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Isolation of Bacteriophages from Group H Streptococci

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Temperate phages were isolated from 5 of the 17 strains of group H streptococci tested.

Streptococci assigned serologically to group H (9) are usually designated as *Streptococcus sanguis* (3-5, 15) and include strains that are highly competent for deoxyribonucleic acid (DNA)-mediated transformation (14, 15). However, no bacteriophages originating from, nor infectious for, such streptococci have been previously reported. It has not been possible, therefore, to utilize genetic interchange by transfection (7) in study of competence, to utilize phage-mediated transduction (18) as in group A streptococci (11) nor to assess the effects of the lysogenic stage on host characteristics as in group A streptococci (17). In a survey which included other putative group H strains, in addition to the few commonly used in transformation studies, we employed mitomycin C as an inducing agent (12) and isolated temperate phages from 5 of the 17 strains tested.

The streptococcal strains, with one exception, were obtained from Roman Pakula (Department of Microbiology, School of Hygiene, University of Toronto, Canada). The exception, strain K208 of Lancefield (10), was obtained from the American Type Culture Collection, Rockville, Md. All strains are listed in Table 1, column 1, together with their code and numbers and original collection sources where known. In the reference column are listed those publications in which a given strain and some of its characteristics (although not always its source) are given.

All incubations were carried out at 37 C. For tests of bacteriophage induction, streptococci were first grown overnight in P broth (8). A 5% inoculum (v/v) of this in fresh P broth was incubated for 1 hr. Mitomycin C was then added to a concentration of 0.1 μg/ml. After further incubation for 120 min (during which lysis was grossly detectable only in those cultures from which phages were subsequently recovered), residual bacteria and debris were sedimented at 12,000 × *g* for 25 min. Each supernatant fluid was tested for lytic activity by spotting 0.3 ml on confluent bacterial growths of the 17 streptococcal strains, each of which was prepared by inoculation on P broth agar (8) 2 hr previously. The plates were incubated overnight.

By this method, the mitomycin-induced lysates of 4 strains (FW227, 8684, E91/46, and Channon) produced areas of lysis on a number of lawns (Table 1). Similarly treated supernatant fluids from the other strains failed to produce lysis on any lawns.

The lytic supernatant fluids of the five strains shown at the top of Table 1, as well as several of those not showing lysis, were centrifuged at 80,000 × *g* for 180 min. Pellets were suspended in 0.25 ml of 1% (w/v) ammonium acetate, resulting in approximately 100-fold concentration of the original fluid. The suspensions, negatively stained on Formvar-coated copper grids with neutral 2% (w/v) potassium phosphotungstate, were examined in a Hitachi electron microscope (model HU-11C) operated at 75 kv. Those from the five lytic supernatant fluids contained abundant bacteriophages of Bradley's type B (14), which were frequently heavily adsorbed to cell wall fragments (Fig. 1). Their appearance and dimensions were very similar to those of other streptococcal phages that have been described (6). No phages were seen in supernatant fluids of six cultures which failed to lyse during mitomycin C treatment and which failed to produce lysis on any lawns. Phage was abundantly present in lysates of strain FW225 but failed to lyse any streptococci tested (Table 1).

As indicated in Table 1, the phages induced from strains FW227, E91/46, and Channon were also capable of growing in, and producing lytic plaques by dilution on, strain Wicky. The necessary conditions were freshly poured plates and use of early- to mid-log bacteria as indicator strains. Plates of P broth with 1.5% agar were poured 2 hr before use. They were then overlaid with P broth-soft agar (0.75%) which had been mixed with a dilution of the mitomycin lysate or supernatant fraction to be tested, and with 0.2 ml of the presumptive indicator strain. Plaques,

TABLE 1. *Lysis on group H streptococcal confluent growth by mitomycin C-induced lysates of group H streptococci*

Streptococci		Lysis[a] on mitomycin C lysate of					References
Strain	Location and other designation	FW227	8684	E91/46	Channon	FW225	
FW227	WFI[b]	−	−	+	−	−	3
8684	Unknown[c]	−	−	+	−	−	
E91/46	NCTC 7870[d]	+	+	−	−	−	15
Channon	NCTC 7869	−	−	+	−	−	15
FW225	WFI	−	−	−	−	−	3, 4
Challis	NCTC 7868	+	+	+	+	−	3–5, 16
Wicky	NCTC 9124	+[e]	+	+[e]	+[e]	−	4, 16
Blackburn	NCTC 10231	+	+	+	+	−	5, 13
SBE I/II	P[f]; ATCC 10558[g]	+	+	+	+	−	14–16
5042/48	383H[d]	+	+	+	+	−	
F90A	ATCC 12396	+	+	+	+	−	4, 16
RB742	WFI	+	+	+	+	−	3
FW229	WFI	+	+	+	+	−	3, 4
3437/48	NCTC 7872	+	+	+	+	−	15
FW35	WFI	−	−	−	−	−	3
84A	Unknown[c]	−	−	−	−	−	3
K208	ATCC 8144	−	−	−	−	−	3, 4

[a] Plus indicates lysis; minus indicates no lysis.
[b] Wright-Fleming Institute, London.
[c] Possibly Wright-Fleming Institute.
[d] National Collection of Type Cultures, Colindale, London.
[e] Also propagated and plaqued on this strain.
[f] Institut Pasteur, Paris (source of Pakula's strain; reference 16).
[g] American Type Culture Collection, Rockville, Md.

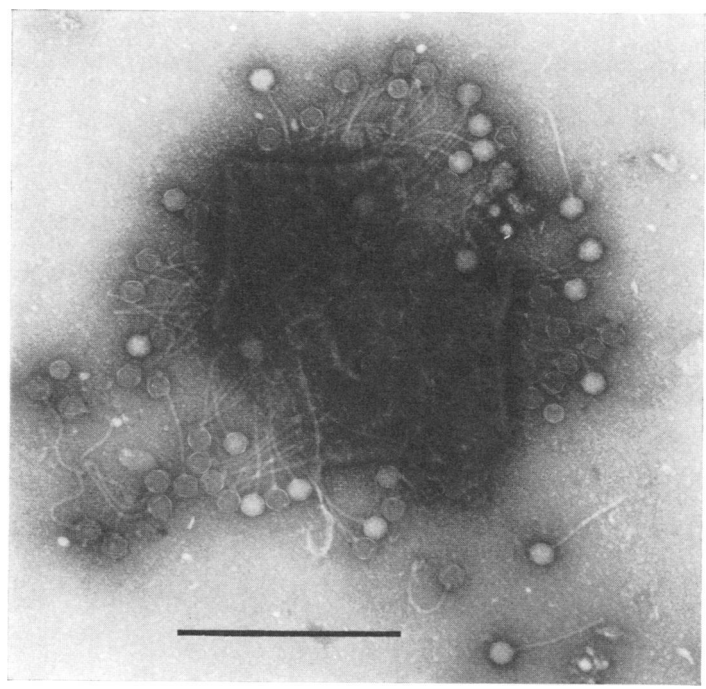

FIG. 1. *Phage readsorbed to cell wall fragments in mitomycin C-induced lysate of strain Channon. Negatively stained with 2% potassium phosphotungstate, pH 7.0. Magnification ×57,500. Marker = 500 nm.*

when present, were turbid and were visible after overnight incubation. If the 1.5% agar plates were made several days in advance and stored at 4 C, or if the indicator strain culture was past the mid-logarithmic phase of growth, results were variable and usually negative, even on strains otherwise positive. By use of these optimal conditions, only strain Wicky has been shown to propagate the three above-mentioned phages, but extensive testing of all strains under varying conditions is not complete. The phage induced from strain 8684 has not yet been propagable on any hosts under any conditions.

As titrated on strain Wicky, the mityomycin-induced lysates of the three propagable strains contained between 10^8 and 5.0×10^8 plaque-forming units (PFU) per ml. Each phage was serially isolated three times from a single plaque and propagated by the described soft agar technique (1). When this method achieved titers of 10^9 PFU/ml, propagation was continued in liquid culture as follows. A 5% (v/v) inoculum from an overnight culture of strain Wicky was made into P broth previously warmed to 37 C. Forty minutes later, phage was added to a final concentration of 10^7 PFU/ml, giving a multiplicity of 1:10 (PFU: colony-forming units). After 2 hr of incubation, chloroform (5%, v/v) was added, and the mixture was shaken vigorously for 3 min to kill the bacteria. The chloroform was allowed to settle out, the broth layer was decanted and centrifuged at 12,000 $\times$ *g* for 30 min, and the phage-containing supernatant fluids were kept for stock. These contained phage titers of 2.0×10^9 to 6.0×10^9 PFU/ml, as determined on strain Wicky.

Preliminary results with some of these phages or with their DNA indicate that lysogeny and transfection of competent cells can be achieved. Studies on details and consequences of these phenomena, as well as on possible transduction, are in progress.

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