THE CLASSIFICATION OF BACTERIOPHAGES LYSING STAPHYLOCOCCI

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INTRODUCTION

Bacteriophages may be classified by their host range, susceptibility to specific antisera, cross-resistance tests, resistance to physical and chemical agents, plaque morphology, and genetical relationships. The system employed for any given group of phages will depend to a large extent on the purpose for which the phages are being used; for the phages used in the type identification of coagulase-positive staphylococci the host range (lytic spectrum) has proved the most useful. In addition, as shown by Rountree (1949*a*), the phage-neutralizing effect of specific antisera is an indication of the stability and degree of specificity of these phages.

The present paper describes a classification of the phages lysing staphylococci based on their lytic spectrum and phage serological group.

METHODS

The phages to be described include those used for routine phage typing and also several phages of other serological groups that have been isolated in this laboratory or have been received from other sources. The phages are designated by numbers, usually given in order of isolation, but certain of the phages not used for typing are designated by the name given them by the isolating laboratory (Wilson & Atkinson, 1945; Rippon, 1952; Williams & Rippon, 1952; Williams, Rippon & Dowsett, 1953).

The strains used to propagate the phages are designated by the same number as the phage, with the prefix P.S., e.g. the strain or culture of staphylococcus used to propagate phage 3A is known as P.S. 3A and P.S. 52A/79 represents the strain used to propagate the two phages 52A and 79.

The culture media used for phage propagation are described in an earlier paper (Williams & Rippon, 1952). In addition Difco nutrient agar, as now used in the routine typing, has been employed for the determination of the lytic spectrum, since reproducible results can only be obtained on a standard medium. This is made up as follows:

Difco dehydrated nutrient broth	20 g.
Sodium chloride	5 g.
Shred agar (Kobe no. 1)	11–12 g.
Water to	1000 ml.

The technique used for routine typing of staphylococci has been described earlier (Williams & Rippon, 1952; Williams *et al.* 1953) and has remained unchanged

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except that the strains not typable at the routine test dilution (R.T.D.) are now examined with phage filtrates at a concentration of 1000 × R.T.D. This gives greater uniformity and removes some of the non-specific reactions due to the 'inhibitory agent' (Williams & Rippon 1952), which occurs when the undiluted phages are used.

Table 1. *An example of the determination of the lytic spectrum of a phage* (79)

Test strain	Undiluted phage	Degree of lysis with phage diluted 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Titre relative to that on the propagating strain P.S. 52A/79
P.S. 3A	±/( )	—	—	—	—	—	—	(10^{-6})
3B	—	..	..	..	..	..	..	—
3C	—	..	..	..	..	..	..	—
6	+/+++	+	±	—	—	—	—	10^{-4}
7	±/++	+	±	—	—	—	—	10^{-4}
29	—	..	..	..	..	..	..	—
29A	CL	(+++)	—	—	—	—	—	( )
31/44	CL	SCL	++	+	±	—	—	10^{-2}
42B/47C	+	±	—	—	—	—	—	10^{-5}
42C	—	..	..	..	..	..	..	—
42D	CL	(+++)	—	—	—	—	—	( )
42E	++	+	±	—	—	—	—	10^{-4}
44A	+/( )	—	—	—	—	—	—	(10^{-5})
47	SCL	++	+	±	—	—	—	10^{-3}
47A	—	..	..	..	..	..	..	—
47B	—	..	..	..	..	..	..	—
51	—	..	..	..	..	..	..	—
52	—	..	..	..	..	..	..	—
52A/79	CL	CL	CL	CL	++	+	±	1
53	—	..	..	..	..	..	..	—
31A	+++	±/( )	—	—	—	—	—	(10^{-5})
54	—	..	..	..	..	..	..	—

Symbols: CL = confluent lysis.
+++ = confluent lysis with secondary growth.
(+++) = strong inhibition.
( ) = slight inhibition.
SCL = semi-confluent lysis.
++ = more than 50 plaques.
+ = 20–50 plaques.
± = less than 20 plaques.
±/+++ = plaques in secondary growth (or area of inhibition).
.. = test not done.

Determination of lytic spectrum

The lytic spectrum is determined on a standard set of strains, namely, the twenty-two propagating strains in routine use in 1949.

The phage filtrate under examination is first tested, undiluted, for its ability to lyse the twenty-two standard strains, and it is then titrated on those that are lysed, using ten-fold steps. The titre on each strain lysed, relative to that on the homologous propagating strain, may then be calculated. An example is given in Table 1. These titrations also indicate which filtrates contain the 'inhibitory agent'.

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The lytic spectrum of a phage on the standard set of twenty-two propagating strains is normally sufficient to decide into which of the various groups of lytic patterns the phage may be placed, but each new phage is also tested by including it in the routine-typing set, and recording the number of strains of staphylococci in each lytic group that are lysed by it. For the analysis only one strain from any given source has been included, although several identical strains may have been typed.

Determination of serological group

The serological methods used are based on those of Rountree (1949*a*). Antisera were prepared in rabbits by the intravenous injection of the undiluted phage filtrates. Neutralization of phage was generally obtained at a serum dilution of the order of 1/1000.

Dilutions of phage, so that a drop from a calibrated pipette (1 drop = 0·02 ml.) gave about 200 plaques on each strain lysed, were set up with ten-fold dilutions of each group antiserum, as illustrated in Table 2 with the serological group B phage 79. When neutralization was not complete with any one antiserum, as on P.S. 6, 7, 31/44 and 52A/79, tests were set up with mixed antisera, using the sera at a constant dilution and varying the phage dilutions so that the serological group could be defined as shown in Table 3. (The preliminary test recorded in Table 2 had given indications that phages of both serological groups B and F were present. A mixture of these two sera was therefore employed in parallel with pure group sera.)

Many of the phage-propagating strains are lysogenic, and the carried phage may therefore contaminate the phage propagated on them. However, the particles of contaminating phage are rarely found in numbers exceeding 10^4 phage particles per ml., whereas particles of the propagated phages usually number 10^8 to 10^9. The contaminating phage can be readily detected if it forms different plaques from those of the propagated phage; should it be of a different serological group, the serological tests, using the undiluted phage filtrate, will indicate its presence. The batch of phage 79 (serological group B) shown in Table 3 was contaminated with a phage of serological group F released from the propagating strain. This contaminating phage lysed the strains P.S. 6, 7 and 31/44; on P.S. 6 and 7 it could be readily picked out, since the plaques formed were clear in comparison to the small overgrown plaques of phage 79.

Some high-titre batches of phage are not completely neutralized by homologous antiserum; yet when the residual plaques are picked and propagated they yield phage identical with the parent stock—'residual' phage. It occurs in stocks of serological group B or F phages more frequently than in those of group A, but the effect is not limited to very highly titred stocks. The batch of phage 79 illustrated in Tables 2 and 3 contains residual phage lysing the propagating strain.

RESULTS

On the basis of their lytic spectra, the phages were allocated to three broad divisions: (*a*) phages with restricted host range lysing some coagulase-positive staphylococci; (*b*) polyvalent phages lysing nearly all coagulase-positive strains, and (*c*) phages lysing only coagulase-negative staphylococci.

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Table 2. *Example of the determination of the serological group of a phage* (79)

Phage dilution	Test strain	Degree of lysis on test strain after incubation of the phage with the specified antisera diluted: A 20	A 200	A 2,000	A 20,000*	B 20	B 200	B 2,000	B 20,000	C 20	C 200	D 20	F 20	F 200	F 2,000	F 20,000	G 20	H 20	Controls: Nutrient broth	Controls: Normal serum
2*	P.S. 52A/79	CL†	CL	CL	CL	+	++	CL	CL	++	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
	6	++	++M‡	++M	++M	+	++	++M	++M	++	++	++M	(++)§	(++)	++M	++M	++	++M	++M	++M
	7	+	++M	++M	++M	+	+M	++M	++M	+	+	++	++	++	++	++M	++M	++M	++M	++M
	31/44	++	CL	CL	CL	+	+	CL	CL	+	+	CL	CL	CL	CL	CL	CL	CL	CL	CL
	42B/47C	±	+	+	+	−	−	±	+	−	−	+	+	+	+	+	+	+	+	+
	42E	−	+	+	+	−	−	±	+	−	−	+	±	+	+	+	+	+	+	+
	44A	−	±	±	±	−	−	±	±	−	−	±	±	±	±	±	±	±	±	±
	47	+	++	++	++	−	−	++	++	−	−	++	++	++	++	++	++	++	++	++
10,000	52A/79	+	++	++	++	−	±	+	++	±	±	++	++	++	++	++	++	++	++	++

* Reciprocal of dilution.
† CL, ++, +, ± = diminishing degrees of lysis.
‡ M = plaques of two types.
§ (++) = overgrown plaques.

Table 3. *Additional serological tests with phage* 79

Test strain	Antisera	Degree of lysis on test strain with phage diluted: 1/2	1/20	1/200	1/2,000	Serological group of phage lysing test strain
P.S. 52A/79	N°*	CL	CL	CL	++	B
	B	CL	++	++	+	.
	F	CL	CL	CL	++	.
	B+F	++	++	+	+	.
P.S. 6†	N°	++	+	±	.	F
	B	++	+	±	.	.
	F	—	.	.	.	.
	B+F	—	.	.	.	.
P.S. 7	N°	++	+	$\pm_1$	$\pm_1$	B and F
	B	++	+	$\pm_2$	.	.
	F	(++)	(+)	.	.	.
	B+F	(±)	.	.	.	.
P.S. 31/44	N°	CL	++	+	$\pm_4$	B and F
	B	+	±	.	.	.
	F	CL	++	+	$\pm_2$	.
	B+F	$\pm_2$	$\pm_2$	.	.	.

* N° = normal.
† The lysis of group B phage was inconstant on this strain.

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The first division of phages, those of restricted host range, were further subdivided into three 'lytic' groups by Williams & Rippon (1952) following the results of Allison (personal communication). These 'lytic' groups (I, II and III) were formulated by analysing the phage patterns occurring when a series of coagulase-positive staphylococci were tested with a set of twenty-four phages at R.T.D. A fourth group (IV), to include those phages lysing predominantly strains of bovine origin, and a 'miscellaneous' or 'unclassifiable' group were later separated (International Subcommittee for Phage Typing of Staphylococci, Rome, 1953, unpublished). When a phage has a very restricted lytic range, lysing three or less of the standard set of strains, it cannot usually be allocated to any of the lytic groups.

The propagating strains of the typing phages are, in most cases, lysed only by phages of the same lytic group as the phage propagated on them, and they are therefore suitable for the determination of the lytic group of newly isolated phages. Similarly, a staphylococcus may be said to be of group I, II, etc., by virtue of its pattern of phage lysis.

The strains P.S. 29A and 31/44 are lysed by phages of both lytic groups I and III, and cannot therefore be used to define the lytic group of a new phage, although they are useful for distinguishing phages.

In addition to the six serological groups of staphylococcal phages described by Rountree, four more, G, H, J and K, have been identified in this laboratory. The group G phages were described in an earlier note (Rippon, 1952), and the groups H, J and K were identified later. Phages comprising groups J and K lyse only coagulase-negative staphylococci. Rountree (1949*a*) was able to divide the serological group B phages into two subgroups which in this paper are designated as B_1 and B_2. The subgroup B_1 contains the typical lytic group I phages, 29, 52, etc., and B_2, 42C and 42D.

In the present work certain minor differences have been noted between the group B phages, but these differences do not appear to be sufficiently regular or significant to be used for finer classification schemes. In general, phages are neutralized only by the homologous antiserum, but weak cross-reactions have been observed between the phages of serological groups A and B; phages of group B are also neutralized with an antiserum prepared against X2, the only group C phage. Some phages of group A are partially inhibited at low phage concentrations by antisera of group H and sometimes of group F as well. These reactions have not been fully studied, and in the present work such minor differences will not be reported. Within the other serological groups all the phages are almost equally neutralized by the homologous antiserum.

Staphylococcal strains belonging to lytic groups I, II and III have been found by Hobbs (1948) and Wahl & Fouace (1952), to correspond with the three groups of staphylococci defined by Cowan (1939) by serological methods. These results have, however, been criticized by Oeding & Vogelsang (1954), who, using the method of serological typing devised by Oeding (1952, 1953*a*, *b*), obtained fairly good correlation between the serological and phage classification with Group III, but not with groups I and II.

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A classification of the staphyloccal phages

It seems convenient to classify the phages, first, on the basis of the broad characteristics of their lytic spectrum; secondly and within these divisions, on their serology; and thirdly, on the particular associations of their host range.

1. Phages of restricted host range:
   Serological group A Lytic groups II, III, IV, Misc.
   Serological group B Lytic groups I, II, III, Misc.
   Serological group F Lytic groups II, III, IV, Misc.
   Serological group C Lyses principally strains of ovine origin.*
2. Polyvalent phages:
   Serological group D
   Serological group G
   Serological group H
3. Phages lysing only coagulase-negative staphylococci:
   Serological group E
   Serological group J
   Serological group K

Tables 4 and 5 show the phages classified in this way. Notes on some of the phages are given below.

Phages of restricted host range (*Table* 4)

The phages used in the routine typing are of serological groups A, B and F. The group A phages are more stable than those of group B (Rountree, 1949*a*), and the phages of serological group F are intermediate. The group B phages have also more exacting growth requirements, notably for calcium; and they seem more prone to produce the 'inhibitory agent'.

The lytic group I phages so far isolated are all of serological group B. The lytic group IV phage of serological group A (phage 42F) was isolated by Smith (1948*a*) by adaptation of the serological group B_2, phage 42D, to the strain E193. This is the only reported instance where adaptation of a phage to a new strain has changed the serological group of the phage. P.S.42D is not known to carry a phage of serological group A, and is, in any case, not demonstrably lytic for E193 (P.S. 42F), so that it is unlikely that a phage carried by P.S.42D was adapted to E193.

Phage 73 is at present unique among the typing phages, since its propagating strain 73 is coagulase-negative. This phage was originally isolated by Fisk. Fisk's number was 10/56-A, but is not described by him in his paper (1942). The phage was propagated in this laboratory in 1949 on the strain F56-A, which had been dried here in 1948, but its earlier history is unknown. It was a mixed culture of coagulase-positive and coagulase-negative staphylococci, but only the coagulase-negative strain was lysed by phage 73. P.S.73 gives rough growth and it is not known whether it is a rough coagulase-negative variant of an originally coagulase-positive strain (as described by Smith, Morrison & Lominski, 1952) or whether it is

* Serological group L, lytic group miscellaneous, has been defined since going to press. The type phage, 187, is phage 735G of Wahl & Fouace (1954).

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an unusual coagulase-negative strain sensitive to the typing phages, many of which lyse it.

Phages 44 and 44A were originally classed as group I phages but they are better grouped either with 42D, or as 'unclassifiable'. They give patterns with group I phages; reactions with group III and/or 'unclassifiable' phages may also be present.

Phage X2 is the only example of serological group C phage in this laboratory; it was isolated by Smith (1948*b*), who found that it lysed staphylococci originating from sheep. It does not lyse human strains when used at the R.T.D.

The first serological group F phages were isolated by Rountree (1949*b*) from lytic group II staphylococci; later it was found that a serological group F variant had appeared in the routine stocks of phage 42D (serological group B_2) (Rippon, unpublished). The serological group F phage is used at Colindale for the routine typing since it is more specific than the group B_2 42D phage. Group F phages belonging to lytic group III have also been isolated.

Polyvalent phages

(*a*) The serological group D phage used by Rountree is that known as K (Krueger), and is identical with the Au2 phage examined by Burnet & Lush (1935). Phages of group D are very stable and easily propagated, and have been studied in many laboratories; all the phages shown in the table have been received from laboratories abroad, and their original source is not certain. Phage A was isolated from sewage. Group D phages differ from those of other serological groups in that none is known to have been isolated from a lysogenic staphylococcus. The 'muscae' phage has a narrower host range than the other phages of this serological group, and was isolated from an extract of house-flies (Shope, 1927).

(*b*) The serological group G phages have been described in an earlier note (Rippon, 1952). No additional phage of this group has been isolated.

(*c*) The serological group H phages 211 and 218 were obtained from an untypable spontaneously-lytic strain (i.e. a strain lysed by its own carried phage), 52.211, which when growing normally did not appear to be lysogenic. These phages were propagated on a single colony of the parent staphylococcus, 52.211a. They were identical in lytic spectrum, lysing nearly all staphylococci to titre. When adapted to P.S.31/44 (to give phage 211A) no significant change in lytic spectrum occurred. Phage 211A has been tested at R.T.D. in the routine typing set and lysed 72% of all strains; it also lyses some coagulase-negative strains. Phages 211, 218 and 211A are difficult to propagate and are unstable, and only a low-titred antiserum could be obtained when they were injected into rabbits. The phages were however all neutralized to the same titre by the antiserum, which also gave a slight inhibition of some serological group A phages when used at a dilution of 1/40.

Phages lysing coagulase-negative strains

The serological group E phage lysing coagulase-negative staphylococci, described by Rountree (1949*a*) is now lost; but although the antiserum remaining has some slight neutralizing action against the group D phages it has no action on

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Table 4. *A classification of staphylococcal phages by serological group and lytic spectrum. Phages of restricted lytic range*

Phage serological group					A																
Phage lytic group					II				III										IV	Misc.		
Phage no.					3C	3B	3A	51	47C	47B	42B	75A	70	42E	75B	6	7	73	47	54	75	42F	78	57	47A
Test strain P.S.	3B				5	5	3	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	3A				4	3	5	2	.	1	.	.	.	1	.	.	.	.	1	.	.	.	.	.	.
	3C				5	5	4	3	.	2	2	.	.	.	.	.	.	.	1	.	.	.	.	1	2
	51				5	.	2	5	.	1	.	2	.	.	2	.	.	.	.	.	.	.	.	.	.
	29				.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	52				.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
	52A/79				.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	31A				.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	1	1	.
	31/44				1	2	.	1	4	4	4	3	5	4	3	.	3	3	3	3	4	3	3	3	2
	44A				.	.	.	.	4	4	1	.	.	1	.	.	4	4	2	2	.	.	.	1	.
	29A				.	.	.	.	3	3	3	.	2	3	3	3	.	4	2	2	.	.	.	3	.
	42C				4	3	.	2	.	.	.	2	3	3	3	.	.	.	2	2	1	.	.	.	.
	42B/47C				.	.	.	.	5	2	5	.	.	2	2	.	2	2	2	1	.	.	2	.	.
	47B				.	.	.	.	1	5	2	.	1	2	.	.	.	2	2	2	.	.	.	.	.
	7				.	.	.	.	4	4	4	5	3	4	5	5	5	4	5	4	5	4	4	3	.
	47				.	.	.	.	4	4	2	4	.	.	5	2	2	2	5	3	4	.	.	.	2
	54				.	.	.	.	4	4	4	5	3	3	5	.	4	4	5	5	5	.	.	.	.
	42E				.	.	.	.	.	2	.	.	.	5	.	.	.	(3)	.	2	.	4	3	.	.
	53				.	.	.	.	.	.	.	5	3	.	5	.	.	.	.	4	4	.	.	.	.
	6				.	.	.	.	4	4	4	5	3	4	5	5	4	4	5	4	4	3	4	3	.
	42D				.	.	.	.	.	.	.	.	.	1	.	.	.	.	1	2	.	.	.	.	.
	47A				.	.	.	.	.	1	1	2	.	1	.	.	.	.	.	1	.	.	.	1	5
Percentage of routine strains lysed at R.T.D. of group	II				72	33	16	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I				1	0	0	0	0	0	4	7	6	1	3	1	1	0	1	0	0	0	3	2	0
	III				0	0	0	0	33	53	14	87	29	31	70	32	33	12	50	45	45	9	7	4	2
	Misc.				4	4	0	8	17	67	33	38	7	39	27	22	22	4	13	17	7	7	6	4	4†
Percentage of total no. of routine strains lysed					13	6	3	3	11	19	6	34	13	18	29	15	17	4	24	22	16	4	4	2	2

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Phage serological group	B																F						C
Phage lytic group	II		I						Misc.					III			II		III		IV	Misc.	
Phage no.	55	71	31A	29A	29	52A	52	79	31	44A	44	69	42C	52B	31B	53	61	62	76	77	42D	58	X2
Test strain P.S. 3B	5	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	4	.	.	.	.	.
3A	4	3	.	.	.	.	.	.	.	.	1	.	.	2	.	.	1	3	.	.	.	.	.
3C	5	5	.	.	.	.	.	.	.	.	1	.	.	1	.	.	3	3	.	.	.	.	.
51	5	5	.	.	.	.	.	.	.	.	1	.	.	.	.	.	5	5	.	.	.	.	.
29	.	.	.	.	5	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.
52	.	.	.	.	.	4	5	.	.	.	1	.	.	2	.	.	.	.	.	.	.	.	.
52A/79	.	.	.	.	.	5	4	5	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.
31A	.	.	5	1	.	.	.	.	2	.	.	.	2	.	1	.	.	.	.	.	.	.	.
31/44	1	2	.	4	4	4	4	4	5	4	5	3	4	4	3	3	.	.	4	4	3	3	.
44A	.	.	.	.	1	4	4	2	.	5	1	4	5	.	.	.	.	.	.	.	1	2	.
29A	.	.	3	5	4	3	3	.	3	.	3	3	4	3	.	.	.	1	3	(3)*	.	.	4
42C	.	4	.	.	.	.	.	.	.	.	.	.	5	.	.	3	.	3	.	.	.	.	.
42B/47C	.	.	.	.	.	.	3	2	.	1	2	.	.	.	.	1	.	.	3	2	.	.	.
47B	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	1	.	.	.
7	.	.	.	4	.	.	3	3	.	.	1	3	4	4	(3)	5	.	.	5	4	( )	3	4
47	.	.	.	3	3	.	3	3	.	.	1	.	.	5	4	5	.	.	5	4	(2)	.	4
54	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	5	.	.	4	4	(2)	3	.
42E	.	.	.	.	.	.	.	3	.	.	.	.	.	(2)	.	2	.	.	.	.	2	.	(3)
53	.	.	.	.	.	.	.	.	.	.	.	.	.	.	(3)	5	.	.	4	4	.	.	.
6	.	.	.	.	.	.	(3)	3	.	.	1	3	4	4	(3)	5	.	1	4	4	( )	3	4
42D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	1	5	.	.
47A	.	.	.	.	.	.	.	.	.	.	1	.	.	1	.	.	.	1	.	.	.	.	.
Percentage of routine strains lysed at R.T.D. of group — II	73	42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61	3	0	0	0	0	0
Percentage of routine strains lysed at R.T.D. of group — I	1	0	2	4	24	66	26	38	0	0	0	5	4	5	1	0	0	0	0	7	0	3	3
Percentage of routine strains lysed at R.T.D. of group — III	1	0	0	0	4	2	1	3	0	0	0	9	0	9	20	47	2	0	41	50	5	4	0
Percentage of routine strains lysed at R.T.D. of group — Misc.	0	0	0	0	35	35	31	50	46	46	35	4	50	7	0	17	8	8	10	19	48	0	0
Percentage of total no. of routine strains lysed	7	5	1	2	12	23	11	15	4	5	3	6	3	6	7	23	13	1	14	20	7	2	1

The figures in the upper part of this table give the relative counts of each phage tested on the various indicator strains. Code number 5 represents the maximum phage count on any propagating strain. Code number 4 a phage count $1/10$–$1/10^2$ of that on the homologous propagating strain. Code number 3 a phage count $1/10^3$–$1/10^4$ of that on the homologous propagating strain. Code number 2 a phage count $1/10^5$–$1/10^6$ of that on the homologous propagating strain. Code number 1 = occasional reaction. ( ) = inhibition by undiluted phage.

* Areas of lysis obscured by secondary growth making an exact reading difficult.

† Strains lysed only by 47A.

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Table 5. *A classification of staphylococcal phages by serological group and lytic spectrum. Polyvalent phages*

Phage serological group		D Polyvalent									G Polyvalent			H Polyvalent		
Phage lytic group																
Phage no.		A	J 11	EW	S 3K	J 10	Gratia	K_2	K_1	Muscae	66	68	65	211	211 A	218
Test strain P.S.	3B	5	5	5	5+*	5	5	5	5	4	3	4	3	5	( )	( )
	3A	5	5	5	5+	5	5	5	5	4	4	4	3	5	5	5
	3C	5	5	5	5+	5	5	5	5	3	4	3	3	( )	( )	( )
	51	5	5	5	5+	5	5	5	5	4	4	4	3	5	5	5
	29	4	5	( )	( )	(3)	4	( )	( )	.	.	.	5	( )	( )	( )
	52	5	5	5	5+	5	4	5	5	.	4	5	4	3	3	( )
	52A/79	5	5	5	5+	( )	5	5	5	4	3	3	3	5	5	5
	31A	5	5	5	5+	5	4	5	5	.	5	5	5	3	3	3
	31/44	5	5	5	5+	5	5	5	5	.	2	2	3	5	5	5
	44A	5	5	5	5+	( )	5	5	5	.	4	3	4	5	5	5
	29A	5	5	5	5+	5	5	5	5	.	5	2	4	5	5	5
	42C	4	(4)	(4)	4	(4)	4	(4)	(4)	4	4	4	(3)	5	5	5
	42B/47C	5	5	5	5+	5	5	5	5	4	5	5	5	5	5	5
	47B	( )	( )	( )	( )	.	.	( )	( )	.	3	2	3	5	5	5
	7	5	5	5	5+	5	5	5	5	.	(4)	2	(4)	5	( )	5
	47	4	( )	5	4	5	3	( )	(2)	.	5	5	4	5	5	5
	54	5	5	5	5+	5	5	5	5	.	( )	(4)	.	5	5	5
	42E	5	5	5	5+	5	5	5	5	4	4	4	4	5	5	5
	53	5	5	5	5+	5	5	4	5	.	(4)	( )	(4)	5	5	5
	6	5	5	5	5+	5	5	5	5	.	(4)	2	(4)	5	5	5
	42D	5	5	5	5+	( )	5	5	5	(3)	3	3	2	5	5	5
	47A	5	5	5	4	5	4	4	5	2	4	5	4	5	5	5
Percentage of routine strains lysed at R.T.D. of group	II	100	88	100	100	81	..	63	56	67	13	0	7		69	
	I	100	93	87	87	36	..	71	64	37	31	31	15		71	
	III	87	70	70	74	87	..	63	50	9	23	12	12		80	
	Misc.	67	67	0	0	100	..	67	67	40	78	67	78		67	
Percentage of total no. of routine strains lysed		85	71	76	75	65	..	58	53	27	28	21	17		72	

The figures in the upper part of this table give the relative counts of each phage tested on the various indicator strains. Code number 5 represents the maximum phage count on any propagating strain. Code number 4 a phage count $1/10$–$1/10^2$ of that on the homologous propagating strain. Code number 3 a phage count $1/10^3$–$1/10^4$ of that on the homologous propagating strain. Code number 2 a phage count $1/10^5$–$1/10^6$ of that on the homologous propagating strain. .. = test not done. ( ) = inhibition by undiluted phage.

* Lysed to a higher titre than the propagating strain.

the phages of serological groups J and K which lyse only coagulase-negative strains.

Three phages of serological group J are known: 'Agressif Gratia' and 'Receptif Gratia' were received from Dr Wahl, who has informed me that these phages are two varieties of phage B.L.S., isolated by Gratia in 1927 from a cow-pox vaccine, coming from Lederle Antitoxin Laboratory, Pearl River, U.S.A. The phage 'J.14' came originally from Dr Boulgakov's collection. The group K phages AG [RG] and EW [RG] were prepared by cross-culturing coagulase-negative staphylococci.

None of the phages in this division lyse coagulase-positive staphylococci and their host range on coagulase-negative strains has not been studied.

DISCUSSION

The serological group of a staphylococcal phage may, to some extent, be related to other properties, as shown in Table 6. The serological groups A, B and F, containing the typing phages, are more heterogeneous than the others. These phages have a limited lytic range, and may be subdivided within these serological groups on the basis of their lytic activity. Phages of lytic groups II and III may be of serological group A, B or F, but no lytic group I phage has been found in the serological groups A and F.

Experience with animal strains has been restricted to those from cattle, and they are lysed by phages of serological groups A, B and F. Most of the phages in these groups have, at one time or another, been found lysing bovine strains. Phages of lytic group II, however, generally only lyse bovine strains that are also lysed by phages of groups I and III; with human strains it is rare to find a group II phage in a pattern with phages of other groups.

Lwoff and his co-workers have shown that phages that can form lysogenic systems, which they call 'temperate', differ in several ways from the 'virulent' phages that cannot (Lwoff, 1953). Boyd (1950, 1951) has used the terms 'symbiotic' for temperate and 'lytic' for virulent types of phage.

The polyvalent staphylococcal phages are usually 'virulent'. The typing phages were all, in the first instance, isolated from lysogenic organisms, and are thus 'temperate' but some have since been 'adapted' to lyse other strains. In some of these instances it seems that virulent mutants of the original typing phage may have been selected, since lysogenic systems cannot be re-formed.

Lowbury & Hood (1953) have studied the secondary growth appearing after lysis by several of the routine typing phages of their respective propagating strains. In only one case (the serological group B phage 69) was the resistant growth not lysogenic; but Lowbury & Hood failed to get any resistant secondary growth with the phages 3A, 3B, 3C, 55, 52, 52A and 42E. Similar results have been obtained with the first four phages in this laboratory, although a few of the colonies picked from the secondary growth after lysis by 3B were resistant on first testing and then reverted to the sensitive state after replating. Smith (1947) has reported the isolation of lysogenic resistant variants of the propagating strains for the phages 3C, 52A and 42E, but in the latter two cases lysogenicity could not be demonstrated at every test.

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Table 6. *The characters of staphylococcal phages of different serological groups*

Serological group of phages	Lytic range of phages	Staphylococci lysed: Coagulase reaction	Staphylococci lysed: Source of strains	Ease of propagation	Stability	Used in routine typing	Ability to form lysogenic systems
A	Restricted II, III, Misc.*	+	Human and bovine	+−+ +†	+ +‡	+	+
B	Restricted I, II, III, Misc.	+	Human and bovine	+−+ +	+	+	+
F	Restricted II, III, Misc.	+	Human and bovine	+ +	+ +	+	+
C	Restricted	+	Animal (sheep)	+ +	+	−	. .§
D	Polyvalent	+ and −	Human and bovine	+ +	+ +	−	−
G	Polyvalent	+	Human and bovine	+ +	+ +	−(+)‖	−
H	Polyvalent	+ and −	Human and bovine	±	±	−	−
J	Restricted	−	.	+ +	. .	−	. .
K	Restricted	−	.	+ +	. .	−	. .

* I, II, III and Misc.: lytic groups I, II, III and Miscellaneous.
† ±−+ +: relative titres on propagation.
‡ ±−+ +: degree of stability.
§ . . = not determined.
‖ Used by Dr Wahl and Dr Wallmark in routine phage typing.

Although the typing phages are 'symbiotic' in Boyd's sense, the 'solid-centre' plaques described by him for the *Staphylococcus typhi-murium* phages are rarely observed. Since secondary lysogenic growth can usually be obtained on agar using strong phages, it may be that a certain multiplicity of infection is necessary for the formation of lysogenic systems with staphylococci.

SUMMARY

Staphylococcal phages may be divided into three broad divisions:

(1) Phages lysing coagulase-positive staphylococci with a restricted host range. Phages in this division may be further divided into five lytic groups I, II, III, IV and Miscellaneous.

(2) 'Polyvalent' phages; phages with a wide host range among coagulase-positive cocci, and sometimes also active on coagulase-negative strains.

(3) Phages lysing only coagulase-negative staphylococci.

Within each division the phages can be further grouped by their serological reactions. Nine serological groups have been defined. Phages of any single serological group also share other characteristics, e.g. stability, and the ability to form lysogenic systems.

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