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Vi I Typing Phage for Generalized Transduction of *Salmonella typhi*

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Salmonella typhi **Vi typing phages were used to transduce temperature-sensitive (Ts) mutants of** ***Salmonella typhi*****. Antibiotic resistance and Ts$^+$ markers were transduced at high frequency (>10^{-4} per virulent phage). Several markers were cotransduced by phage Vi I, suggesting that it may be useful for mapping studies of the** ***S. typhi*** **genome.**

It has been more than one century since *Salmonella typhi* was recognized as the causative agent of typhoid fever (7), but the genetics of this microorganism have not been studied systematically, in part because of the lack of a convenient gene transfer system. Baron et al. were the first to demonstrate generalized transduction of *S. typhi* with the typing phage Vi II (2, 3). Mise and coworkers (9, 10) showed that phage j2 of *S. typhi* is a generalized transducing phage similar to P1 of *Escherichia coli*; however, it has a very short half-life, which limits its utility. We recently looked again at the feasibility of using the lytic typing phages for transduction and report here generalized transduction in *S. typhi* by one of them, Vi I.

Bacteria and media. The bacterial strains used in the present study were all derived from *S. typhi* Ty2, which was obtained from the American Type Culture Collection (Rockville, Md.) (Table 1). Temperature-sensitive (Ts) mutants of *S. typhi* that are able to grow at 28°C but cannot complete more than one round of replication at 37°C were isolated following nitrosoguanidine mutagenesis as described previously (11). The location of the Ts lesions on the *S. typhi* chromosome is not known, but the conditions used for the isolation of the mutants favored the selection of strains with Ts mutations in essential genes (such as the genes for DNA polymerase, RNA polymerase, and ribosomal proteins). The reversion rate of each Ts mutation was estimated by plating a large number of cells and incubating them at 37°C. Spontaneous antibiotic-resistant mutants of *S. typhi* were isolated after large numbers (ca. 10^9 CFU) of the wild-type strain were plated on tryptic soy agar containing antibiotics (all from Sigma Chemical Co., St. Louis, Mo.). The concentration of each antibiotic used and the spontaneous mutation rates to resistance are listed in Table 1. Bacteria were grown in LB broth or on LB agar (8); when required, LB agar was supplemented with antibiotics.

Bacteriophages. Vi phages I to V, the gift of H.-W. A. Ackermann (Laval University, Montreal, Quebec, Canada) were propagated on antibiotic-resistant Ts$^+$ *S. typhi* strains in broth culture for 3 h at 37°C in a New Brunswick Scientific environmental shaker incubator. The lysates were pelleted by centrifugation, and the supernatants were sterilized by filtration (0.45-μm-pore-size Nalgene syringe filter; Nalge Company, Rochester, N.Y.).

Plaque assay. Vi I phage formed tiny plaques (ca. 0.1 mm in diameter) on *S. typhi* Ty2 suspended in soft LB agar layers. In preliminary experiments, we determined that the latent period was about 15 min and that the burst size was approximately 10 (data not shown). The numbers of PFU harvested were never higher than 5 × 10^7/ml, and the addition of 2.5 mM $CaCl_2$ to the medium did not improve the titer. However, the number of virulent phage must have been at least 2 log units higher, because when estimated multiplicities of infection of 10^{-2} were used in preliminary transduction experiments, 98% of the bacteria were killed. We reduced the bacterial killing to about 50% by using an apparent multiplicity of infection of 10^{-4} (with an estimated 2 × 10^7 virulent phage in each transduction mixture) and by keeping the transduction mixture on ice after the attachment incubation until the samples were transferred to melted agar. On this basis, we calculated the titer of virulent phage as the PFU titer × 2,000 in all lysates.

Transduction protocol. Equal volumes (0.4 ml) of bacteria (10^8 CFU/ml) and phage (apparent titer of 10^4 PFU/ml [but an estimated 2 × 10^7 virulent phage] pooled from eight lysates of the antibiotic-resistant Ts$^+$ *S. typhi* strains) were mixed, and the mixture was incubated for 10 min at 37°C. Immediately after the attachment period, the mixture was placed on ice to inhibit phage replication; at least three separate samples of 0.1 ml each were added to 10 ml of melted LB agar at 45°C and poured into a sterile petri dish. The agar concentration was increased to 1.6% to minimize infection of adjacent bacteria and loss of potential transductants. The plates were incubated at 37°C for 24 h when transduction of the Ts$^+$ phenotype was desired; when transduction of the antibiotic resistance genes was performed, the plates were incubated at 28°C for 3 h (to allow for expression of the transduced genes) before being overlaid with 10 ml of LB agar containing the appropriate antibiotic. The plates were incubated again at 28°C for an additional 21 h. Bacterial viability was monitored by plating appropriate dilutions of the transduction mixture on LB agar immediately after the incubation for phage attachment. Transduction frequencies were calculated by dividing the number of transductants first by 8 (because the transducing phage were pooled from eight

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TABLE 1. Bacterial strains used in this study

Strain	Antibiotic resistance (concn in μg/ml)	Mutation frequency[a]	Temp phenotype
S. typhi Ty2	Sensitive to all antibiotic tested	NA[b]	+
Sm^r	Streptomycin (300)	6×10^{-9}	+
Rif^r	Rifampin (200)	6×10^{-9}	+
Nb^r	Novobiocin (3,000)	4×10^{-8}	+
Ksg^r	Kasugamycin (500)	3×10^{-8}	+
Nal^r	Nalidixic acid (75)	1×10^{-8}	+
Em^r	Erythromycin (200)	5×10^{-9}	+
Spc^r	Spectinomycin (300)	8×10^{-9}	+
Mgo^r	Methylglyoxal (1,500)	1×10^{-9}	+
Ts mutants[c]	Sensitive to all antibiotics tested	NA	−

[a] The frequency of resistant mutants was calculated from the number of antibiotic-resistant clones per CFU of the wild-type strain plated on medium containing the antibiotic.
[b] NA, not applicable.
[c] Individual Ts mutants of *S. typhi* are listed in Table 2.

lysates) and then by the estimated number of virulent phage in the transduction mix (9, 10).

Fifty-six potentially independent Ts mutants of *S. typhi* were transduced with a pool of equal volumes of Vi I phage propagated on eight antibiotic-resistant Ts$^+$ *S. typhi* strains. Transductants with the Ts$^+$ or one of the eight antibiotic resistance phenotypes were obtained with all of the Ts mutants tested, and both types of gene were transduced at high frequencies (Table 2). Vi II, III, and IV phages also transduced antibiotic resistance and *ts*$^+$ genes into Ts *S. typhi* (data not shown); however, the frequencies were not as high as those seen with Vi I, at least under the conditions that we were using, and we therefore decided to concentrate on Vi I. Transduction experiments with Vi V were unsuccessful.

TABLE 1. Cotransduction of *S. typhi* with Vi I phage

Mutant strain[a]	Spontaneous-revertant frequency[b]	Transduction frequency[c]	Linkage[d]	Percent
A115	10^{-8}	8×10^{-5}	Spc^r	3
A212	10^{-7}	1×10^{-4}	Sm^r	52
A217	10^{-8}	1×10^{-4}	Em^r	24
A335	10^{-8}	1×10^{-4}	Rif^r	44
B4	$<10^{-7}$	1×10^{-4}	Mgo^r	12
	$<10^{-7}$	1×10^{-4}	Ksg^r	2
B8	10^{-7}	1×10^{-4}	Mgo^r	12
B10	10^{-7}	1×10^{-4}	Rif^r	2
B18	10^{-7}	1×10^{-4}	Em^r	2
B19	$<10^{-7}$	4×10^{-4}	Mgo^r	30
B20	$<10^{-7}$	1×10^{-4}	Mgo^r	8
C120	$<10^{-7}$	3×10^{-4}	Nb^r	15
C317	10^{-7}	1×10^{-4}	Rif^r	4
D125	10^{-6}	1×10^{-4}	Mgo^r	92
D25	$<10^{-7}$	1×10^{-4}	Nb^r	20
D219	$<10^{-7}$	1×10^{-4}	Mgo^r	45
E15	$<10^{-7}$	1×10^{-4}	Mgo^r	2

[a] The Ts mutants were isolated from cultures distributed into five different flasks immediately after mutagenesis.
[b] The frequencies of spontaneous revertants were calculated from the numbers of Ts$^+$ clones per CFU of the Ts strain incubated at the nonpermissive temperature.
[c] Transduction frequencies were calculated as the number of transductants per estimated virulent phage. The frequencies shown here are for transduction of the Ts$^+$ marker. Experiments in which antibiotic resistance was selected gave similar results.
[d] Transductants selected at 37°C (Ts$^+$) were sequentially streaked on plates containing antibiotics. The simultaneous acquisition of resistance to an antibiotic was calculated and expressed as percent linkage.

Cotransduction. Each Ts mutant was transduced with a pool of Vi I phage grown on the Ts$^+$ antibiotic-resistant strains. Transductants that appeared after 24 h of incubation at 37°C were sequentially streaked on agar plates containing different antibiotics, and the plates were incubated at 37°C for 18 h. Percent linkage to the antibiotic resistance genes was calculated from the number of antibiotic-resistant clones per 100 Ts$^+$ transductants (after division of the original number of Ts$^+$ transductants to account for the fact that we were using a pooled lysate). In other experiments, antibiotic-resistant transductants scored after incubation at 28°C for 24 h were replica plated, and the plates were incubated at 37 and 28°C to detect cotransduction of the *ts*$^+$ gene.

Cotransduction of the *ts*$^+$ and antibiotic resistance genes allowed us to determine the linkage of the Ts mutations to selectable chromosomal markers in 25 of the 56 strains; 16 of these mutants had different linkage patterns (Table 2). Linkage established by selecting for Ts$^+$ and scoring for simultaneous acquisition of antibiotic resistance was in good agreement with that determined by selecting for antibiotic resistance and scoring for loss of the Ts phenotype.

The first system reported for generalized transduction in *S. typhi* used the typing phage Vi II (2, 3). Subsequently, Mise and coworkers described the j2 phage and its utility in generalized transduction (9). Transduction frequencies in the former studies were on the order of 10^{-6}/PFU, while in the latter they ranged from 6×10^{-6} to 2×10^{-5}/PFU; although these appear to be lower than the frequencies reported in the present study (10^{-4} per virulent phage), it must be kept in mind that the efficiency of the plaque assay that we used leaves much to be desired. The major disadvantage of the j2 system is the very short half-life of the phage (about 3.5 days at 4°C), which would probably limit its practical application. The Vi I preparations used in our study were stable at 4°C for several months without detectable loss of plaque-forming or transducing ability (data not shown).

Transduction of auxotrophic markers (*ΔaroA his Δpur*) into *S. typhi* has been achieved with P22 phage propagated on *Salmonella typhimurium* (4, 6); although this method is useful for transferring genes from *S. typhimurium*, it cannot be applied to the exchange of genetic material between *S. typhi* strains.

Vi phages have long been used for typing *S. typhi* in epidemiological studies (1, 5), and Baron et al. (2, 3) used the typing phage Vi II for generalized transduction of Sm^r and nutritional markers. In the work reported here, Vi I phage transduced different *ts*$^+$ and antibiotic resistance genes at high frequencies, indicating that it is also a generalized transducing phage for *S. typhi*. Cotransduction of *ts*$^+$ and antibiotic resistance genes suggests that the size of the phage would make it useful for mapping studies of the *S. typhi* genome.

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