SEROLOGY AND TRANSDUCTION IN STAPHYLOCOCCAL PHAGE

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ABSTRACT

DOWELL, C. E. (The University of Texas, Dallas) AND E. D. ROSENBLUM. Serology and transduction in staphylococcal phage. J. Bacteriol. **84**:1071–1075. 1962.—A triply lysogenic strain of *Staphylococcus aureus* was shown to carry a serological group B phage capable of transduction. Three typing phages (53, 80, 42D), either belonging to serological group B or having a close association with it, were also shown to have transducing ability. A rapid screening method was used to isolate two new transducing phages, both of which belonged to serological group B. Propagating strain 42B/47C was found to carry a transducing phage that was neutralized by both group B and group F antisera. Nine other phages belonging to serological groups other than group B did not have generalized transducing ability, nor did three group B typing phages that were atypical in their calcium requirement. It was postulated that transducing ability is associated with staphylococcal phages of serological group B and with related phages of group F.

Morse (1959) described the transduction of the streptomycin and novobiocin markers, using typing phage 53. In later work, Ritz and Baldwin (1961) and Pattee and Baldwin (1961) reported transduction by typing phages 29, 52A, 79, 53, and 80. Earlier work in our laboratory (Dowell and Rosenblum, 1961*b*) had shown that only one phage from a triply lysogenic staphylococcal strain was capable of transduction. This phage belonged to staphylococcal serological group B (Rountree, 1949). Since the typing phages used by other workers to transduce also belonged to serological group B, tests were undertaken to determine whether any relationship existed between transduction and phage serology.

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MATERIALS AND METHODS

Media. Trypticase Soy Agar (BBL) containing 0.004 M $CaCl_2$ was used for maintenance of stock cultures, propagation of phage stocks, and phage assays. Soft agar for phage propagation and assay was prepared by adding 0.7% (w/v) agar to nutrient broth containing 0.004 M $CaCl_2$, and dispensed in 2.5-ml amounts. For some transduction experiments, Brain Heart Infusion Agar containing 0.5% (w/v) sodium citrate was employed. Soft agar to be used in certain transduction experiments was prepared by adding 0.7% (w/v) agar to Brain Heart Infusion Broth containing 0.5% (w/v) sodium citrate, and dispensed in 2.5- to 3.0-ml amounts. Sterile Trypticase Soy Broth was used as a diluent in making phage counts, resuspending cells from slants, etc.

Antibiotic media for selection of transductants and mutants were prepared in the usual manner. To guard against loss of antibiotic activity, media containing antibiotics were usually prepared the day before use. The level of antibiotic used was 500 and 2 μg/ml for streptomycin and novobiocin, respectively.

Bacterial strains. The propagating strains of *Staphylococcus aureus* for the staphylococcal typing phages were obtained from John Blair (Hospital for Joint Diseases, New York, N.Y.) and E. T. Bynoe (Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada). Other strains of *Staphylococcus aureus* used in this study were obtained from the stock collection of the Department of Microbiology, The University of Texas Southwestern Medical School, Dallas. Most of these strains were originally isolated from clinical sources.

Antibiotic-resistant mutants were selected by spreading 1–2 $\times$ 10^9 cells on a suitable antibiotic medium and incubating 1 to 2 days at 37 C. Mutant colonies were streaked on antibiotic-free plates, and isolated colonies were tested for antibiotic resistance and stored on slants.

Phages. The typing phages used in this study

were obtained from Drs. Blair and Bynoe. Additional phages were isolated from lysogenic staphylococcal strains by methods previously described (Rosenblum and Dowell, 1960).

Phage stocks were prepared in one of two ways. The agar layer method was routinely used for the propagation of phage stocks (Swanstrom and Adams, 1951; Blair and Carr, 1953). The ultraviolet induction method (Lwoff, Siminovitch, and Kjeldgaard, 1950; Welsch, Cavallo, and Cantelmo, 1953) was adapted for certain experiments to obtain phages from lysogenic strains. In this procedure, the lysogenic strain to be induced was grown to approximately 10^8 cells/ml in 10 ml of Trypticase Soy Broth. The culture was then centrifuged for 15 min at 2,200 $\times$ g and the supernatant fluid discarded. The cells were resuspended in 5 ml of saline, transferred to a petri dish, and the uncovered dish was irradiated for 10 sec at 60 cm from a 15-w General Electric germicidal lamp. After irradiation, the cell suspension was transferred to a sterile tube, and 5 ml of double-strength Trypticase Soy Broth were added. The tube was incubated with shaking at 37 C for 3 hr; then, after remaining overnight at room temperature, the lysate was filtered through an ultrafine sintered-glass filter, tested for sterility, and stored at 4 C.

Preparation of phage antisera. Antisera against three serological groups of staphylococcal phages (Rountree, 1949) were prepared in the following manner, using typing phages 6, 29, and 77 as the immunizing agents for serological groups A, B, and F, respectively. Rabbits, usually a group of three for each phage, were injected subcutaneously with 5 ml of high-titer phage filtrate at 5-day intervals for a total of six injections each; 1 week after the last injection, a test rabbit was bled from the ear vein and a phage neutralization test (Rountree, 1949) was performed on the serum. If the test gave a satisfactory neutralization titer (1:1,000 or better), 50 ml of blood were taken by cardiac puncture from each rabbit and 50 ml of saline were injected intraperitoneally. The next day, the rabbits were exsanguinated and the sera from both bleedings were stored in the frozen state in screw-cap tubes.

Density-gradient studies. Analytical density-gradient centrifugation was carried out by the procedures of Weigle, Meselson, and Paigen (1959). Phage stocks in broth were concentrated either by differential centrifugation (Seto, Kaesberg, and Wilson, 1956) or by centrifugation in the no. 40 rotor of a Spinco (model L) preparative ultracentrifuge for 90 min at 10,300 $\times$ g. The final phage pellet was resuspended in a suspension medium described by Weigle et al. (1959). A portion of phage (1–7.2 $\times$ 10^{10} particles) in a suspension medium was added to a stock solution of cesium chloride buffered at pH 8.0 with 0.015 M tris(hydroxymethyl)aminomethane to give a final density of 1.50 $\pm$ 0.01 g/cm^3, as determined with an Abbe refractometer. The mixture was centrifuged at 27,690 rev/min at 20 C in a Spinco (model E) analytical ultracentrifuge, and ultraviolet (UV) absorption photographs were taken after 16 hr.

Transduction techniques. Two methods were used to demonstrate transduction in staphylococcal systems.

(A) The liquid culture method (Watanabe and Watanabe, 1959) was used in early studies of the transduction of novobiocin and streptomycin resistance. In this method, 5 $\times$ 10^9 phage of a transducing lysate (grown on an antibiotic-resistant donor strain) were mixed with approximately 10^9 cells of the appropriate recipient strain in 15 ml of adsorption medium (7.5 ml of Trypticase Soy Broth and 7.5 ml of nutrient broth containing 0.008 M $CaCl_2$). The mixture was incubated at 37 C for 6 hr and was plated on antibiotic medium for the assay of transductants. Controls were run in exactly the same manner except that nontransducing phage (grown on an antibiotic-sensitive donor) was employed. In some cases, an equal number of uninfected cells was plated as a control. Assays of phage adsorption and surviving cells were also made. Transductant colonies were counted after incubation for 1 to 2 days at 37 C.

(B) Morse (1959) noted that approximately 50% of novobiocin-resistant transductants are expressed immediately. We took advantage of this fact in designing a simple test for the transduction of novobiocin resistance. A number of phage particles (usually 1–5 $\times$ 10^8) were mixed with approximately 10^9 cells of a suitable recipient strain in 1 ml of adsorption medium (see above). After a 25-min adsorption period at 37 C, the total mixture was plated in nutrient broth-soft agar on Trypticase Soy Agar plates containing novobiocin. Transductant colonies were enumerated and controls were prepared as described in method A.

RESULTS

Transducing phage from a triply lysogenic strain. An induced lysate from a lysogenic staphylococcal strain gave variable positive results in the transduction of streptomycin resistance. Closer examination revealed that this strain carried at least three phages. The results of transductional experiments with these phages (designated T, D, and C) are presented in Table 1. It is evident that only phage C is capable of generalized transduction. This phage belonged to serological group B; phages T and D were not members of groups A, B, or F.

Isolation of new transducing phages by means of the "Accu-Drop" spotter. The use by Hartman (*unpublished data*) of the "Accu-Drop" unit (The Sylvania Co.) in studying complementation in *Salmonella* suggested a method for detecting transducing phage. Preliminary experiments had shown that a cluster of novobiocin-resistant colonies developed within the area on a novobiocin agar plate where transducing phage C had been spotted on a sensitive strain. It thus seemed possible that additional transducing phages might be detected by a similar means.

Accordingly, novobiocin-resistant mutants were derived from 24 staphylococcal strains which represented all phage-typing groups and also included four nontypable strains. The novobiocin-resistant mutants were induced with UV irradiation, and the lysates were sterilized by filtration. Eighty-five staphylococcal strains were grown 4 to 5 hr in 2 ml of Trypticase Soy Broth, and individually spread on novobiocin agar plates. Each plate was then spotted with the 24 lysates by means of the "Accu-Drop" unit.

The plates were incubated at 37 C and examined after 1 to 2 days for clusters of novobiocin-resistant colonies. Two lysates gave such clusters. These phages, designated 5 and 15, were derived from propagating strain 75 and strain 2676. Serological studies showed that the number of transductions obtained with these lysates was markedly reduced by pretreatment of these phages with serological group B antiserum. For both phages, some of the recipient strains transduced were not sensitive to lysis by the phage preparation. The method described has the disadvantage of not detecting transducing phage that are noninducible and are therefore present in only small amounts in the induced lysates, but it does, however, permit the rapid screening of a large number of induced lysates for transducing activity against many possible recipient strains.

TABLE 1. *Results of transductional studies with phages T, D, and C**

Phage	Colonies on 2 μg novobiocin/ml agar		Colonies on 500 μg streptomycin/ml agar	
	Experimental	Control	Experimental	Control
T	62	59	29	22
D	30	25	12	22
C	26,150	15	257	17

* Recipient strains sensitive to <10 μg streptomycin/ml and to 0.2 μg novobiocin/ml. Experiments were performed as described in transduction method A.

Isolation of phage β. Ritz and Baldwin (1961) reported that "mutants" of phages 42B, 47C, and 52 were capable of transduction. Since phages 42B and 47C belong to serological group A and since we had been unable to show transduction with them, we examined the propagating strain of these phages (P. S. 42B/47C) for the presence of a carried phage which might be the "mutant" described.

A novobiocin-resistant mutant was isolated from P. S. 42B/47C, and a UV-induced lysate was prepared. Two different phages could be demonstrated in this preparation. One phage, a turbid plaque-former, seemed devoid of transducing activity, but the other phage, a clear-plaque type, was able to transduce novobiocin resistance; this phage was designated β. The results of a transduction experiment with β and typing phages 42B and 47C are presented in Table 2. Serological studies of a single plaque isolate revealed that phage β is neutralized by both B and F antisera. The results suggest that the "mutant" phage isolated from typing phages 42B and 47C by Ritz and Baldwin (1961) may in reality be a phage carried by the propagating strain for these phages.

Transduction by staphylococcal typing phages. Because of the strong reaction of phage β with group F antisera, transduction with two group F typing phages (42D and 77) was attempted. Typing phage 42D is a serological variant of a phage originally belonging to group B (Rippon, 1952). The results of these studies showed that only phage 42D was able to transduce novobiocin resistance. In addition, four group A and four

TABLE 2. *Transduction experiments with phages β, 42B, and 47C**

Phage	Novobiocin-resistant colonies	
	Experimental	Control
47C	13	6
42B	6	4
β	116	1

* Experiment performed as described in transduction method B, except that 1.56×10^7 particles of phage β were used.

TABLE 3. *Transduction by staphylococcal typing phages**

Phage	Serological group	Total colonies on novobiocin agar	
		Experimental	Control
54	A	15	25
81	A	45	85
3C	A	0	0
3A	A	0	0
44	B	0	0
31	B	0	0
53	B	1,675	5
42C	B	15	100
42D	F	94	23
77	F	2	18

* Experiments performed as described in method A, except 42D and 77, tested by method B.

group B typing phages (including phage 53) were tested for their ability to transduce novobiocin resistance (Table 3). Of the eight group A and B phages tested, only phage 53 was capable of transduction. Typing phage 80 (serological group B) was later tested and found to have transducing activity, as reported by Ritz and Baldwin (1961). The three group B phages that did not transduce (42C, 44, and 31) differ from most other group B phages in that they are able to lyse cells on Brain Heart Infusion Agar. Ritz and Baldwin (1961) reported that Brain Heart Infusion or Heart Infusion Agar is necessary for the detection of transductants. We have found that Brain Heart Infusion Agar acts by selectively inhibiting the adsorption of group B phages, owing to lack of calcium available in the medium (Dowell and Rosenblum, 1961*a*). A summary of all tests for transducing ability is given in Table 4.

Density-gradient studies. The buoyant densities of phages C and 53 were compared with the densities of nontransducing phages from serological groups A (typing phage 54) and B (phage 44). The buoyant densities of phages C and 53 were the same and significantly greater than that of phage 54. Phage 44, however, had the same density as phages C and 53. There seems to be no correlation, therefore, between buoyant density and transducing ability, although buoyant density and serology may be correlated. Additional phages should be examined, however, to confirm this possibility.

TABLE 4. *Summary of tests of transduction with staphylococcal phages*

Serological group	Phages
Transducing phages	
B	C, 15, 5, 53, 80 (29, 79, 52A),* (52)†
B and F	β
F	42D (derived from B)
Nontransducing phages	
A	42B, 47C, 54, 81, 3A, 3C
B	44, 31, 42C
F	77
Not A, B, or F	T, D

* Pattee and Baldwin (1961); not tested by us.
† Ritz and Baldwin (1958); not tested by us.

DISCUSSION

For quite some time it has been evident that staphylococcal phages do not comprise a homogenous group (Burnet and Lush, 1935). Probably the most significant division of these phages has been made on the basis of serology (Rountree, 1949; Rippon, 1956), which seems to reflect antigenic differences in the protein coat and is also correlated with differences in size (Seto et al., 1956; Ortel, 1959), calcium requirements (Rountree, 1951), stability (Rountree, 1949; Rippon, 1956), and other properties.

The present results indicate that transducing ability is also associated with serology, being a property primarily of group B phages. Edgar and Stocker (1961) also reported transduction by phage 53 and "other typing phages serologically related to it." With the limited number of phages tested, however, it cannot be stated that this is the only group that contains competent transducing phage. Typing phage 42D, one of the transducing phages described, is a serological group F but it originated from a group B phage

(Rippon, 1952). Phage β, neutralized by both B and F antisera, also suggests a close relationship between B and F and the possibility that phages of serological group F might also be capable of transduction. Indirect evidence in support of the relationship between serology and transduction is the fact that the two additional phages isolated by the screening procedure were group B, although all or almost all the lysates contained inducible phage. The reason for the association between serological group and transducing ability has not been established.

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