Intergroup Lysis and Transduction by Streptococcal Bacteriophages

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Bacteriophages from streptococci of groups A, E, and G lysed streptococci distributed among serogroups A, C, G, H, and L; propagated in some, but not all, of these; and transduced streptomycin resistance within group A or (by A phages only) from group A to group G streptococci.

We previously reported transduction among group A streptococci by group A streptococcal phages (1, 5) and by a temperate group G phage (2). The latter report included evidence of intergroup lysis and lysogenization by several phages. The present report extends the survey of intergroup lysis and adds the first evidence of intergroup transduction as well as of intragroup (A) transduction by phages induced from streptococci of other serological groups.

A total of 191 streptococcal strains was tested, by the agar surface method (2), for lysis by 8 different streptococcal phages. The streptococci came from our stock collection and include our own isolates as well as prototype strains used for production of grouping antisera (received originally from the Diagnostic Reagents Branch, Center for Disease Control, Atlanta, Ga.). Sources of some strains, and of most of the phages, are given in previous publications (1, 2, 5). In some, but not all, instances, serogroup classification was confirmed in our laboratory by the usual method (2). Phage ATCC 19953B-1 was received, together with its propagating strain ATCC 19953 (Group D) from the American Type Culture Collection, Rockville, Md. Phage ET42 was induced from a group E *Streptococcus*, originally received as ATCC 12390 (Lancefield's grouping strain K129) (4).

Among 138 strains representing streptococcal groups A, C, G, H, and L, a number of instances of lysis by the eight phages of both homologous and heterologous serogroups occurred (Table 1). Among 53 strains representing groups B, D, E, F, K, M, N, P, Q, and R, no lysis by these phages was demonstrated (*not tabulated*). These observations extend the lytic range of virulent phage A25 to two additional groups (H and L). However, propagation of these phages in representatives of these groups, or of group G, has not been demon-

strated. On the other hand, a group E temperate phage was shown to lyse, and was subsequently propagated in, streptococci of groups A and C. The results also expand the number of strains of groups C and G streptococci lysed by phages A25 and GT234 over that previously reported (2).

Lysis as shown by the agar surface method of spotting supernatant materials on lawns can conceivably be caused by (i) high phage multiplicities, leading to lysis-from-without; (ii) cell wall enzymes sometimes found in phage-containing crude supernatant fluids; (iii) infection by low phage multiplicities followed by lytic cycles and release of phage which can be subsequently propagated in the same strain; or (iv) as in iii, but followed by release of phage progeny that are mutated, defective, or otherwise unable to propagate in the same strain.

In our initial studies, the agar surface contained in an 8.5-cm-diameter Petri dish was spread with 0.1 ml of a suspension containing 5×10^8 colony-forming units (CFU) of the *Streptococcus* to be tested. On this lawn were spotted 0.01-ml drops containing 10^7 plaque-forming units (PFU) of each phage to be tested. The results obtained by this method are shown in Table 1. Similar spotting of each phage suspension, but after it was freed of phage (as determined on known indicator strains) did not cause lysis on such lawns. Clearly, when phage was present, the ratio of PFU to CFU was large and could be expected to cause lysis-from-without of sensitive streptococci. To examine this possibility further, agar surfaces were spotted with 0.01-ml drops containing 10^3 , 10^7 , and 10^8 CFU of the streptococci shown to be sensitive in the initial screening studies. A 0.01-ml drop containing 10^7 PFU of the appropriate phage was then placed on each dried drop of streptococci. The PFU-CFU ratios were then approximately 1:10, 1:1, and 10:1, respectively. All positive results

TABLE 1. *Intergroup lysis by several bacteriophages^a*

Phages ^b	Group ^c of phage origin	No. positive/no. tested in streptococcal host group						
		A	C	D	E ^d	G	H	L
A25	A	12/14	51/69	0/13	0/1	16/26	1/11	8/12
AT298	A	6/14	15/69	0/13	0/1	0/26	0/11	0/12
AT650	A	5/14	3/69	0/12	0/1	0/26	0/11	0/12
AT3657	A	7/14	3/69	0/12	0/1	0/26	0/11	0/12
C1	C	0/14	65/69	0/13	0/1	0/26	0/11	0/12
19553B	D	0/14	0/10	1/13 ^e	0/1	0/16	0/9	0/7
ET42	E	3/14	2/10	0/13	0/1	0/16	0/9	0/7
GT234	G	3/14	19/69	0/13	0/1	5/26	0/11	0/12

^a Lysis was determined on streptococcal lawns by the agar surface method (2) by using about 10^7 PFU/spot. Materials and methods were as previously described (1, 2, 5).

^b The group A, E, and G phages were propagated in group A strain 56 $\times$ 188 str^r; the C1 phage in group C strain 26RP66; and the group D phage in group D strain 19553.

^c Serogroup of streptococcal strain originally found as indicator strain, or (for temperate phage) as lysogenic strain.

^d Only strain available was Lancefields K129 (4), lysogenic for phage ET42.

^e Only positive was the propagating strain, ATCC 19553.

(Table 1) were confirmed by this method in all ratios used, which suggests that lysis-from-without was not a factor. In addition, pelleted and washed phages, when resuspended and similarly tested in a PFU-CFU ratio of 1:10, gave the same results. Furthermore, crude phage-containing supernatant materials heated to 45 C for 15 min to inactivate phage-associated lysins (3) caused lysis. Also, phage A25 adsorbed to streptococci of groups A, C, G, H, and L in less than 15 min. These results appeared to rule out lysis by free enzyme and implicated the phage directly, as did the use of antiphage sera (in A and G only), which prevented lysis. The results then suggest that an infectious cycle was responsible for the lysis shown, but we have as yet no explanation for the inability of some phages to propagate on the strains that they initially lyse by the agar surface method.

Nevertheless, after these demonstrations of intergroup lysis and (in some instances) propagation, we tested 123 strains representing groups A, C, G, H, and L for transduction of streptomycin resistance by some of the phages shown in Table 1. Streptomycin-resistant strains of groups A, C, and G were infected with phages that were able to propagate therein; the phage progeny were used to infect known susceptible streptomycin-sensitive strains, and the survivors were examined for transductants by methods previously described (1, 5). No strain was found capable of serving as a donor except group A strain 56 $\times$ 188 str^r. Accordingly, four group A-propagable phages were grown in either 56 $\times$ 188 str^r or (as control) 56 $\times$ 188 str^s to titers of about 10^9 PFU/ml. The phage preparations were irradiated with ultraviolet light to about 90% inactivation as previously described (1) and were subsequently tested for transduction

by the plate method (5). Results are shown in Table 2. As previously shown (1, 5), a group A virulent phage (A25 = ATCC 12204) and a group A temperate phage (AT298) transduced within group A. In addition, however, these same phages

TABLE 2. *Transduction from group A Streptococcus (56x188 str^r)^a to strains of the same and other serogroups^b*

Phage	No. positive/no. tested in streptococcal strains from group				
	A	C	G	H	L
A25 (ATCC 12204)	14/24 ^c	0/63	1/23 ^d	0/18	0/11
AT298	4/4 ^e	0/3	1/23 ^f	NT ^g	NT
ET42	2/2 ^h	0/14	0/23	NT	NT
GT234	2/3 ⁱ	0/2	0/1	NT	NT

^a Phages A25, AT298, and GT234 propagated in other streptomycin-resistant strains of group A, or of groups C and G, failed to transduce streptomycin resistance to streptomycin-sensitive strains of the homologous or heterologous serogroups.

^b Tested by the plate method (5).

^c Transduced strains included K56 and S43G (2), as well as other strains of various serotypes. In K56, transductants/PFU = 3.5×10^{-5} .

^d Transduced strain was 54x264 (2). Transductants/PFU = 3.5×10^{-6} .

^e Transduced strains included K56 and S43G. In K56, transductants/PFU = 3.6×10^{-6} .

^f Transduced strain was 54x264. Transductants/PFU = 3.1×10^{-6} .

^g Not tested.

^h Transduced strains were K56 and S43G. In K56, transductants/PFU = 3.4×10^{-6} .

ⁱ Transduced strains were K56 and 57x243. In K56, transductants/PFU = 10^{-6} .

were capable of transducing streptomycin resistance to one strain of group G. Furthermore, temperate phages from groups E and G, propagable in a group A donor, transduced the marker to other Group A strains.

These findings emphasize the previously discussed problems (2, 3, 5) of streptococcal phage receptors and of prior assumptions of streptococcal group specificity for phages. They also make possible, by transduction and lysogenization (2) studies, more meaningful investigations of variations in cell surface antigens and other components among streptococcal serogroups.

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