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Transduction of Linked Chromosomal Genes between *Pseudomonas aeruginosa* Strains during Incubation In Situ in a Freshwater Habitat

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Both transduction of single chromosomal loci and cotransduction of closely linked loci were observed between lysogenic and nonlysogenic strains of *Pseudomonas aeruginosa* in a freshwater habitat. Transductants were recovered at frequencies of 10^{-6} to 10^{-5} transductants per CFU. Transductants of lysogenized strains were recovered 10- to 100-fold more frequently than were transductants of nonlysogenic parents. Lysogens are thus capable of introducing phages which mediate generalized transduction into the natural microbial community and serving as recipients of transduced DNA. It would appear that lysogeny has the potential of increasing the size and flexibility of the gene pool available to natural populations of bacteria. The ability to generate and select new genetic combinations through phage-mediated exchange can be significant in the face of a continually changing environment and may contribute to the apparent fitness of the lysogenic state in natural ecosystems.

Horizontal gene transmission under natural conditions is important to the evolution and genetic diversity of bacteria. Questions about the potential for the transfer of genetic material in microbial populations have received relatively little attention until recently. Recent developments in genetic engineering of microorganisms have spawned interest in providing biotechnological answers to environmental problems, raising concerns over the significance of transmission of the recombinant DNA from genetically engineered microorganisms to environmental populations. These concerns have prompted a number of investigators to begin to examine specific modes of horizontal gene transfer in natural habitats.

Transduction of chromosomally encoded genes is routinely performed in many laboratories for a number of purposes, including strain construction and gene mapping. However, as a mechanism of gene dispersal in nature, transduction has not been well characterized, and little is actually known about the potential for bacteriophage-mediated gene transfer in the environment. We have previously demonstrated the transduction of plasmid DNA (14) and chromosomally encoded streptomycin resistance (11) between strains of *Pseudomonas aeruginosa* in a freshwater lake. In addition, we have shown that *P. aeruginosa* bacteriophages are abundant in fresh water and that these phages apparently exist in a dynamic equilibrium with the host population under both lytic and temperate conditions (O. A. Ogunseitan, G. S. Sayler, and R. V. Miller, Microb. Ecol., in press). This report investigates the transduction of linked chromosomal genes between lysogenic and nonlysogenic strains of *P. aeruginosa* PAO as well as between two lysogens during incubation in a freshwater reservoir.

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MATERIALS AND METHODS

Bacteria, bacteriophages, and plasmids. All of the bacterial strains used in these studies were derivatives of *P. aeruginosa* PAO and are listed in Table 1. F116L (8) was a gift of J. Shapiro. Bacteriophage DS1 (14), a variant of F116L, was isolated from RM2008, a clinical isolate that was a gift from G. Jacoby. E79 is a virulent bacteriophage of *P. aeruginosa* (4). The plasmid Rms149, also isolated from RM2008, is Tra^- Mob^- and encodes resistance to carbenicillin (Cb^r), streptomycin (Sm^r), gentamicin (Gm^r), and sulfonamide (Su^r).

Media, cultivation of strains, and preparation of phage stocks. Bacteria were maintained in Luria broth or on L-agar (9). *Pseudomonas* minimal medium (PMM) and PMM agar (9) containing 0.4% glucose or 10 mM acetamide were used as selective media in transduction experiments. Antibiotics were added to selective media in the following concentrations: streptomycin, 250 μg/ml; nalidixic acid, 500 μg/ml; and carbenicillin, 500 μg/ml. Amino acids were supplied at 25 μg/ml.

Phage lysates were prepared by the method of Miller and Ku (9). Phage titers were determined in top agar overlays (9) on L-agar plates containing strain PAO1 as an indicator.

Source of bacteriophages and transducing particles in microcosms and environmental test chambers. Bacteriophage virions were not introduced directly into the systems. Instead, lysogens of the *Pseudomonas*-specific, temperate, generalized transducing bacteriophage F116L or its variant DS1 (14) were present. Virions of F116L were produced in the system through the induction of the resident prophage to lytic growth either by spontaneous induction or in response to some external stimulus during incubation. Transducing particles containing host DNA mistakenly packaged into phage capsids may be formed during induction of the prophage or during primary lytic infection of a nonlysogenic member of the test community.

Chromosomal transduction in microcosms and in situ. Microcosms were prepared as described previously (14). *P. aeruginosa* PAO was maintained in sterile lakewater chambers incubated at a previously described field study site on

TABLE 1. Bacterial strains used in this study

Strain	Genotype[a]	Source or reference
PAO1	Prototroph	9
PAO303	*argB21*	7
PAO303L	As for PAO303 but F116L lysogen	This study
PAO515	*met-9011 nalA5 amiE200*	12
PAO515L	As for PAO515 but F116L lysogen	This study
PAO660	*phe-2*	2
RM40	*lys-12 met-28 trpC6 pur-600*	9
RM40L	As for RM40 but F116L lysogen	This study
RM278	As for PAO1 but DS1 lysogen	This study
RM759	As for RM761 but DS1 lysogen	This study
RM761	As for PAO515 but *eseA901*	This study
RM762	As for PAO660 but *str-951*	This study
RM763	As for RM762 but DS1 lysogen	This study
RM2008	*leu-10 ilvB rif* Rms149 DS1 lysogen	14
RM2140	As for PAO1 but containing Rms149	14
RM2141	As for PAO515 but containing Rms149	This study
RM2234	As for RM40 but containing Rms149	This study

[a] Nomenclature follows the rules of Demerec et al. (3). Abbreviations: *ami*, unable to utilize acetamide as a sole carbon and energy source; *arg*, arginine auxotrophy; *ese*, resistant to infection by phage E79; *ilv*, isoleucine-valine auxotrophy; *leu*, leucine auxotrophy; *lys*, lysine auxotrophy; *met*, methionine auxotrophy; *nal*, nalidixic acid resistance; *phe*, phenylalanine auxotrophy; *pur*, purine (adenine) auxotrophy; *rif*, rifampin resistance; *str*, streptomycin resistance; *trp*, tryptophan auxotrophy.

Fort Loudon Lake (15–18). The test chambers (1 liter) were Lifecell tissue culture flasks, which are flexible, gas-permeable, plastic bags that were purchased sterile from Fenwal Laboratories (Morton Grove, Ill.). The chambers were filled with sterilized lake water through the attached tubing, which was then sealed. Access into the chambers for inoculating and sampling was done by way of sampling site couplers (Fenwal Laboratories) designed specifically for use with the Lifecell chamber.

Virions of phage were present at concentrations of up to 10^6 PFU/ml in mid-log-phase cultures of lysogenic strains owing to spontaneous induction of a small fraction of the growing bacteria (10). A standard plate count for each strain was used to determine cell numbers. Microcosms and test chambers were inoculated at approximate cell densities comparable to levels found at the field study site (14). Viable-cell counts for Fort Loudon Lake water were 3×10^4 CFU/ml when plated on yeast extract-peptone-glucose agar (17). The ranges of the physical and chemical parameters monitored during the field trials were: temperature, 20 to 31°C; conductivity, 200 to 275 μS; dissolved oxygen, 5.4 to 14.5 mg/liter; and pH, 7.3 to 8.4.

Primary selection and enumeration of transductants. The primary selection of transductants was performed on PMM agar supplemented with glucose (0.4%). Amino acids or antibiotics were added when necessary.

Determination of free bacteriophage titers. Cell-free filtrates were serially diluted in Luria broth, and the dilutions were mixed with PAO1 in lambda top agar overlays. Phage were enumerated by plaque formation. The detection limit was 10 PFU/ml.

Genetic and molecular screening of potential transductants. To verify that the putative transductants exhibited the transductant phenotype, individual markers were checked by replica plating onto L-agar containing the appropriate antibiotics or onto PMM agar supplemented with acetamide (10 mM) or glucose (0.4%) as the carbon source. Auxotrophic markers were verified on PMM agar containing glucose and, when necessary, the appropriate combinations of amino acids. Resistance to virulent phage E79 ($E79^r$) was scored as previously described (11).

TABLE 2. Transduction of RM40 with cell-free phage lysates[a]

Transducing phage	MOI (PFU/CFU)	No. of transductants/10^7 PFU			
		Chromosomal allele			Plasmid Rms149
		met-28	*trpC6*	*lys-12*	
F116L	0.02	3.6	4.6	0.2	3.3
	0.2	2.2	1.8	0.2	0.9
	2.0	0.3	0.2	0.01	0.3
DS1	0.02	4.3	4.7	0.8	8.2
	0.2	2.4	2.3	0.4	1.8
	2.0	0.3	0.2	0.03	0.6

[a] Phage lysates were prepared on RM2140, a nonlysogenic strain which contains Rms149.

RESULTS AND DISCUSSION

Relative efficiency of plasmid and chromosomal transduction with cell-free lysates. To determine whether chromosomal markers were transduced as efficiently as plasmid markers, cell-free lysates of phage F116L were prepared on RM2140, a prototrophic strain carrying the plasmid Rms149, and used to transduce strain RM40 in laboratory transduction protocols (14). No significant difference in the frequency of plasmid and chromosomal transduction was observed (Table 2). The frequency of transduction for the *lys-12* allele in RM40 was 10-fold lower than that of the other markers studied at each of the multiplicities of infection (MOIs) used. The *lys-12* allele consistently transduced at lower frequencies than other chromosomal alleles present in the same strain. The reason for this has not been determined.

Chromosomal transduction in microcosms. Three paradigms for the source of the transducing particles were examined both in laboratory microcosms and in environmental test chambers incubated in a freshwater reservoir. These were (i) transduction from a genetic donor lysogenic for the mediating phage to a non-lysogenic recipient, (ii) transduction from a nonlysogenic donor to a lysogenic recipient, and (iii) transduction between two lysogens.

(i) Virion production in microcosms. The MOI, the ratio of bacteriophages to cells, is an important parameter in laboratory transductions, but it is not directly applicable to the paradigms being tested since bacteriophage particles are not added directly to the system. To avoid confusion, the ratio of phage to bacteria will be referred to as the phage/bacteria ratio (PBR). The PBR was calculated from the bacteriophage titers and total cell counts determined at each sampling period. After an initial rise, the PBR in microcosms remained low and relatively constant during the entire incubation period (Table 3). The amplitude of the initial rise in PBR may be artifactual, since we washed the cells exhaustively before inoculation into the test chambers to avoid introduction of previously formed phage particles into the system (13).

(ii) Reciprocal exchange of markers in microcosms. Transduction was observed in test chambers designed to test each

TABLE 3. Transduction between lysogens and nonlysogens in microcosms.

Expt no.	Parent 1[a]	Parent 2[a]	Avg PBR[b]	Maximum transduction frequency (10^{-7}/CFU)[c]	No. of transductants/10^7 CFU[d]								
					Parent 1 alleles				Parent 2 alleles				
					ilvB112	*leu-10*	*met-9011*	*amiE200*	*met-28*	*trpC6*	*lys-12*	*rif*	Rms149
1	PAO515L (Lyso)	RM40 (Nonlyso)	0.005 (±0.001)	5.2	—	—	1.1	6.4	<0.1	0.1	<0.1	—	—
2	PAO515 (Nonlyso)	RM40L (Lyso)	0.002 (±0.001)	12.1	—	—	11.8	0.1	<0.1	<0.1	0.4	—	—
3	PAO515L (Lyso)	RM40L (Lyso)	0.008 (±0.0006)	0.8	—	—	0.3	0.7	<0.1	<0.1	0.2	—	—
4	RM2008 (Lyso)	RM40 (Nonlyso)	0.1 (±0.06)	3.7	2.1	0.9	—	—	0.6	0.1	0.1	ND[f]	ND
5	RM2008 (Lyso)	RM278 (Lyso)	0.007 (±0.003)	2.6	2.4	0.3	—	—	—	—	—	1.0	29.1

[a] Lyso, Lysogen; Nonlyso, nonlysogen.
[b] Average (± standard deviation) of all sampling times.
[c] Transductants per 10^7 CFU for all genotypes. Maximum combined transduction frequency observed during trial.
[d] Transductants of the parental genotype. A value of <0.1 indicates that the transduction frequency was below the level of detection.
[e] —, Allele not present.
[f] ND, Not scored.

of our three paradigms (Table 3). Chambers containing a lysogen and nonlysogen showed higher frequencies of total transductants than chambers containing two lysogenic strains.

Transduction of individual chromosomal alleles was observed at frequencies of 10^{-8} to 10^{-6} transductants per CFU (Table 3). Reversion of these markers was determined by incubating each strain alone and by inoculating the microcosm with two nonlysogenic strains. Reversion frequencies were below the level of detection (<10^{-8} revertants per CFU). This is consistent with the reversion frequencies of these alleles measured by standard laboratory methods (<10^{-9} revertants per CFU).

In systems containing both a lysogenic strain and a nonlysogenic strain, transduction of chromosomal alleles in both the lysogenic and nonlysogenic strains was observed (Table 3). These data indicate that both primary infection of the nonlysogen and spontaneous induction of the prophage from lysogens generated sufficient numbers of transducing particles to allow transduction. Reciprocal exchange of alleles was also observed in chambers inoculated with two F116L lysogens.

In general, more transductants of the lysogenic parent than of the nonlysogen were recovered. We have observed previously (14) that higher frequencies of plasmid transduction are detected in situ when the recipient strain is a lysogen. Lysogenic transductants may be more easily recovered from model environmental systems owing to their immunity to superinfection and killing by phage virions. As absorption of DNA by F116L lysogens is not inhibited (1), the potential for transduction is not reduced but the probability of survival of the transduced lysogen is increased.

Transduction of chromosomal alleles within the same strain was observed at similar frequencies (Table 3). However, in experiments which measured the frequency of both chromosomal and plasmid Rms149 transduction (Table 3), transductants of plasmid DNA were recovered more frequently than transductants of chromosomal DNA. This difference did not manifest itself in standard laboratory transductions with phage lysates (Table 3). Rms149 transductants were consistently recovered at higher frequencies in environmental simulations.

The reciprocal exchange of markers in chambers containing a lysogen and a nonlysogen indicates that the lysogen can serve as both the source of the phage capable of mediating transduction and the recipient of transduced DNA. This reciprocal exchange also demonstrates that transducing particles can be produced in these environmental simulations either by the primary infection of a nonlysogenic cell or during the induction of a prophage from a lysogen.

Microcosm studies with mixed populations. In another experiment, three strains, each having a single unique auxotrophy, were incubated together in the same microcosm. Only one of the three strains was a lysogen. Five microcosms were prepared. Chambers 1 to 3 were inoculated with PAO303L, PAO515, and PAO660, respectively, The two nonlysogenic strains were inoculated together in chamber 4, and chamber 5 was inoculated with all three strains. The number of prototrophic colonies which developed in each chamber was scored by plating on PMM containing glucose. In chambers 1 to 4, these prototrophs could arise only through reversion. In chamber 5, however, transduction could also contribute to the production of prototrophs. Reversion frequencies measured in chambers 1 to 4 were calculated to be between 10^{-9} and 10^{-10} prototrophs per CFU. In chamber 5, where transduction was free to act upon the population, prototrophs were detected at frequencies of 6.5×10^{-7} prototrophs per CFU or higher. These frequencies were 100 times greater than those observed in any of the control chambers.

The phenotypes of representative prototrophs in chamber 5 were examined for nonselected markers (Table 4). Putative PAO515 transductants were scored for the presence of the *amiE200* and *nalA5* alleles. They represented between 6 and

TABLE 4. Distribution of various phenotypes among prototrophic transductants recovered from microcosms inoculated with mixed populations[a]

Incubation time (h)	% in phenotypic class		
	Prototrophic	Ami$^+$ *nalA5*	*amiE200 nalA5*
3	93	0	7
19	90	0	10
25	100	0	0
43	47	33	20
69	40	40	20

[a] At least 10 well-isolated transductants were scored at each time point.

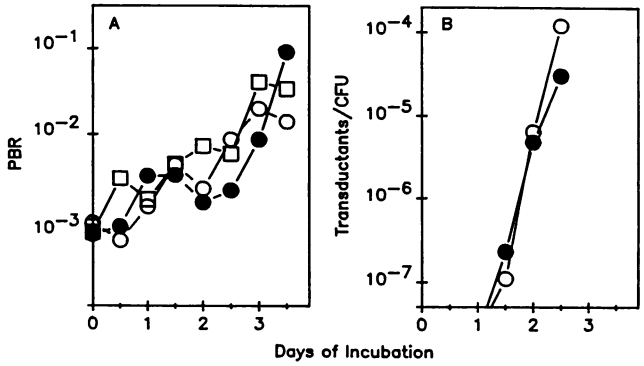

FIG. 1. Population dynamics in chromosomal transduction experiments in situ. (A) PBR during incubation in situ. (B) Met$^+$ derivatives of PAO515L recovered. Symbols: ○ and ●, chambers inoculated with PAO515L and RM40; □, chamber inoculated only with PAO515L.

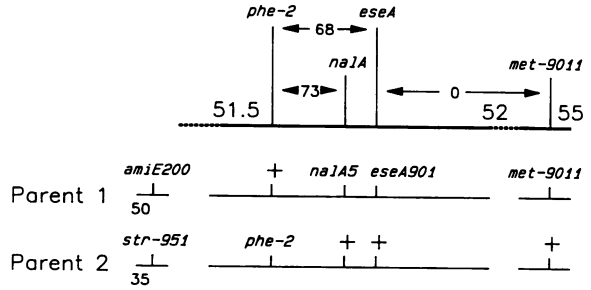

FIG. 2. Genotypes of strains used in cotransduction experiments. Map positions (6) of loci scored are indicated by the large numbers and are given in minutes. Cotransduction frequencies of loci (in percent) are indicated by smaller numbers between arrows.

20% of the transductants examined. PAO303L and PAO660 transductants were represented by Ami$^+$ Nal^s prototrophic clones and could not be conclusively differentiated. Differences in colony morphology associated with these two parental strains suggested that this class of transductants contained both lineages. Ami$^+$ and Nal^r prototrophs were detected after extended incubation of the microcosm. They most likely originated from the cotransduction of the prototrophic allele of *phe-2* and the *nalA5* allele from PAO515 to RM754. The loci *phe-2* and *nalA* have been shown to be 67% cotransducible in standard laboratory transductions (2). Thus, it appears that all members of a mixed population of *P. aeruginosa* can serve as sources of transducing particles and as recipients of transduced DNA when one or more members of the community is lysogenic for a phage capable of mediating generalized transduction.

Estimation of transduction under in situ environmental conditions. The transduction of chromosomal markers between PAO515 and RM40 was investigated in situ by incubating the strains in environmental chambers immersed in Fort Loudon Lake. A total of eight chambers were used. Duplicate chambers containing either two nonlysogenic strains, a lysogen and a nonlysogen, or a lysogen and a nonlysogenic plasmid donor were analyzed. In control chambers, PAO515L and RM40 were incubated alone. The plasmid transduction system was included as a positive control. Plasmid transduction from a nonlysogen to a lysogenic recipient had been demonstrated previously (14). In this study, transductants receiving the Tra$^-$ Mob$^-$ plasmid Rms149 ranged from 1.3×10^{-7} to 2.1×10^{-8} transductants per CFU during the course of this experiment. These data are consistent with the frequencies reported previously.

(i) Changes in PBR during incubation in situ. The PBR in the test chambers incubated in situ increased from less than 10^{-3} to as much as 10^{-1} PFU/CFU over the course of this study. Unlike in the microcosms, PBRs increased at a steady rate during the course of this experiment (Fig. 1A). The control chamber containing only the lysogenic strain showed a similar increase in PBR, indicating that induction of the prophage contributes significantly to the phage numbers.

(ii) Transduction of chromosomal DNA in situ. Large numbers of PAO515 that were now prototrophic for the *met-9011* allele were detected in chambers inoculated with the lysogenic strain (PAO515L) and the nonlysogenic strain (RM40), indicating that chromosomal transduction had taken place (Fig. 1B). Significant increases in the number of cells possessing the transductant phenotype were detected after 36 h in both chambers. The frequencies of recovery at 36 h were 1.1×10^{-7} and 2.3×10^{-7} transductants per CFU, respectively. By 60 h of incubation in situ, the number of Met$^+$ Nal^r Ami$^-$ bacteria in both chambers had increased to greater than 10^{-5}/CFU. Transductants of the multiply auxotrophic RM40 strain were not detected.

The control chamber containing the PAO515 lysogen exhibited an extremely high frequency of reversion of the *met-9011* marker. This phenomenon had not been observed in microcosm studies and suggested that the increase in reversion frequency may be a response to environmental stress or stimuli. This unexpected result made it difficult to be certain that the observed increases in the number of Met$^+$ cells in the chambers containing both lysogens and nonlysogens was due solely to the transduction of chromosomal DNA. This problem was addressed in a second field trial.

Estimation of the frequency of cotransduction of chromosomal genes in situ. To circumvent the problems encountered in our initial field experiments, genetically marked strains were constructed which allowed the scoring of the cotransduction of adjacent genetic markers (Fig. 2). The probability of simultaneous reversions in adjacent markers is extremely low (the product of the reversion frequencies of the two alleles), and thus the background of cells which obtain the recombinant phenotype by reversion as opposed to transduction is reduced to below the level of detection.

The cotransduction frequencies of the various genes present in these strains was determined in standard laboratory transductions (9). In these experiments, the cotransduction frequency of *phe-2* and *nalA* was determined to be 73% in this genetic background. This agreed well with the previously published frequency of 67% (2). The cotransduction frequency of *phe-2* and *eseA* was 68% in this genetic background.

Selection of primary transductants was based on transduction to prototrophy of the auxotrophic markers. Transductants were detected by filtering the sample through a membrane (0.45-μm pore size) and growing on PMM agar containing glucose as the carbon source. Well-separated isolates were scored for the unselected markers (Nal^r, Sm^r, and E79^r) and the ability to use acetamide as a carbon and nitrogen source (Ami$^+$).

(i) Changes in PBR. The PBR in each chamber increased dramatically during the first 12 h of incubation, coinciding with a rapid drop in cell numbers (Fig. 3). After the first 12 h of incubation, both the cell density and the PBR increased, as was noted in the first set of environmental experiments

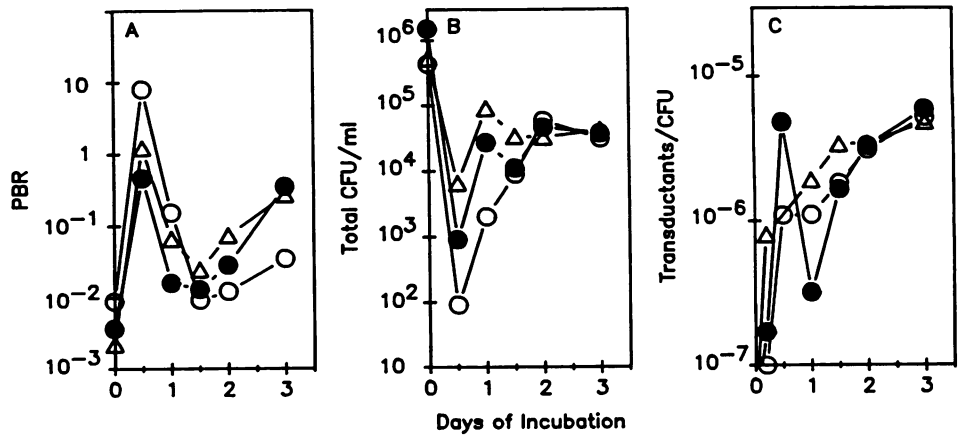

FIG. 3. Comparative population dynamics in cotransduction experiments of *P. aeruginosa* in situ. (A) PBR during incubation in situ. (B) Total CFU of all genotypes recovered. (C) Frequency of transductants recovered per total CFU of all genotypes. Symbols: ○, chamber inoculated with a lysogen of parent 1 (RM759) and a nonlysogen of parent 2 (RM762); ●, chamber inoculated with a nonlysogen of parent 1 (RM761) and a lysogen of parent 2 (RM763); △, chamber inoculated with two lysogens (RM759 and RM763).

(Fig. 1A). The initial decrease in cell numbers was significantly less in the chamber containing the two lysogens, suggesting that primary infection of the nonlysogens contributed, at least in part, to the observed loss of viable bacteria when both lysogens and nonlysogens are present.

(ii) Frequency of cotransduction in situ. The frequency of prototrophic transductants in chambers containing nonlysogenic and lysogenic parents and the frequency of transductants in the chamber containing two lysogenic parents are shown in Fig. 3C.

Well-isolated colonies from the primary selection plates were scored for cotransduction of unselected markers. Cotransduction was detected in chambers containing a lysogen and a nonlysogen and in chambers containing two lysogenic strains. The percentages of transductants which exhibited alteration at one, two, and three loci are shown in Table 5. The cotransduction frequency observed in situ for *phe-2* and *nalA5* was 64.4%, while the frequency for *phe-2* and *eseA901* was 34.4%. Although both of these frequencies are lower than those determined in laboratory transductions, they are consistent with the predicted gene order on the *P. aeruginosa* chromosome (2, 6).

Isolates altered at more than one locus were not detected in chambers containing two nonlysogenic strains, nor were they found in control chambers containing the individual test strains RM759, RM761, and RM762 alone. The control chamber which was inoculated with RM763 (*phe-2 str-951*, DS1 lysogen) did exhibit an increased frequency of reversion to prototrophy, as had been observed in the initial field experiments.

TABLE 5. Distribution of classes of transductants isolated from test chambers incubated in situ[a]

Expt no.	Parent 1[b]	Parent 2[b]	% of transductant type and no. of recipient loci transduced			
			From parent 1 to parent 2			From parent 2 to parent 1 (single locus)
			Single locus	Two loci	Three loci	
1	RM759 (Lyso)	RM762 (Nonlyso)	0	0	0	100
2	RM761 (Nonlyso)	RM763 (Lyso)	0	75	17	8
3	RM759 (Lyso)	RM763 (Lyso)	11	78	7	5

[a] At least 100 well-isolated colonies were examined for unselected markers.
[b] Lyso, Lysogen; Nonlyso, nonlysogen.

Transduction on the membrane filter. To verify that the observed transduction had actually occurred in the chamber during incubation in situ and not on the membrane filter during laboratory analysis of the sample, experiments were performed to determine the probability of transduction by bacteriophages retained on the filter and between two lysogenic strains when filtered together onto the same filter. At phage concentrations and cell densities comparable to those in the in situ experiments, the probability of transduction by a retained transducing particle was well below our level of detection (<1 transductant per 1,000 filters). When donor and recipient strains were filtered together, transduction was not detected at the cell densities that were representative of those in the chambers. Thus, there is little or no chance that the transductants observed in our experiments originated during anlaysis of the field samples.

This study clearly shows that transduction of chromosomal DNA occurs between strains of *P. aeruginosa* PAO both in laboratory simulations and in situ in a freshwater lake. Transduction of chromosomal DNA occurs reciprocally between lysogens and nonlysogens. Reciprocal transduction also occurs between lysogens.

It has been suggested that the frequency of lysogeny in natural populations of *P. aeruginosa* may approach 100% (5). In the study reported here, the ability of lysogens to serve as a source of phages capable of mediating transduction as well as recipients of transduced chromosomal DNA was demonstrated. Both induction of prophages from lysogens and primary infection of nonlysogenic bacteria are potential sources of transducing particles. It would appear that the lysogenic state increases the size and flexibility of the gene pool available to natural populations of bacteria. This may lead to an increased fitness of the lysogenic strain in natural habitats. Consistent with this hypothesis, we found that approximately 45% of the *Pseudomonas* isolates from Fort Loudon reservoir tested positive in colony hybridization with DNA of a naturally occurring phage from the field site (Ogunseitan et al., in press). In addition, when this

phage was introduced into microcosms containing a sensitive host population, over 90% of the recoverable CFU were carriers of phage DNA after 45 days of incubation (Ogunseitan et al., in press).

Diversity generated by the phage-mediated exchange of DNA may be significant in the face of a continually changing, complex environment. We must consider transduction, as a mechanism of genetic exchange in natural habitats, to be far more environmentally significant than has traditionally been envisioned.

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