Transduction in Group A Streptococcus

Carmen Gomez Leonard, Alba E. Colón, and Roger M. Cole

National Institutes of Health, Bethesda, Maryland

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Transduction, a phage mediated genetic transfer, has been reported to occur in several species of bacteria (Hartman and Goodgal, 1959; Thorne, 1962; Allen et al., 1963).

This report presents evidence for the transduction to streptomycin resistance of Group A streptococcus by several phages. To our knowledge transduction has not been reported previously in Group A streptococcus.

Materials and Methods

Streptomycin-sensitive streptococci belonging to serological group A were employed. Strain 61 X 101, type 12 (K 56) was obtained from Dr. Ebbe Kjems (Staatenserum-institut, Copenhagen). Strain 56 X 188, type 6 and strain 60 X 80, type 11 were isolated in our laboratories from throat cultures. Strain 60 X 298, type 13, strain 52 X 32, type 25, and strain 60 X 303, type 25 were obtained from the Communicable Disease Center, USPHS, Atlanta. Strain 650, type 1 was isolated by Dr. Griffith in 1930. Mutants resistant to 2 mg streptomycin per ml were isolated from strain 56 X 188 by exposure to streptomycin and from strain 61 X 101 by isolation of a streptomycin-resistant transductant.

The medium used for cell production, phage propagation and for transduction experiments was that described as P-broth by Friend and Slade (1966): it was composed of Brain Heart Infusion broth (Difco), 0.2% yeast extract (Difco), 10 μg DL-tryptophan per ml, and 10^{-4} M $CaCl_2$. Solid media were prepared by adding 1.5% (P-hard agar) or 0.75% (P-soft agar) Difco Agar to P-broth.

Stock cultures were maintained on slants of Trypticase Soy agar (Baltimore Biological Laboratory) reinforced with 5% sheep blood. The cultures were transferred to P-broth prior to their use.

Both strain 61 X 101 and strain 56 X 188 were used as indicators of the various phages tested. Temperate phages 60 X 298, 61 X 100, and 60 X 80 were isolated after chloroform induction of their respective cultures. Virulent phages 12203 (A6), 12204 (A25), and 12206 (A5) came originally from Mr. W.R. Maxted's collection (National Collection of Type Cultures, London, England) and the numbers in parenthesis are those given by Mr. Maxted.

Phage was propagated and titrated by the agar layer technic (Adams, 1959) using either streptomycin-sensitive or streptomycin-resistant cells. The cells for propagation of the phage were grown in P-broth for 4 hours, then the cells were mixed with phage using a phage to cell ratio of 10:1 (10^8 PFU to 10^7 cells) in 3 ml of P-soft agar, and the mixture was poured over P-hard agar plates. The plates were incubated overnight. The phage lysate was harvested in P-broth, centrifuged, and filtered (Millipore filter, 0.45μ). The phage suspensions were then plated on P-hard agar to insure they were free of cells.

For propagation and titration of virulent phages, hyaluronidase (Nutritional Biochemical Corp.) was added to the medium (Maxted, 1955).

Phage antiserum was prepared in New Zealand white rabbits by intravenous injections of phage three times per week. This treatment was repeated for three weeks. Sera were collected a week after the last injection. Neutralization of phage by phage antiserum was done as described by Adams (1959).

The following procedure was used in plate transduction experiments: cultures were grown for 18 hr. in P-broth using a 10% inoculum. Then 1 ml of culture suspension was mixed in test tubes with 3 ml of P-soft agar and varying amounts of phage suspension. The tubes were mixed and poured over P-hard agar plates and incubated for $3\frac{1}{2}$ hr. The plates were overlaid with 8 ml of Brain Heart Infusion Agar (Difco) containing 300 μg dihydrostrepto-

mycin per ml. The transductants to streptomycin resistance were scored after 40 hr. incubation at 37 C.

Tube transduction was done as follows: the cultures were grown as described above, then 1 ml was mixed in test tubes with varying amounts of phage suspension and 0.5 ml normal rabbit or horse serum. The tubes were incubated for 25 min, then 3 ml of P-soft agar were added and the mixture poured over P-hard agar plates. Transductants were selected for and scored as described above.

Appropriate controls of cells without phage and of cells with phage propagated on strain 56 X 188 or 61 X 101 streptomycin-sensitive were always included.

Deoxyribonuclease preparations and other materials were those previously described (Leonard et al., 1967).

Results and Discussion

Several temperate and virulent phages were isolated by us or were obtained from various sources as described under Materials and Methods. These phages were screened for their transducing ability against various strains of group A streptococci using the plate transduction method. Of the various strains tested with these phages (strains 56 X 188, 52 X 32, 60 X 303, 60 X 80, 650, and 61 X 101) only strain 61 X 101 was transduced.

Table 1 shows the results of plate transduction of strain 61 X 101 by various phages propagated on strain 56 X 188 S^R (streptomycin-resistant). This preliminary screening showed that a temperate phage, 60 X 298, and two virulent ones, 12204 and 12206, were the most efficient transducing phages under the conditions tested. Further testing is needed to develop optimum conditions for phage propagation and for transduction by each of these phages.

Table 2 shows plate transduction of 61 X 101 by different preparations of phage 12204 propagated on strain 56 X 188 either resistant (S^R) or sen-

Table 1. Plate transduction of group A streptococcus, strain 61 X 101 by different phages*

Phage	Ratio of PFU to cells	Transductants per plate	Transductants per PFU
12203	50:1	37	1.4×10^{-8}
12204	5:1	250	1.0×10^{-6}
12206	10:1	73	1.5×10^{-7}
60 X 80	60:1	5	1.7×10^{-9}
60 X 298	10:1	111	2.2×10^{-7}
650	10:1	0	
61 X 100	10:1	0	

*The phages were propagated on strain 56 x 188 S^R. The recipient cells (5×10^7 cells per plate) were grown in P-broth for 18 hr. Controls of recipient cells alone and of cells with the various phages propagated on strain 56 X 188 S^S gave no colonies.

Table 2. Plate transduction of group A streptococcus, strain 61 X 101 by phage 12204*

Phage prepn.	Strain used for phage propagation	Ratio of PFU to cell	Transductants per plate	Transductants per PFU
P-1	56 X 188 S^R	5:1	246	1.0×10^{-6}
P-3	"	2:1	25	2.5×10^{-7}
P-7	"	5:1	85	3.3×10^{-7}
P-8	"	20:1	119	1.2×10^{-7}
P-11	"	40:1	121	6.0×10^{-8}
P-12	"	16:1	78	1.0×10^{-7}
P-16	"	40:1	57	2.8×10^{-8}
P-17	"	4:1	35	1.7×10^{-7}
P-18	"	40:1	65	3.2×10^{-6}
P-19	"	3:1	24	1.8×10^{-7}
P-1S	56 X 188 S^S	1000:1	0	
"	"	100:1	0	
"	"	40:1	0	
"	"	20:1	0	
"	"	5:1	0	
"	"	1:10	0	

*The phage was propagated on strain 56 X 188 streptomycin-resistant or streptomycin-sensitive. The recipient cells (5×10^7 cells per plate) were grown in P-broth for 18 hr. Controls of recipient cells alone and of phage alone gave no colonies.

sitive (S^S) to streptomycin. Recipient cells without phage or with varying amounts of phage (10^6 to 10^{10} PFU per plate) propagated on 56 X 188 S^S gave

no colonies. Streptomycin-resistant colonies grew only from the samples which contained recipient cells and phages grown on 56 X 188 S^R, indicating that transduction had occurred. The number of transductants found was proportional to the amount of phage added. The highest efficiency of transduction obtained to date has been 3.4×10^{-6} per PFU. In all, 22 preparations of phage 12204 (propagation in 56 X 188 S^R) have been tested and all have transduced strain 61 X 101.

Additional proof that this genetic transfer is mediated by phage and is indeed transduction was obtained by the addition of deoxyribonuclease to the transducing system. No inhibition of the number of colonies was obtained indicating that free DNA was not involved and therefore it was not transformation. On the other hand, Table 3 shows that addition of antiserum to phage inhibited this genetic transfer. Table 3 also shows that this antiserum combined with phage 12204, since it inhibited plaque formation.

Table 3. Inhibition of transduction and of plaque formation by phage antiserum.*

Phage 12204 PFU per plate	Transductants per plate		Plaques per plate	
	Normal serum	Phage antiserum	Normal serum	Phage antiserum
2.5×10^8	270	0	>1000	0
2.5×10^7	32	0	>1000	0
1.0×10^5	0	0	>1000	0
1.0×10^3			1000	0
1.0×10^2			112	0

* One ml of pre-immune serum or antiserum to phage 12204 plus 0.1 ml of phage 12204 (2.5×10^9 PFU per ml) were held 25 min at 37 C. The mixture was then diluted in P-broth and tested for transduction and for plaque formation with cells of strain 61 X 101.

Transduction of strain 61 X 101 by phage 12204 was also obtained by the tube transduction method. Recently we found that the addition of rabbit or horse serum to this mixture resulted in much better transduction. Under these conditions, serum probably enhanced hyaluronic acid capsule formation during growth of the recipient cells. Maxted (1955) showed that the presence

of this capsule partially inhibited adsorption of virulent phages, since these phages do not produce any hyaluronidase. When we added hyaluronidase to the transducing mixture of phage 12204 and cells of strain 61 X 101 (either by the plate or tube transduction method) few or no transductants were obtained. It seems probable that formation of the capsule during growth of the transductants protects them from further infection and lysis by any new phage released into the transducing mixture.

Transformation or other systems of genetic exchange has not been reported to occur in group A streptococcus (Perry and Slade, 1966). The availability now of a transducing system makes feasible genetic studies on virulence factors such as M protein, capsules, and also of many other antigens and properties of these organisms.

Further work is in progress to obtain other transducible strains and to obtain higher efficiencies of transduction.

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