N} , four were of B serotype: 52A, 55, 39, 523 (the fifth was phage 187, L serotype). Phage 79 (lytic group I) and phage 42D (lytic group IV) were exceptions and tended to plate with high EOP K_{1N}/H values. (2) Regardless of serotype, and like the B phages, all phages of lytic group II also had low EOP K_{1N}/H values, indicating a low potential for forming plaques on host K_{1N} . This low plaque-forming ability resembles that of the restricted form of the host-modifiable phage K_{14} (Ralston & Baer, 1964*a*). (3) Most of the lytic group III phages and those classified as miscellaneous of A serology tended to plate with EOP K_{1N}/H values close to or greater than 1. Phage 47 (lytic group III, of A serotype) was different, however, and formed 100-fold more plaques on strain PS 47 than on K_{1N} . (This phage produced a mixture of plaques on both hosts—90% large and 10% small—the significance of which has not been ascertained.) (4) Only one serotype F phage was tested, phage 77 (lytic group III). This phage had a low EOP K_{1N}/H value, i.e. formed relatively few plaques on host K_{1N} as compared with host PS 77.

Because of the proven lysogenicities of many of the parent cultures from which the phage stocks were obtained, a possibility existed that high-titre phage stocks which formed low numbers of plaques on strain K_{1N} were really contaminated with phages carried lysogenically by the propagating hosts. However, a number of observations make this quite unlikely: (1) separate stocks of the same phage + host system showed fairly constant EOP K_{1N}/H values. The EOP K_{1N}/H values remained constant when assays were performed on lysates whose titres had dropped several-fold on storage or on phage suspensions from single plaques. It would seem unlikely that contaminants would be present in constant proportions under so many conditions. (2) Different hosts had produced each phage; and therefore, to produce low titres on host K_{1N} , all the different hosts would have to be lysogenic and carry a phage capable of forming plaques on host K_{1N} . Tests of several propagating strains failed to indicate that they all harboured phages capable of forming plaques on host K_{1N} . (3) According to Rippon (1956) spontaneously induced contaminants are rarely present in titres as high as 10^4 in lysates containing 10^8 particles/ml. Several of our phage stocks had titres for host K_{1N} which were 10^{-2} to 10^{-3} of the titres for their propagating host. This would indicate an unusually high percentage of lysogenic phage in the phage stocks. Because of these considerations we are more inclined to consider that phage stocks which plated at comparatively low titres on strain K_{1N} contained a single phage which possessed a low potential for plating with strain K_{1N} rather than a mixture of phages one of which was capable of forming plaques on this strain.

Comparative assays on strain K_{1N} and K_{1HI} . Upon performing titrations of the restricted form of phage K_{14} on the two types of *Staphylococcus aureus* K_1 , we found previously that this phage plated with low efficiency on strain K_{1N} but with high efficiency on strain K_{1HI} (Ralston & Baer, 1964*a*). We have now determined the EOP K_{1N}/K_{1HI} values of several of the typing phages. All group II typing phages of both A and B serology behaved similarly to the host-restricted phage K_{14} , forming many plaques on strain K_{1HI} and few plaques on strain K_{1N} , whereas the other phages tended to form plaques equally well on both hosts, their EOP values approaching 1. At present we do not know what metabolic characteristic distinguishes the two K_1 forms or what property causes both phage K_{14} (a broad host-range

phage) and the lytic group II phages (narrow host-range phages) to exhibit similar relationships in their plaque-forming potentials for the two hosts. We wish to point out that it may be significant that these phages have all undergone at least one cycle of infection in a host of phage lytic group II and that phage K₁₄ has been shown to be host-restricted by all strains belonging to this phage group.

*Restrictions of host range of typing phages by
Staphylococcus aureus K₁ cultures*

Notwithstanding its wide susceptibility to many typing phages, the K₁ strain is capable of producing restrictions in the host range of many phages. Strain 73, which is also susceptible to plaque formation by a wide number of phages, produces similar host-modifications as demonstrated by the following tests. Several typing phages were plated to obtain isolated plaques on their propagating host (H), on strain K_{1N} and on strain 73. The progeny from single plaques were each suspended in TS broth and assayed again on the three hosts (Table 2). From the titration values, two calculations were made: (1) EOP K_{1N}/H, to determine what happened to the ability of the phage to form plaques on its common propagating host after it had been produced on (H), on host K_{1N}, and on host 73; and (2) EOP K_{1N}/73, to compare the plating efficiency of phage from each passage for two hosts each known to be susceptible to many phages. The calculations of EOP K_{1N}/H indicated that: (1) passage of a phage on its usual propagating host resulted in progeny with an EOP K_{1N}/H much like the parent stock; (2) passage of each phage on host K_{1N} resulted in host-modified particles. These had a low efficiency of plating for their respective hosts (H). Phage 70 appeared to be an exception; (3) passage of each phage on PS 73 also resulted in host-modified particles. These particles, like those produced on host K_{1N}, also had a low efficiency of plating for their usual propagating host. (Phage 70 was host-restricted by strain 73.) The EOP K_{1N}/73 values indicated that in most instances the phages formed equal numbers of plaques on both hosts, but there were enough exceptions to make it appear that strains K_{1N} and 73, while very similar, often differed with respect to their responses as phage modifiers and as indicator hosts.

In other studies, strain K_{1HI} was tested for ability to host-modify the typing phages. Lysates were prepared on this strain in TS broth and samples were titrated on the propagating host (H) and on strain K_{1HI}. From the titres, an EOP K_{1HI}/H was calculated. The following values were obtained: 3C = 16,000; 79 = 10,000; 70 = 500; 77 = > 5000; 53 = 33; 42D = 1,000,000. It can be concluded that both forms of strain K₁ host-restrict many different typing phages. In these tests phage 70, which was not altered during passage on strain K_{1N}, was host-restricted by strain K_{1HI}. In each instance of host-restriction the lytic specificity of the phage was either greatly reduced or abolished, i.e. the phage no longer possessed a high efficiency of plating for its usual propagating host.

In the present studies, one-step burst tests were not performed on the phages during their replication in the K₁ and 73 hosts. These tests would have provided more reliable evidence for a true host-controlled modification of the phages. Our conclusions are based upon changes occurring within a single plaque passage. However, a detailed study of phage 3C will be presented separately (Ralston & Baer, 1964b), showing that a phenotypic modification of the phage occurs within a single

burst on cocci of strain K_{1N} and K_{1H}, and that the host range of the phage is changed from one predominantly lytic for hosts of phage group II to one almost exclusively lytic for host K_{1N}, K_{1H}, strain PS 73 and for certain *Staphylococcus aureus* strains of

Table 2. *Host-induced modifications of the plaque-forming abilities of specific staphylococcal typing phages, as shown by single plaque passage on strains K_{1N} and 73*

Stock phages were propagated on TS agar on each of three strains. Contents of isolated plaques were diluted in TS broth and assayed on each of the three strains. The relative efficiencies of plating (EOP) were calculated from the resultant titres.

Phage group	Phage	Sero-type of phage	Passage on host	Plaque contents assayed (plaques/ml. on strain)			EOP* (approximate values)	
				(H)†	K _{1N}	73	K _{1N} /H	K _{1N} /73
I	79	B	79	125	244	55	2	5
			K _{1N}	0	25	0	> 25	> 25
			73	0	5,000	466	> 5000	10
II	3A	A	3A	5,000	16	10,000	0.003	0.0001
			K _{1N}	0	9	5	> 9	1
			73	0	5,000	5,000	> 5000	1
	3C	A	3C	191	13	200	0.07	0.06
			K _{1N}	0	170	199	> 170	1
			73	n.t.	5,000	5,000	n.t.	1
III	7	A	7	124	149	91	1	1
			K _{1N}	2	37	41	18	1
			73	13	2,000	2,000	154	1
	42E	A	42E	3,000	5,000	5,000	2	1
			K ₁	45	10,000	10,000	220	1
			73	312	> 10,000	> 10,000	> 30	1
	70	A	70	50,000	70,000	70,000	1	1
			K _{1N}	30,000	30,000	192	1	15
			73	100	15,000	15,000	150	1
	53	B	53	1,500	10	596	0.006	0.02
			K _{1N}	n.t.	988	922	n.t.	1
			73	44	22	> 50,000	> 0.5	< 0.0004
	83	B	83	100,000	1,000	3,000	0.01	0.3
			K _{1N}	0	190	303	> 190	0.6
			73	0	5,000	5,000	> 5000	1
	73	A	73	10,000	10,000	—	1	1
			K _{1N}	—	588	517	1	1

* EOP designates efficiency of plating, determined from the ratio: titre/ml. on host K_{1N} ÷ titre/ml. on host H, etc. Values are preceded by signs > or < where plaque contents provided insufficient phage to make more accurate calculations.

† H designates propagating host, commonly used for propagating the specific phage.

phage lytic group III. Yet its group II lytic host range is restored within a single burst cycle by those few cocci of strain 3c which are successfully infected by the host-modified particles of phage 3C. Most of the changes of Table 2 are probably similar in basic mechanism.

DISCUSSION

According to the present typing scheme (Blair & Williams, 1961) certain phages are considered to be related and are placed in broad lytic groups because they tend to form patterns of lysis in common. The extent to which the classification relates to phage or host genetic structure is not yet apparent. Most of the phages originated from lysogenic strains or were obtained by 'adaptation' (Fisk, 1942; Williams Smith, 1948) and are temperate (Rippon, 1956). In general they lyse strains only within their broad lytic group, but each lytic group of phages embraces a variety of kinds. Their serology does not correlate with their lytic specificities; neither does the serology of the host surface structure relate to their lytic range (Oeding & Williams, 1958), though vague relationships have been reported (Hobbs, 1948; Wahl & Fouace, 1952). Phages of a given serotype, however, share characteristics in common, and our studies make evident certain patterns of plaque-forming abilities with respect to a common host, *Staphylococcus aureus* K_{1N}. These patterns appear to be dependent upon phage serology but are also related to the lytic classification and previous passage history. It has also become clear from our tests that, for these characteristics to be demonstrable, the phages need to be propagated on their recommended hosts; for, immediately after they are produced on strain K_{1N}, they lose their lytic specificity for their usual propagating host.

Hosts which type primarily with phages of lytic group II have been shown to modify (restrict) the phage K₁₄ for host K_{1N} (Ralston & Baer, 1964*a*), and these same hosts have recently been found capable of restricting the plaque-forming efficiency of phage 3C for host K_{1N}. The present studies indicate that all phages of group II show low potentials for infecting host K_{1N}. Based on these considerations, it may be speculated that group II cocci possess an unusual ability to modify certain phages. Certain differences between the group II and group III phages, particularly those of A serotype, may depend upon changes imposed by the different propagating hosts.

It has long been recognized that staphylococcal phages are subject to host-controlled modifications (Rountree, 1956), but, in general, the changes reported heretofore have been confined to hosts within a given broad lytic phage group. Results of our tests with these phages indicate that by propagation on cultures of *Staphylococcus aureus* K₁ many phages can be modified so that their original group lytic specificity is abolished. This is not the only kind of change which can be imposed by the K₁ host, for this strain is known to release the polyvalent K₁₄ phage from the restriction contributed to it by hosts of phage group II (Ralston & Baer, 1964*a*). It may be significant that strain K₁, which performs both restrictive and release modifications, is, itself, susceptible to a broad range of phages. More detailed analyses of the kind and extent of restrictions that can be performed by different hosts might aid in further defining the genetic structure of both phage and host and bring more meaning to the present staphylococcal classification schemes.

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