Brief Definitive Report

TRANSDUCTION OF BACTERIOCIN DETERMINANTS IN GROUP A STREPTOCOCCI*

By JOHN R. TAGG,‡ STEPHEN SKJOLD, AND LEWIS W. WANNAMAKER§

(From the Departments of Pediatrics and Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455)

Investigations of gene transfer between Group A streptococci are at present limited to the use of transduction. Since the prototype studies by Leonard and associates (1) on the transducibility of streptomycin resistance there have been a number of reports of the transfer of resistance markers to antibiotics (2–10). Recently, Malke (9) has also shown that the virulent phage A25 is able to transduce the genome of P56188, an unrelated temperate bacteriophage. Continued progress in the genetic analysis of Group A streptococci is to a great extent dependent upon the identification and utilization of new marker genes. In previous studies we have reported the production, purification, and physicochemical characteristics of streptococcin A-FF22 (SA), a bacteriocin of Group A streptococcus strain FF22 (11, 12). Indirect evidence has suggested that the genetic determinants of SA production and producer cell immunity may be plasmid borne.[1] In the present study we demonstrate transduction of these determinants to three different recipient strains.

Materials and Methods

Phages and Bacteria. The virulent transducing phage A25 was provided by Dr. W. R. Maxted, Central Public Health Laboratory, Colindale, England, and its double temperature-sensitive mutant A25ts1-2 (2) was received from Dr. H. Malke, Institut für Mikrobiologie und experimentelle Therapie der Deutschen Akademie der Wissenschaften, Jena, E. Germany. Group A streptococcus strain FF22 (SA^+) (type M-52) (11) and its spontaneously cured (SA^-) derivative strain SPON1[1] have been used in previous studies, as have Group A streptococci strains K56 (type M-12) and GT-9440 (type M-6) (6). Spontaneous mutants resistant to 2 mg/ml streptomycin were selected from the appropriate strains. *Micrococcus luteus* was used as the indicator organism in the assay of SA activity (13).

Media. The composition of the media has been described previously (6). No. 1 broth was used for phage propagation and no. 3 broth for growing the recipient strains in transduction experiments. Triple-layer plates used for the selection of transductants contained in the top layer either 1,500 μg/ml streptomycin or 15 U/ml of SA (12).

Phage Propagation and Transduction Procedures. Phage lysates were prepared and titered by previously described techniques (6).

The plates, designated as N6 plates, used for titering phage were modified (P. Cleary, Z.

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‡ Career Investigator Fellow of the American Heart Association. Present address: Department of Microbiology, University of Otago, Dunedin, New Zealand.

§ Career Investigator of the American Heart Association.

[1] Tagg, J. R., and L. W. Wannamaker. 1976. Studies of the genetic basis of streptococcin A-FF22 production. *Antimicrob. Agents Chemother.* In press.

Johnson, and N. Laible, personal communication) from those previously described for use in host range experiments (6). They contained proteose peptone no. 3 (Difco Laboratories, Detroit, Mich.), 4%; NaCl, 0.6%; yeast extract (Difco Laboratories), 2%; Na_2HPO_4, 0.07%; agar, 1%; $CaCl_2$, 0.02%; glucose, 0.05%; and hyaluronidase (Type 1 from bovine testes; Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 68 μg/ml. The propagation of A25 phage on strain FF22 was facilitated by predigestion of bacteria with hyaluronidase and adsorption of phage at concentrated amounts of host cells and phage. 5 ml of an overnight culture of the propagating strain grown at 30°C in no. 1 broth (3×10^8 colony-forming units/ml) were treated with hyaluronidase (Sigma Chemical Co.), final concentration 450 μg/ml, in a water bath at 29.5°C for 15 min. 8 ml of filtered phage lysate in no. 1 broth (1×10^9 plaque-forming units/ml) were added and incubation was continued for an additional 30 min. Then no. 1 broth was added to a total vol of 100 ml and incubation was continued for another 4–5 h.

The optimal conditions for transduction of SA were similar to those used for the transduction of streptomycin resistance in earlier studies (6). Ultraviolet irradiation was for 8 min at an intensity of 7,000 ERG/s/cm^2. Transduction by phage A25ts1-2 was conducted at the nonpermissive temperature 37.5°C (2). Controls consisted of the transducing lysate alone, the recipient streptococci alone, and in some experiments the use of lysates prepared on streptomycin-sensitive (str^S) or non-SA-producing (SA^-) donor strains. To examine the possibility that free DNA was responsible for the observed genetic changes, in some experiments donor phage lysates were pretreated with 50 μg/ml DNase I (Worthington Biochemical Corp., Freehold, N. J.) at 37°C for 30 min before testing for transducing ability.

Test for Transductants. The selection of SA^+ transductants was initially on the basis of their host cell immunity to the homologous bacteriocin. Transductants growing in the presence of SA were then examined for ability to produce SA (SA^+ phenotype) by picking onto a plate seeded with *M. luteus* and observing the production of inhibition zones.[1] The selected transductants for SA^+ and also for streptomycin resistance (str^R) were replica picked onto both streptomycin plates and *M. luteus*-seeded SA indicator plates to determine the frequency of cotransduction of str and SA markers. The stability of transductants was tested by first streaking individual SA^+ clones onto blood agar plates to obtain single colonies. Confirmed SA^+ (or str^R) subclones were then restreaked for single colony isolation and three SA^+ (or str^R) colonies were dispersed in 1-ml vol of saline and streaked onto blood agar. Individual colonies were tested (culture 1 in Table II) and one positive clone from each of the three lines was dispersed in saline and restreaked as above. This procedure was repeated four times to give the serial cultures numbered 2–5 in Table II.

Results

By use of phage A25 it was possible to transduce the determinants of SA and str^R from the donor Group A streptococcus strain FF22 (SA^+ and str^R) to either its spontaneously cured (SA^-) derivative, SPON1, or to the unrelated strains, GT-9440 and K56 (Table I). The frequency of transduction of SA^+ was of the same order of magnitude or slightly less than that of str^R. No co-transduction of SA^+ and str^R determinants was observed in any of the individual SA^+ or str^R transductants.

Controls established that the transducing lysates were bacteria free and also that both the lysate and the recipient streptococci must be present to give rise to SA^+ derivatives. No mutation to SA^+ was observed in recipient cultures, but some low frequency mutation to str^R was observed and was corrected for in the tabulated results. Lysates prepared on SA^- strains failed to give rise to SA^+ transductants. Serological typing confirmed the identity of SA^+ or str^R transductants with the appropriate recipient strains. When phage lysates were pretreated with DNase, transduction of the SA^+ marker was still observed.

Variation of the time of irradiation of the phage lysate from 0 to 16 min indicated that the optimal time for subsequent transduction of both SA^+ and str^R determinants was approximately 8 min, this dose inactivating 90–99% of the

TABLE I
Transduction Experiments

Donor strain	Phage	Recipient strain	Transduction frequency*	
			Streptococcin A-FF22	Streptomycin resistance
FF22 str^R SA^+	A25	SPON1	1.4×10^{-8}	3.0×10^{-8}
		K56	1.1×10^{-8}	5.0×10^{-8}
		GT-9440	5.0×10^{-8}	ND‡
GT-9440 str^R SA^+	A25	K56	3.0×10^{-8}	ND
	A25ts1-2	K56	4.0×10^{-6}	5×10^{-6}

* Transductants per plaque-forming unit.
‡ ND, not done.

phage as determined by plaque-forming ability. Use of an SA^+ transductant of strain GT-9440 as the donor in transduction experiments gave a slightly higher transduction frequency of SA^+ (Table I). Furthermore, when the temperature-sensitive phage A25ts1-2 was used in this system a 100-fold increase in the transduction frequency occurred.

Tests of the stability of the various SA^+ transductants (Table II) indicated that whereas SPON1 SA^+ strains appeared to be quite stable, SA^+ transductants of K56 and GT-9440 gave rise to both SA^+ and SA^- progeny. There was no evidence of reversion of the SA^- segregants to SA^+. Growth of the K56 SA^+ or GT-9440 SA^+ strains on medium supplemented with bacteriocin (14 U/ml) selected for SA^+ organisms. Similar tests indicated no apparent instability of the str^R transductants of either K56 or SPON1.

Discussion

This study extends the range of Group A streptococcus marker genes that are known to be transducible by phage A25. Determinants of SA production and producer cell immunity have been cotransduced into a spontaneous SA^- derivative of the parent M-type 52 strain and also into two unrelated Group A streptococci of M-types 12 and 6. This emphasizes that SA is independent of the M antigen of the host strain. Transduction of str^R was also demonstrated but clearly was not cotransducible with SA^+ determinants. The demonstrated ability of strain FF22 to act both as a donor and as a recipient in A25-mediated transduction extends the rather limited number of strains able to serve these functions (8). The availability of the SA^+ marker for transduction experiments increases the versatility of this system of genetic exchange.

Strain GT-9440 has previously been shown to be an excellent donor in A25 transduction (6) and this was confirmed in the present study by using SA^+ transductants of GT-9440 as donors in transduction mediated by both A25 and its temperature-sensitive derivative. Use of the double temperature-sensitive phage mutant, A25ts1-2, at a nonpermissive temperature greatly increased the frequency of transduction, confirming with this system the importance of protecting transductants from lysis by superinfecting-free phage particles.

The observed enhancement of SA^+ transduction frequency on exposure of lysates to ultraviolet irradiation was of interest, since this has sometimes been taken as suggestive evidence of a chromosomal location for the selected marker (10, 14). This then does not support our previous observations that the SA determinants may be plasmid located.[1] However, as pointed out by Lacey (15),

TABLE II
Frequency of SA$^+$ Derivative Clones Obtained from Serial Cultures of SA$^+$ Transductants

Transduction strain	Colony no.	Culture no.				
		1	2	3	4	5
SPON1 SA$^+$	1	100*	100	100	100	100
	2	100	100	100	100	100
	3	100	100	100	100	100
K56 SA$^+$	1	60	80	30	64	42
	2	24	32	40	36	50
	3	8	24	56	38	80
GT-9440 SA$^+$	1	6	32	56	36	52
	2	36	88	98	90	96
	3	60	64	74	60	80

* Percent SA$^+$ of 50-100 colonies tested.

the ultraviolet effect may be misleading at times and it seems that the final proof of the location of the SA determinants requires more definitive experiments.

The instability of SA$^+$, but not str^R, transductants of heterologous strains points to some basic difference between the two determinants. McKay and associates (16) noted the instability of lactose-positive transductants of *Streptococcus lactis* C2 and suggested that this is supportive evidence of an extrachromosomal site for the determinants of lactose metabolism.

Summary

Determinants of streptococcin A-FF22 (SA) production and host cell immunity have been transduced to three serologically distinct Group A streptococci. Streptomycin resistance markers were not cotransducible with bacteriocin determinants. SA$^+$ transductants of strains unrelated to the parent SA$^+$ strain were unstable but SA$^+$ transductants of a spontaneous SA$^-$ derivative of the parent appeared to be stable.

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