INFECTION AND IMMUNITY, Feb. 1981, p. 737-741
0019-9567/81/020737-05$02.00/0

Vol. 31, No. 2

General Transduction in *Vibrio cholerae*

JAMES E. OGG,* TERRY L. TIMME, AND MOHAMMAD M. ALEMOHAMMAD†
Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523

Evidence was obtained for general transduction in *Vibrio cholerae*. Transduction of three amino acid markers and three antibiotic resistance characters was demonstrated using strains of biotype *eltor* and biotype *cholerae*. Some of the genetic characters were transduced from a biotype *eltor* donor (and its mutant derivatives) to biotype *cholerae* and *eltor* recipients. For the genetic traits examined, the frequencies of transduction ranged between 10^{-5} and 10^{-8}. Maximal frequencies were obtained with transducing phage lysates that were irradiated with ultraviolet light. The development of a system of general transduction will now aid in fine structure analysis and detailed mapping of the chromosome of *V. cholerae*.

Plasmid-mediated sexual conjugation has been the only reported method of gene exchange and of chromosome mapping in *Vibrio cholerae*, the causative agent of Asiatic cholera (7, 8, 13, 18). More rapid progress could be made on mapping the chromosome of this important human pathogen if gene transfer by transduction were available. General transduction, which has been the principal source of data for mapping *Escherichia coli* K-12 (4) and *Salmonella typhimurium* (19), would permit an accurate analysis of the fine structure of short genetic segments of the chromosome of *V. cholerae* and also make possible more precise placement of genetic markers mapped by conjugational analysis.

While investigating antigenic changes resulting from infection of a biotype *cholerae* strain of *V. cholerae* with phage from a biotype *eltor* lysogen, it was noted that the polymyxin resistance character (a differential characteristic of biotype *eltor*) was expressed in some survivors of a phage-infected culture (17). The results of those studies suggested transduction of the polymyxin resistance trait from the biotype *eltor* donor to the biotype *cholerae* recipient strain.

In this paper we present evidence for general transduction of a number of genetic markers from donor *eltor* strains to recipient strains of either biotype *eltor* or biotype *cholerae*. Ultraviolet (UV) irradiation of transducing phage lysates before their absorption by recipient cells resulted in an increase in the number of transductants appearing on the selection medium, a phenomenon reported to occur with other transducing phage (3, 12). A preliminary report on the results of transduction of the methionine marker and the polymyxin resistance character from a donor biotype *eltor* strain to recipient strains of biotype *cholerae* was presented previously (T. L. Timme, J. E. Ogg, M. Alemohammad, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, D61, p. 48).

† Present address: College of Science, Jundishapur University, Ahwaz, Iran.

MATERIALS AND METHODS

Bacterial strains. The characteristics of the *V. cholerae* strains are given in Table 1. The first three strains listed were described previously (17). The auxotrophic mutants and antibiotic-resistant mutants were derived by mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, according to the general procedure described by Adelberg et al. (2). The stability of the mutant strains was confirmed through several passages in broth and plating on selective medium. Working stocks of the *V. cholerae* strains and their mutants were established from single colonies and maintained on agar slants at room temperature.

Media. The bacteria were normally cultivated at 35°C in L broth (14) with the pH adjusted to 7.6 with NaOH. T1N1 broth (20) plus 1.5% agar was used for the routine plating medium. In preparing an antibiotic selection medium, T1N1 agar was supplemented with either 200 IU of polymyxin B sulfate, 50 μg of rifampin, or 100 μg of streptomycin sulfate per ml of medium. In the overlay technique (1) for phage titrating, T1N1 agar was used; the overlay consisted of T1N1 broth plus 0.7% agar. M9 minimal glucose broth and agar (16), supplemented with required amino acids at 40 μg/ml, were used as the synthetic media. For some experiments M9 agar contained rifampin.

Preparation of transducing phage. Bacteriophage was isolated from the lysogenic strain 1633 by UV induction and single-plaque purified on strain 1621. The progeny of this plaque was designated CP-T1. High-titer transducing phage lysates were prepared by the soft agar overlay technique, using strain 1621 and its mutants. The lysates obtained usually had titers of about 10^{10} plaque-forming units per ml. The phage preserved over chloroform at 4°C were stable for 6 months or longer. The cell-free condition of the phage was determined by placing samples in L broth and plating samples onto T1N1 agar. For some

TABLE 1. *Characteristics of V. cholerae strains*

Strain	Biotype[a]	Phenotype[b]	Source[c]
1621	*eltor*	Prototroph	Nepal
1633	*eltor*	Prototroph	Nepal
029	*cholerae*	Prototroph	Nepal
14035	*cholerae*	Prototroph	ATCC
903	*cholerae*	Met^-	Strain 029
904	*eltor*	Str	Strain 1621
905	*eltor*	Met^-	Strain 1621
906	*eltor*	Met^- Lys^-	Strain 905
907	*eltor*	Met^- Trp^-	Strain 905
908	*eltor*	Met^- Rif	Strain 905

[a] Biotype *eltor* strains are resistant to polymyxin B (200 IU/ml or 50 IU by the disk test); biotype *cholerae* strains are sensitive to the same levels of polymyxin B.

[b] Met^-, auxotroph for methionine; Lys^-, auxotroph for lysine; Trp^-, auxotroph for tryptophan; Str, resistance to streptomycin (100 μg/ml); Rif, resistance to rifampin (50 μg/ml).

[c] ATCC, American Type Culture Collection; Nepal strains (17). Strains 903–908 were derived by mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

experiments phage CP-T1 was propagated on strains 904 and 908.

UV-irradiated phage suspensions were prepared by making appropriate dilutions in 0.67 M phosphate buffer (pH 7.0) and exposing a preparation to doses of UV calculated to reduce the surviving fraction to between 10^{-1} and 10^{-3}.

Procedure for transduction experiments. Recipient strains were grown overnight on a water bath shaker at 35°C from a small inoculum in L broth to a concentration of about 2×10^9 to 4×10^9 cells/ml. A 1:2 dilution was made in L broth, and $CaCl_2$ was added to 2.0×10^{-3} M. The cultures were incubated with shaking for an additional 30 min. Bacteria were mixed with suitably diluted unirradiated and UV-irradiated phage lysates calculated to give a multiplicity of infection of about 0.1. After incubation for 20 min at 35°C, the suspensions were chilled in an ice bath. The cells were sedimented in a centrifuge, and the supernatant was assayed for free phage to determine the amount of phage absorbed by the recipient cells. The controls consisted of phage lysate in L broth to confirm the cell-free condition of the phage preparation and a tube with only recipient cells to detect spontaneous mutations. The phage in L broth was incubated for 72 h, and samples were plated onto T1N1 agar.

In screening for auxotrophy to prototrophy transductants, the pellet of phage-treated cells (and the untreated cell control) was suspended in saline (0.85% NaCl). The cells were washed twice with saline, and samples were spread on M9 minimal glucose agar with and without the suitable amino acid or antibiotic supplement. Plates were incubated for 72 h and examined.

Two methods were used to permit phenotypic expression when scoring for antibiotic-resistant markers acquired by recipients from donor strains through transduction. After centrifugation of the phage-treated cultures and controls, the cells were washed twice with saline. An approximation of the frequency of transduction of a drug-resistant marker was obtained by the liquid culture method. Cells were suspended in L broth and incubated with shaking for 90 min. Samples were then spread onto T1N1 agar containing the appropriate antibiotic, and the plates were incubated for 72 h. Another estimate of transduction frequency of drug-resistant markers was obtained with a modification (9) of the delayed selection technique (6, 23). Samples of the phage-treated and control cells in saline were spread onto T1N1 agar. After the surface of the agar was dry, 3.0 ml of T1N1 soft agar was added to a plate. At zero time and after 2.5 h of incubation at 35°C, duplicate plates were overlaid with a 0.7% agar solution containing either 1,000 μg of streptomycin, 2,000 IU of polymyxin B, or 500 μg of rifampin per ml. The plates were held in the refrigerator for 24 h to permit diffusion of the antibiotic, then incubated for 72 h, and scored. Both methods have limitations (23) in studying the kinetics of transduction for an antibiotic resistance character such as streptomycin.

The transduction data given in the tables are corrected for spontaneous mutations (or revertants) found in the corresponding controls. (In the majority of experiments no colonies were detected after plating 1×10^8 to 6×10^8 cells of controls of the recipient strains on selective media.)

Assay for transformation. In some experiments phage lysates were treated with 400 μg of deoxyribonuclease (DNase) I per ml (Sigma Chemical Co., St. Louis, Mo.) for 4 h at 37°C in 4 mM $MgSO_4$. The transducing activities of DNase-treated lysate and untreated phage lysate were compared.

RESULTS

The approximate frequencies of transduction for three amino acid markers and three antibiotic resistance characters (calculated on the basis of phage particles absorbed by recipient cells) are listed in Tables 2 and 3. Overall, a higher number of transductants appeared on the selection plates with recipient cells treated with UV-irradiated phage lysates compared with unirradiated phage. As noted for other transducing phage (3, 12), we found a relationship between phage UV-inactivation kinetics and the number of transductants; maximum numbers were usually obtained with recipient cells treated with lysates having phage surviving fractions between 0.01 and 0.1.

The two mutant strains auxotrophic solely for methionine were transduced with phage grown on the prototrophic biotype *eltor* donor strain 1621 (Table 2). For the biotype *eltor* recipient strain 905, the maximum frequency of transduction was about 1×10^{-5} using irradiated phage; for the biotype *cholerae* recipient strain 903, the maximum frequency of transduction was approximately 2×10^{-6}. Phage CP-T1 propagated on strain 908 yielded a lysate capable of trans-

TABLE 2. *Frequency of tranduction of auxotrophs using unirradiated and UV-irradiated phage*

Recipient strain	Donor strain	Selected marker[a]	Approximate frequency of transduction[b]			
			Unirradiated phage	Irradiated phage (surviving fraction)		
				0.46 (± 0.13)	0.10 (± 0.07)	0.02 (± 0.019)
903	1621	Met	5.8×10^{-8} (± 4.3)	1.9×10^{-7}[c]	2.3×10^{-6} (± 0.3)	2.7×10^{-6} (± 0.8)
903	904	Met	1.8×10^{-8} (± 1.3)	1.3×10^{-7} (± 0.7)	2.3×10^{-7} (± 1.2)	1.0×10^{-7}[c]
903	908	Met	1.0×10^{-8}[c]	ND[d]	ND	4.8×10^{-7}[c]
905	1621	Met	3.6×10^{-6} (± 0.8)	6.6×10^{-6} (± 1.6)	1.0×10^{-5} (± 0.1)	1.2×10^{-5} (± 0.1)
905	904	Met	4.6×10^{-7} (± 1.9)	7.4×10^{-7} (± 1.5)	2.4×10^{-6} (± 1.9)	2.6×10^{-6} (± 0.8)
906	1621	Met	2.6×10^{-8} (± 1.8)	ND	3.5×10^{-7} (± 2.0)	3.7×10^{-6}[c]
906	1621	Lys	8.0×10^{-8} (± 4.2)	ND	5.4×10^{-7} (± 4.1)	1.8×10^{-6} (± 1.3)
907	1621	Trp	2.0×10^{-7} (± 0.7)	ND	1.5×10^{-6} (± 0.6)	2.3×10^{-6} (± 1.0)
908	1621	Met	1.0×10^{-6} (± 1.8)	4.8×10^{-6}[c]	ND	4.3×10^{-6} (± 2.7)

[a] Lys, lysine independence; Met, methionine independence; Trp, tryptophan independence.

[b] Frequency of transduction per phage absorbed; the standard error, expressed by the figures in parentheses, was calculated from data from two to five independent experiments, except where indicated. The multiplicity of infection ranged from 0.05 to 0.35.

[c] Based on only one experiment.

[d] ND, Not done.

TABLE 3. *Transduction of antibiotic resistance in V. cholerae*

Recipient	Donor	Selected marker	Method[a]	Frequency with unirradiated phage[b]	Maximum frequency with irradiated phage[c]
903	908	Rif	Broth	5.0×10^{-8}	5.7×10^{-7}
904	908	Rif	Agar	2.5×10^{-8}	4.0×10^{-7}
905	908	Rif	Broth	1.0×10^{-8}	5.1×10^{-7}
905	908	Rif	Agar	2.0×10^{-8}	6.0×10^{-7}
905	904	Str	Agar	2.0×10^{-8}	5.0×10^{-7}
906	904	Str	Agar	5.0×10^{-8}	1.3×10^{-7}
029	1621	Pmr[d]	Broth	1.3×10^{-8}	ND[e]
14035	1621	Pmr	Broth	2.8×10^{-8}	ND

[a] Described in the text.

[b] Approximate frequency of transduction per phage absorbed.

[c] Phage irradiated to yield a surviving fraction ranging between 10^{-1} and 10^{-2}.

[d] Pmr, Resistance to polymyxin B (200 IU/ml).

[e] ND, Not done.

ducing strain 903 to methionine independence (Table 2, line 3) but, as was expected, it did not transduce strain 905. This result indicates that the location of the mutational site for the methionine requirement is different in strains 903 and 905.

The data on the frequency of transduction of the Lys^+ and Trp^+ characters from donor strain 1621 to auxotrophic mutant recipients of strain 905 are given in Table 2, lines 7 and 8, respectively. Phage-treated populations of strain 906 plated on M9 agar supplemented with lysine to select Met^+ transductants provided data (line 6) for a comparison of the efficiency of transduction of these three amino acid markers. The frequency of transduction was approximately the same for the three amino acid markers when using the double auxotrophic mutant strains (906 and 907) and irradiated phage lysates.

Counterselection for Met^+ transductants of the double mutant strain 908 (Met^- Rif) on M9 agar containing rifampin yielded approximately the same number of methionine-independent colonies as did recipient strain 905 treated with the same phage preparation and plated on M9 agar (Table 2, lines 4 and 9). The results of these experiments rule out the possibility of the contamination of phage lysates with donor cells, since the donor strain is sensitive to rifampin.

The frequencies of transduction of the three antibiotic resistance markers appear in Table 3. With unirradiated phage, all three were transduced at approximately the same frequency. With UV-irradiated phage and the delayed selection technique, the estimated highest mean frequency of transduction of rifampin and streptomycin resistance characters was 6.0×10^{-7} and 5.0×10^{-7}, respectively. In general, the maximum level of transduction of the two antibiotic resistance markers, using UV-irradiated phage lysates, was lower than that obtained for the amino acid markers.

DNase treatment of CP-T1 phage lysates obtained from donor strain 904 did not appear to influence the transduction of the polymyxin resistance character to recipient strains 029 and 14035. When strains 029 and 14035 were tested independently, the average frequency of transduction with DNase-treated phage was 1.2×10^{-8} and 1.7×10^{-8}, respectively. The average frequency with the untreated phage was 1.5×10^{-8} and 2.7×10^{-8} for strains 029 and 14035. These results indicate that the observed changes to drug resistance in the recipient strains were

due to the phage and not to free transforming deoxyribonucleic acid (DNA) that could be present in phage lysates.

A limited number of transductants were screened for cotransduction of an unselected marker. No cotransduction was found for Met and Str on scoring the Met^+ transductants (218 of recipient strain 905 and 137 of strain 903) that were isolated after treatment with phage grown on the streptomycin-resistant strain 904. Str transductants of strains 905 and 906 (Table 3, lines 5 and 6) retained their respective growth factor requirement(s), suggesting that there is no joint transduction of Str and Met or Str and Lys. These initial results, although not conclusive, indicate no cotransduction for the markers tested.

DISCUSSION

Phage-mediated transduction of amino acid and drug resistance characters was demonstrated in *V. cholerae*. Since a number of donor genetic traits could be transferred to suitable recipients by the temperate phage used in these experiments, this system would be classified as general transduction. Also, the frequency of transfer of the characters tested is of the same order (10^{-5} to 10^{-8}) as that reported for genetic markers in *E. coli*, using generalized transducing phage P1, and in *S. typhimurium*, using phage P22 (3, 14, 23, 25).

When lysates of phage CP-T1 were irradiated with UV doses inactivating 90 to 99% of the plaque-forming ability of the phage, there was an increase in the frequency of transduction. A decrease in transduction titer occurred with heavier doses of radiation. UV irradiation of phage lysates has been reported to increase the frequency of transduction for chromosomal genes with other transducing phage such as P1 (3, 15, 24), P22 (12), PBS1 (22), and SPP1 (11). The UV effect on transducing phage preparations has been attributed, in part, to an increase in the efficiency of stable integration of the exogenote into the endogenote chromosome (3). Inactivation of infectious phage genomes by the UV treatment also may contribute to some increase in the number of transductants appearing on selection plates.

There was a difference in the maximum frequencies of transduction of the antibiotic resistance characters compared with the amino acid markers. The lower frequency of transduction for a drug resistance character may be due, in part, to the recessive nature of many antibiotic resistance alleles. Thus, a delay in phenotypic expression and the selective disadvantage of the drug-resistant, slow-growing transductants (6, 23) would lead to a lower observed frequency. A mechanism similar to that proposed for phage P22 also may account for some of the observed differences in transduction titers between the amino acid markers and the drug resistance characters. According to this model, the formation of transducing DNA fragments by sequential encapsulation from a small number of preferred starting points in the host chromosome (10) could explain the observation that certain bacterial markers are transduced by wild-type phage P22 at much higher efficiency than others (21). Further studies of the transduction mechanism with phage CP-T1 must await more detail on the chromosomal location of genetic markers in biotype *eltor*.

The results of preliminary experiments to detect unselected markers in the transductants indicated no cotransduction for Met and Str or Lys and Str. However, the results are inconclusive since a limited number of transductants were scored and we did not use donor and recipient strains carrying several markers, which would have permitted the scoring of more unselected markers in the transductants. It is noted, however, that the linkage map constructed from conjugational analysis of *V. cholerae* biotype *cholerae* strain 162 designates the relative distance of the Str locus from the Met alleles to be about 3.5 and that of Str from Lys alleles to be about 2.7 (18). The distance between the Str and Met or Lys markers may exceed the length of pieces of host DNA that can fit into the protein coat of phage CP-T1, assuming the relative positions of these markers on the chromosome are the same in biotype *eltor* strains. Measurement of frequencies of cotransduction mediated by phage CP-T1 should be an invaluable tool for providing better genetic estimates of the distances between two markers on the chromosome of *V. cholerae*.

The ability of biotype *cholerae* strains (029, 14035, and 903) to be transduced by phage grown on a biotype *eltor* strain affirms the close genetic relationship between strains of these two biotypes of *V. cholerae*. Thus, transduction may play a role in the evolution of strains of *V. cholerae* and may be partly responsible for the appearance of new types being reported in nature. Transduction of genetic material from biotype *eltor* donors to biotype *cholerae* recipients, and vice versa, could assist in studies on the function of the gene(s) determining specific biotype characteristics.

Judging from the numerous reports of isolation of lysogenic *V. cholerae* during the last 50 years, it is likely that other transducing phage will be isolated from this organism. We believe

our success in demonstrating transduction in *V. cholerae* may have been due to the use of essentially fresh clinical isolates which might have increased the probability of isolating a transducing phage. The range of occurrence of transducing choleraphage may follow a pattern similar to that reported for *Salmonella*, where the highest incidence of transducing phages was among strains obtained from human sources (5).

ACKNOWLEDGMENT

We thank Betty J. Ogg for her secretarial and technical assistance.

LITERATURE CITED

1. **Adams, M. H.** 1959. Bacteriophage, p. 450–451. Interscience Publishers, New York.
2. **Adelberg, E. A$_2$, M. Mandel, and G. C. C. Chen.** 1965. Optimal conditions for mutagenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **18**:788–795.
3. **Arber, W.** 1960. Transduction of chromosomal genes and episomes in *Escherichia coli*. Virology **11**:273–288.
4. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
5. **Bailey, W. R.** 1956. Studies on the transduction phenomenon. II. The occurrence of *Salmonella* transducing phages in nature. Can. J. Microbiol. **2**:555–558.
6. **Banic, S.** 1959. Transduction to penicillin and chloramphenicol resistance in *Salmonella typhimurium*. Genetics **44**:449–455.
7. **Bhaskaran, K.** 1960. Recombination of characters between mutant stocks of *Vibrio cholerae* strain 162. J. Gen. Microbiol. **23**:47–54.
8. **Bhaskaran, K., V. B. Sinha, and S. S. Iyer.** 1973. Chromosome mobilization in *Vibrio cholerae* (biotype *eltor*) mediated by sex factor P. J. Gen. Microbiol. **78**: 119–124.
9. **Chang, W., and J. E. Ogg.** 1970. Transduction in *Vibrio fetus*. Am. J. Vet. Res. **31**:919–924.
10. **Chelala, C. A., and P. Margolin.** 1974. Effects of deletions on cotransduction linkage in *Salmonella typhimurium*: evidence that bacterial chromosome deletions affect the formation of transducing DNA fragments. Mol. Gen. Genet. **131**:97–112.
11. **Ferrari, E., U. Canosi, A. Galizzi, and G. Mazza.** 1978. Studies on transduction process by SPP1 phage. J. Gen. Virol. **41**:563–572.
12. **Garen, A., and N. D. Zinder.** 1955. Radiological evidence for partial genetic homology between bacteriophage and host bacteria. Virology **1**:347–376.
13. **Johnson, S. R., and W. R. Romig.** 1979. Transposon-facilitated recombination in *Vibrio cholerae*. Mol. Gen. Genet. **170**:93–101.
14. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
15. **Luria, S. E., J. N. Adams, and R. C. Ting.** 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. Virology **12**:348–390.
16. **Miller, J. E.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
17. **Ogg, J. E., M. B. Shrestha, and L. Poudayl.** 1978. Phage-induced changes in *Vibrio cholerae*: serotype and biotype conversions. Infect. Immun. **19**:231–238.
18. **Parker, C., D. Gauthier, A. Tate, K. Richardson, and W. R. Romig.** 1979. Expanded linkage map of *Vibrio cholerae*. Genetics **91**:191–214.
19. **Sanderson, K. E., and P. E. Hartman.** 1978. Linkage map of *Salmonella typhimurium*, edition V. Microbiol. Rev. **42**:471–519.
20. **Smith, H. L., and K. Goodner.** 1965. On the classification of vibrios, p. 4–8. *In* Proceedings of the Cholera Research Symposium, 24–29 January 1965. U.S. Government Printing Office, Washington, D.C.
21. **Susskind, M. M., and D. Botstein.** 1978. Molecular genetics of bacteriophage P22. Microbiol. Rev. **42**:385–413.
22. **Takahasi, I.** 1963. Transducing phages for *Bacillus subtilis*. J. Gen. Microbiol. **31**:211–217.
23. **Watanabe, T., and M. Watanabe.** 1959. Transduction of streptomycin resistance in *Salmonella typhimurium*. J. Gen. Microbiol. **21**:16–29.
24. **Wilson, D. E.** 1960. The effects of ultraviolet light and ionizing radiation on the transduction of *Escherichia coli* by phage P1. Virology **11**:533–546.
25. **Zinder, N. D., and J. Lederberg.** 1952. Genetic exchange in *Salmonella*. J. Bacteriol. **64**:679–699.