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Potential for Transduction of Plasmids in a Natural Freshwater Environment: Effect of Plasmid Donor Concentration and a Natural Microbial Community on Transduction in *Pseudomonas aeruginosa*

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Transduction of *Pseudomonas aeruginosa* plasmid Rms149 by the generalized transducing bacteriophage ϕDS1 was shown to occur during a 9-day incubation of environmental test chambers in a freshwater reservoir. Plasmid DNA was transferred from a nonlysogenic plasmid donor to a ϕDS1 lysogen of *P. aeruginosa* that served both as the source of the transducing phage and as the recipient of the plasmid DNA. When the concentration of donors introduced into the chambers was varied while the recipient concentration in each chamber was at a level equivalent to natural concentrations of *P. aeruginosa*, the concentration of plasmid-containing donor cells introduced was shown to affect the frequency of transduction significantly. Transduction was observed both in the absence and in the presence of the natural microbial community. The presence of the natural community resulted in a rapid decrease in the numbers of the introduced donors and recipients and a decrease in the number of transductants recovered. These results demonstrate the potential for naturally occurring transduction in aquatic environments and indicate that donor load may be an important parameter in assessing this potential.

Transfer of genetic material between bacteria mediated by transducing bacteriophages is a widely used standard technique in microbial genetics. Whereas bacteriophages exhibiting transductional capabilities have been shown to occur in a number of environmental systems (21), relatively little is known about the potential for gene transfer mediated by bacteriophages in the environment.

Transduction of drug resistance genes encoded by plasmid DNA has been well documented when performed under laboratory conditions in *Escherichia coli* and *Salmonella typhimurium* (8, 19, 28). We have previously demonstrated transduction of chromosomal genes of *Pseudomonas aeruginosa* in a freshwater environment by the temperate phage F116 (20). Both cell-free lysates grown on the donor strain and F116 lysogens were shown to mediate transduction despite the nutritive, thermal, and ionic conditions encountered in a natural setting.

With concern over introduction of genetically altered bacteria into the environment, an evaluation of the potential for transfer of genes carried on extrachromosomal elements in the environment seems timely. The purpose of this study was to investigate the propensity for transduction of plasmid DNA in a natural freshwater habitat. We examined a number of systems which seem representative of those which potentially would allow gene transfer by transduction in a natural setting. The effects of the donor/recipient ratio on detectable transduction frequencies both in the presence and in the absence of the natural community were assessed. Our investigation demonstrated that plasmid transduction can occur in freshwater environments.

MATERIALS AND METHODS

Bacteria, bacteriophages, and plasmids. All of the bacterial strains used in these studies are derivatives of *P. aeruginosa* PAO and are listed in Table 1. The plasmids used are described in Table 2. Bacteriophage F116L (16) was a gift of J. Shapiro. Phage ϕDS1 was isolated from RM2008. It is a variant of F116L, which transduces the *P. aeruginosa* PAO genome with higher efficiency (D. J. Saye and R. V. Miller, manuscript in preparation).

Media, cultivation of strains, and preparation of phage stocks. Bacteria were maintained in Luria broth or on L agar (18). *Pseudomonas* minimal medium (PMM) and PMM agar (18) containing 0.4% glucose or 10 mM acetamide were used as selective media in transduction experiments. For strains with selectable markers (plasmid or chromosomal), antibiotics were used at final concentrations (per milliliter of medium) of 500 μg of carbenicillin, 500 μg of nalidixic acid and 1,000 μg of streptomycin. Amino acids were supplemented at 25 μg/ml.

Lysates of F116L and ϕDS1 were prepared on strain PAO1 or the indicated plasmid-containing strain by the method of Miller and Ku (18). Phage were titered in lambda top agar overlays (18) on L agar plates with PAO1 as an indicator strain.

In vitro transduction protocols. (i) Cell-free lysate method. Unless indicated otherwise, recipient bacteria were grown to a cell density of 10^8 CFU/ml in Luria broth, centrifuged at 5,000 × *g* for 5 min, and suspended in TNM buffer (18). Suspended cells were mixed with phage lysates at a multiplicity of infection (MOI) of 0.1 and kept for 10 min at 37°C to allow adsorption of phage. Cells were pelleted in an Eppendorf microcentrifuge for 90 s, and the supernatant which contained unadsorbed phage was discarded. The cell pellet was suspended in TNM buffer and plated on selective medium, and transductants were enumerated after incubation for 2 days at 37°C.

Plasmid-containing transductants were selected on L agar supplemented with carbenicillin and nalidixic acid. Transductants were then screened for Sm^r, and the ability to use

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TABLE 1. Bacterial strains

Strain (plasmid)	Genotype[a]						Prophage[b]	Response to infection[c]	Source or reference
	ami	*ilv*	*leu*	*met*	*nal*	*rif*			
PAO1	+	+	+	+	+	+		S	18
PAO515	E200	+	+	−9011	A5	+		S	24
PU21	+	B112	−1	+	+	−		S	9
RM272	E200	+	+	+	A5	+	φDS1	I	PAO515
RM273	+	+	+	+	A910	+		S	Spontaneous mutant of PAO1
RM2008(Rms149)	+	B112	−1	+	+	−	φDS1	I	G. A. Jacoby[d]
RM2139(pME292)	+	+	+	+	+	+		S	PAO1
RM2140(Rms149)	+	+	+	+	+	+		S	PAO1
RM2142(R2)	+	+	+	+	+	+		S	PAO1
RM2143(pME294)	+	+	+	+	+	+		S	PAO1
RM2161(Rms149)	+	+	+	+	+	+	F116L	I	RM2140
RM2162(Rms149)	+	+	+	+	+	+	φDS1	I	RM2140

[a] The genotype symbols are as recommended by Demerec et al. (7). The abbreviations used are those of Bachmann (1).
[b] Prophage presence is indicated by the name of the phage.
[c] S, Sensitivity to infection; I, immunity to infection by F116L and φDS1.
[d] Jacoby et al., 18th ICAAC.

acetamide as the sole carbon source (4–6, 20). Selection for transduction of the chromosomal marker *met-9011* of PAO515 to prototrophy was made by plating the transduction mixture onto PMM agar.

(ii) Culture method. Evaluation of the potential of lysogenic strains to serve as the source of the transducing bacteriophage by spontaneous induction was carried out in 1-liter volumes of PMM supplemented with glucose. RM272 was used as the recipient. Either RM2140 (nonlysogenic) or RM2162 (lysogenic) was used as the donor strain. Both donor and recipient were inoculated to levels of 10^3 CFU/ml and incubated at 37°C. Samples were taken and evaluated at 24-h intervals.

Total cell counts were made by plating appropriate dilutions on L agar. Donor and recipient concentrations were determined by plating onto L agar containing carbenicillin or nalidixic acid, respectively. Transductants were estimated by (i) plating 0.1 ml onto L agar containing carbenicillin and nalidixic acid or (ii) filtering 100 ml of sample through a 0.45-μm (pore size) membrane filter (Millipore Corp., Bedford, Mass.) and placing the filter on the surface of the selective plate. Plates were incubated at 37°C for 2 days.

In situ transduction. (i) Field site. Lake water was collected in sterile 20-liter Nalgene carboys at a fixed sampling site located at the Singleton terminal of the Little River embayment on Fort Loudoun reservoir, Knoxville, Tenn. Extensive microbiological background data exist on this reservoir concerning bacterial densities, activities, biodegradation of polluting substances, gene screening, and plasmid occurrence (22, 25–27). Physical and chemical attributes of the native water were measured at the site of collection, and the water was transported to the laboratory within 1 h of collection. Viable cell counts of Fort Loudoun Lake water were 3×10^4 CFU/ml when plated on yeast extract-peptone-glucose agar (26). Full data on the physical and chemical parameters at each sampling time are available on request. The ranges for these parameters were as follows: air temperature, 17 to 34°C; water temperature, 19 to 32°C; dissolved oxygen, 2.8 to 9.9 mg/liter; conductivity, 120 to 260 μmhos; and pH 7.1 to 9.2.

TABLE 2. Plasmids

Plasmid	Phenotype[a]	Molecular size (kb)[b]	Copy no.	Reference(s)
pME292	Cb^r Km^r Mob^- Tra^-	6.8	2	12–14
pME294	Cb^r Km^r Mob^- Tra^-	6.8	14	12–14
Rms149	Cb^r Gm^r Sm^r Su^r Mob^- Tra^-	55	?	9, 15
R2	Cb^r Km^r Sm^r Su^r Mob^+ Tra^+	67.5	?	15

[a] Abbreviations used: Cb^r, carbenicillin resistance; Gm^r, gentamicin resistance; Km^r, kanamycin resistance; Sm^r, streptomycin resistance; Su^r, sulfonamide resistance; Mob, mobilization; Tra, transmissible; +, proficient; −, deficient.
[b] kb, Kilobases.

(ii) Preparation of bacterial cultures. Cultures to be used for inoculation of environmental chambers were grown overnight in 1 liter of Luria broth under appropriate selection at 37°C with shaking. Cells were harvested by centrifugation at 5,000 × *g* for 5 min and suspended in a total volume of 200 ml of sterile (autoclaved) Fort Loudoun Lake water (optical density, 110 Klett units at 660 nm). The suspended cells were transported on ice to the field site, where the environmental test chambers were inoculated.

(iii) Preparation and incubation of environmental test chambers. The test chambers (8 liters total volume) were purchased from Plastic Film Enterprises (Royal Oak, Mich.) and consisted of Teflon film bags (Tedlar) double sealed at the edges, with a single sealable valve. A length of Tygon tubing was securely attached to the valve, and a tubing flow valve was attached at the distal end of the tubing. Access into the chambers for filling, inoculation, and removal of samples was by way of the Tygon tubing.

The chambers and the Fort Loudoun Lake water were sterilized separately by autoclaving, and the chambers were aseptically filled to a volume of 7 liters. Chambers for the natural community were separately autoclaved and then filled with 7 liters of fresh lake water. The filled chambers were transported to the field site and inoculated in situ.

The chambers were secured with polyester netting and suspended in the lake at a depth not exceeding 0.5 m. Two 125-ml samples were aseptically removed from each chamber. One of these samples was used to determine physical and chemical parameters. The second sample was transported on ice to the laboratory for microbiological determinations (within 6 h). Sampling was performed at 0, 12, 24, 36, 48, and 72 h and at 5 and 7 days. Two field trials were conducted.

TABLE 3. Frequency of transduction of various plasmids as a function of molecular size

Plasmid	No. of transductants/10^7 PFU[a]					
	F116L			ϕDS1		
	Chromosomal (*met-9011*)	Plasmid (Cb^r)	P/C ratio[b]	Chromosomal (*met-9011*)	Plasmid (Cb^r)	P/C ratio[b]
pME292	1.0	<0.2[c]		14.2	0.2	0.01
pME294	4.5	<0.2[c]		23.6	0.9	0.04
Rms149	2.5	0.3	0.12	1.9	18.5	9.7
R2	1.0	0.5	0.5	19.5	42.7	2.2

[a] PAO515 was infected at a MOI of 0.1 in TNM buffer as described in the text. Transduction of chromosomal DNA was monitored by selecting for methionine prototrophy. Plasmid DNA transduction was monitored by selection for Cb^r.

[b] To show the relative transduction potentials of the various plasmids, transduction frequencies are expressed as the ratio of plasmid transductants (P) to chromosomal (*met-9011*) transductants (C). All donor strains are transformants of PAO1; therefore, the frequency of *met-9011* transduction to prototrophy should be equal for each of the donor strains and characteristic of the transducing phage used. Ratios were not calculated for entries below the limit of detection of our assay.

[c] These values indicate the limit of detection in our assay. No transductants were observed.

(iv) Primary selection and enumeration of transductants. From each experimental sample, 1-, 10-, and 100-ml volumes were filtered through 0.45-μm membrane filters (Millipore). Before filtration, nalidixic acid was added to a final concentration of 400 μg/ml to halt DNA metabolism in the donor (2). Filters were extensively washed with phosphate-buffered saline to remove bacteriophage. Preliminary laboratory data have shown that these steps preclude the possibility of transduction occurring on the membrane surface and assure that only transductants formed before filtration appear on the membrane after incubation. Each membrane was transferred to the surface of an L agar plate supplemented with carbenicillin and nalidixic acid and incubated for 2 days at 37°C. Total viable cell counts were obtained on L agar, and total *Pseudomonas* counts were estimated on *Pseudomonas* isolation agar (Difco Laboratories). Donor and recipient cell numbers were estimated on L agar plates containing carbenicillin and nalidixic acid respectively.

(v) Determination of free bacteriophage titers. Five milliliters of each sample was filtered through 0.45-μm membrane filters, and the filtrate was serially diluted in Luria broth. The dilutions were mixed with PAO1 in a lambda top agar overlay, and phage were enumerated by plaque formation. The detection limit for free phage was 100 PFU/ml.

Genetic and molecular screening of potential transductants. To verify that the putative transductants contained both the plasmid markers associated with Rms149 and the appropriate chromosomal markers unique to the recipient strain, transductants were replica plated onto L agar containing appropriate antibiotics and onto PMM supplemented with acetamide (10 mM) or glucose (0.4%) as the sole carbon source (4–6, 20). To verify further that Rms149 was present in the transductants, plasmid DNA was isolated by rapid alkaline lysis (17), digested with *Eco*RI (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) as recommended by the manufacturer, and electrophoresed on 0.7% agarose gels (17). Digestion patterns were analyzed by comparing them with that of authentic plasmid DNA isolated from RM2140 (9).

RESULTS

Transduction efficiency as a function of plasmid molecular size. A series of experiments was conducted in the laboratory to compare transduction of plasmids of different sizes by the temperate *P. aeruginosa* bacteriophages F116L (16) and the ϕDS1 variant. Plasmids of both small and large molecular sizes were tested (Table 2). Transductions were carried out, as previously described, by the cell-free lysate method (Table 3). Transduction of the chromosomal marker *met-9011* to prototrophy was used as an internal control for efficiency of transduction. F116L-mediated transduction of plasmid DNA was detectable only for plasmids of high molecular weight. ϕDS1 was able to transduce plasmids of both sizes, although higher frequencies were observed for the larger plasmids. The frequencies of transduction of both chromosomal and plasmid DNAs by ϕDS1 were consistently greater than comparable F116L transduction frequencies.

Rms149 was chosen for subsequent experiments since this plasmid is not self-transmissible (G. A. Jacoby, T. R. Korfhagen, and L. Sutton, Program Abstr. 18th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 93, 1978), and our preliminary data indicated that both F116L and ϕDS1 were able to mediate its transfer. The multiple antibiotic resistances conferred by Rms149 also improved our ability to select and identify putative transductants.

Evaluation of Fort Loudoun Lake water as a transducing medium. As a first step in evaluating freshwater environments as a medium for transduction of plasmid DNA, we substituted sterile Fort Loudoun Lake water for TNM in the standard cell-free lysate transduction protocol. Although overall ϕDS1-mediated transduction frequencies were reduced slightly (an average of threefold) when compared with transduction frequencies observed with TNM buffer as the suspending medium, the plasmid/chromosome ratio remained constant.

Laboratory evaluation of transduction by the culture method. To investigate the potential for transduction with lysogens as the source of transducing phage, two 1-liter volumes of sterile lake water were inoculated with a lysogenic or nonlysogenic donor and a lysogenic recipient

TABLE 4. In vitro experiments with lysogens as a source of transducing particles in culture method transductions

Incubation time (h)	Flask 1[a]			Flask 2[a]		
	Plasmid donor RM2140 (NL) (CFU/ml)	Recipient RM272 (ϕDS1) (CFU/ml)	Transductants (CFU/ml)	Plasmid donor RM2162 (ϕDS1) (CFU/ml)	Recipient RM272 (ϕDS1) (CFU/ml)	Transductants (CFU/ml)
24	1.1×10^4	1.0×10^4	0.01	2.0×10^3	1.7×10^4	<0.01[b]
48	3.4×10^7	3.6×10^7	2.2×10^3	1.7×10^4	3.4×10^6	0.18
72	2.4×10^8	6.2×10^8	1.5×10^3	6.0×10^6	5.2×10^8	<10[b]

[a] Donors and recipients were inoculated into each flask at 10^3 CFU/ml. NL, Nonlysogenic.

[b] These values indicate the limit of detection in our assay. No transductants were observed.

TABLE 5. Description of in situ experimental chambers

Expt and chamber no.	Plasmid donor[a]	Recipient[a]	Donor/recipient ratio	Free phage	Natural community
Field trial 1[b]					
I	—	RM273 (NL)	1:1		Absent
II	RM2140 (NL)	RM273 (NL)	1:1		Absent
III	—	RM273 (NL)	1:1	φDS1	Absent
IV	—	RM273 (NL)	1:1	F116L	Absent
V	RM2162 (φDS1)	RM273 (NL)	1:1		Absent
VI	RM2161 (F116L)	RM273 (NL)	1:1		Absent
VII	RM2162 (φDS1)	—			Absent
VIII	RM2140 (NL)	RM272 (φDS1)	1:1		Absent
IX	RM2140 (NL)	RM272 (φDS1)	1:1		Present
Field trial 2[c]					
I	RM2140 (NL)	RM273 (NL)	35:1		Absent
II	RM2140 (NL)	RM272 (φDS1)	1:5		Absent
III	RM2140 (NL)	RM272 (φDS1)	2:1		Absent
IV	RM2140 (NL)	RM272 (φDS1)	20:1		Absent
V	RM2140 (NL)	RM272 (φDS1)	200:1		Absent
VI	RM2140 (NL)	RM273 (NL)	35:1		Present
VII	RM2140 (NL)	RM272 (φDS1)	1:5		Present
VIII	RM2140 (NL)	RM272 (φDS1)	2:1		Present
IX	RM2140 (NL)	RM272 (φDS1)	20:1		Present
X	RM2140 (NL)	RM272 (φDS1)	200:1		Present
XI	—	—			Present

[a] Designations in parentheses indicate the presence of prophage. —, not present; NL, nonlysogen.
[b] In field trial 1 (4–13 June 1986), both the donor and recipient strains were inoculated at 10^4 CFU/ml. Phage were inoculated at 5×10^3 PFU/ml.
[c] In field trial 2 (13–20 August 1986), the recipient was inoculated at 10^4 CFU/ml. The donor strain was inoculated at a concentration sufficient to give the donor/recipient ratio indicated. Free phage were not added to any of these chambers.

(Table 4). Each system was supplemented with 0.4% glucose as a carbon source and incubated at 37°C. The system containing the nonlysogenic donor with the lysogenic recipient showed the largest numbers of transductants. Subsequent trials produced similar results.

In situ transduction models (field trial 1). Nine chambers were introduced into Fort Loudoun Lake (Table 5). These were designed to test the following three paradigms for the source of transducing phage: (i) cell-free lysates—chambers III and IV; (ii) lysogenic plasmid donors—chambers V and VI; and (iii) lysogenic, plasmid-free recipients—chambers VIII and IX. In model iii, the presence of the natural microbial community (chamber IX) was also evaluated.

Only chamber VIII yielded detectable levels of transductants. Four transductants were isolated from chamber VIII. Two were recovered from the 24-h sample, and two were taken from the 48-h sample. The four transductants from chamber VIII were analyzed genetically and found to be Cb^r and Sm^r (plasmid markers), as well as Nal^r, and unable to utilize acetamide as a carbon source (chromosomal markers unique to the recipient). Isolation of plasmid DNA from these transductants and restriction analysis with *Eco*RI confirmed that these isolates indeed harbored plasmid Rms149 (Fig. 1).

Chamber IX, which differed from chamber VIII only by containing the natural microbial community, did not produce detectable transductants. The concentration of viable donors and recipients in chamber IX fell to 100 CFU/ml after 24 h and less than 10 CFU/ml by 72 h of incubation even though the total cell count (indigenous community) remained constant. The number of donors and recipients in chamber VIII remained at or above the level of inoculation through 72 h, and 10^2 CFU/ml was recoverable after 5 days of incubation. There appeared to be a significant effect exerted by the natural community on the ability of inoculated organisms to survive within the test environment.

Phage titers in chambers VIII and IX were both 10^3 PFU/ml at the 0- and 1-h samplings. The number of phage in chamber VIII increased to 10^4 PFU/ml by 6 h, while the number of phage in chamber IX decreased to 5×10^2 PFU/ml. After 24 h of incubation, phage in chamber IX

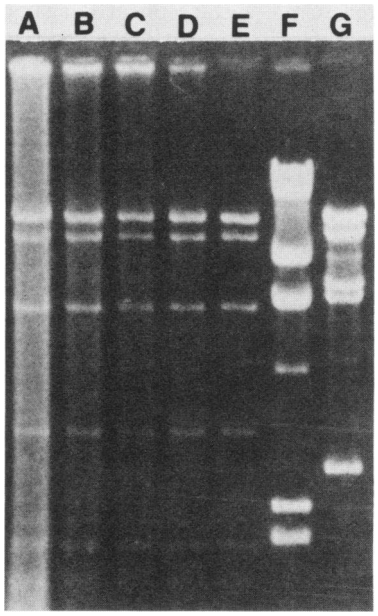

FIG. 1. *Eco*RI restriction patterns of plasmid DNA from transductants isolated from environmental test chambers. Transductants recovered after 24 (lanes A and B) or 48 (lanes C and D) h of incubation in situ are compared with authentic Rms149 DNA purified from RM2140 (lane E). *Hin*dIII fragments of phage lambda (lane F) and an *Eco*RI digest of φDS1 DNA (lane G) are also shown.

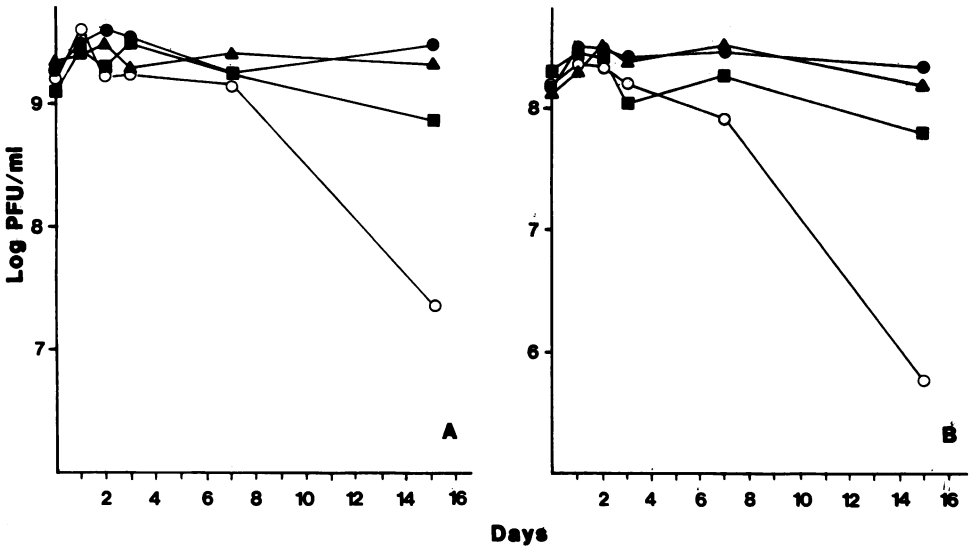

FIG. 2. Stability of ϕDS1. The ability of ϕDS1 to remain infective was evaluated by incubating the phage suspension at room temperature (25 to 26°C) in Luria broth (●), PMM (■), and sterile (▲) and nonsterile (○) Fort Loudoun Lake water. Phage were enumerated by plaque formation in lambda top agar overlays containing PAO1. Panels: A, lysates grown on PAO1; B, lysates grown on RM2140.

could not longer be detected (<100 PFU/ml). The titer in chamber VIII remained at 10^4 PFU/ml or greater through 7 days of incubation and was still 8×10^3 PFU/ml at 9 days of incubation.

Effects of the natural microbial community on the stability of transducing particles. The stability of the free bacteriophage ϕDS1 was subsequently investigated in the laboratory. Cell-free lysates of ϕDS1 were prepared after growth on PAO1 and RM2140. The lysates were inoculated at 10^9 and 10^8 PFU/ml, respectively, into sterile L broth, sterile PMM, sterile Fort Loudoun Lake water, and nonsterile Fort Loudoun Lake water. The concentrations of PFU were determined over a 15-day period. In the sterile media, phage titers remained relatively stable over this period, but in nonsterile lake water the titers fell 100-fold over the 15-day period beginning at 48 h (Fig. 2).

The frequency of transduction produced by these phage lysates was evaluated at each sampling period. The number of Rms149 transductants produced decreased in the first 24 h and then remained relatively constant (data not shown).

Effect of plasmid donor concentration and the natural microbial community on in situ frequencies of transduction of plasmid DNA (field trial 2). In field trial 1, only paradigm iii produced detectable levels of transductants. Field trial 2 was designed to confirm this observation and evaluate the model further. Eleven test chambers were used (Table 5). Each of the experimental chambers was inoculated with a nonlysogenic, plasmid-containing donor and a lysogenic recipient. Free phage particles were not added to any of these chambers. They were designed to test (i) the effect of varying the concentration of plasmid-containing cells on the rate and frequency of transduction of plasmid DNA to the recipient and (ii) the effect of the natural microbial community in our test system.

Transduction of Rms149 showed a significant response to the donor/recipient ratio. In the absence of the natural microbial community, both the maximum concentration of transductants and the time at which maximum levels were recovered varied (Fig. 3). When the natural community was present, there was a general depression in the number of

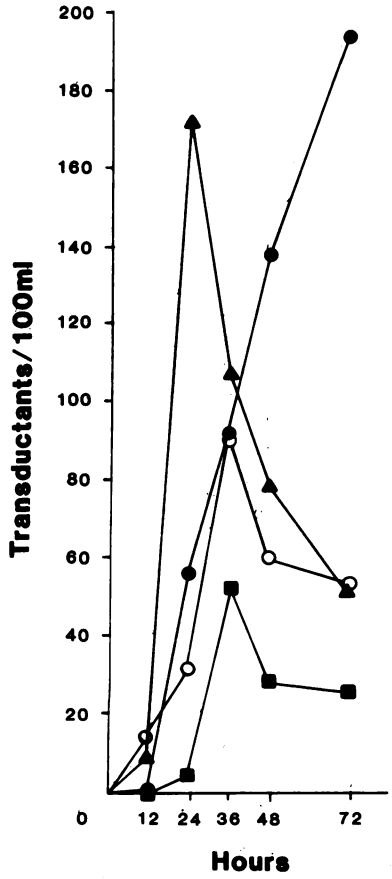

FIG. 3. Transductants detected in environmental chambers as a function of the donor/recipient ratio. The yield of transductants per 100 ml at each sampling interval was determined as described in the text. The donor/recipient ratios were as follows: chamber II (●), 1.5; chamber III (■), 2:1; chamber IV (▲), 20:1; and chamber V (○), 200:1. These chambers did not contain the natural microbial community.

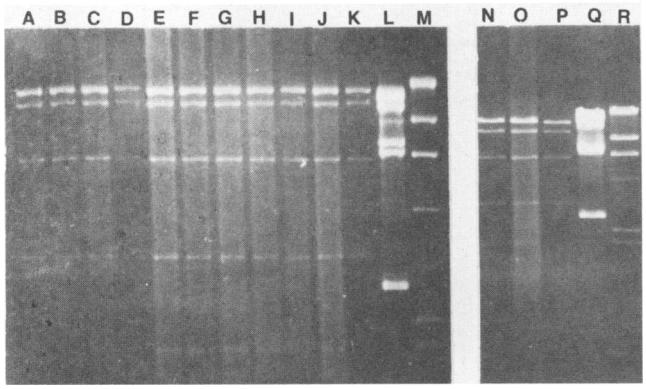

FIG. 4. Restriction analysis of transductants. *Eco*RI restriction patterns of plasmid DNA isolated from transductants recovered from chamber II (lanes A and B), chamber III (lanes C and D), chamber IV (lanes E and F), chamber V (lanes G and H), chamber VIII (lane N), and chamber IX (lanes I and O) were compared with those of Rms149 DNA isolated directly (lanes J and P) and CsCl-purified DNA (lane K) isolated from the donor strain RM2140. *Hin*dIII digests of lambda DNA (lanes M and R) and an *Eco*RI digest of ϕDS1 (lanes L and Q) are also shown.

transductants recovered, as had been seen in the first field trials. Transduction was detected only in chambers VII (at 7 days) and IX (at 24 h and 7 days).

Selected transductants from each chamber were confirmed as *P. aeruginosa* by plating on *Pseudomonas* isolation agar and analyzed genetically and molecularly to confirm transfer of plasmid Rms149. Genetic screening of the presumptive transductants revealed that they were not only Cb^r and Nal^r but also Sm^r and unable to use acetamide as a carbon source (Ami^-). *Eco*RI digestion patterns of plasmid DNAs isolated from these transductants were identical to patterns of authentic Rms149 isolated from RM2140 (Fig. 4).

Bacteriophage titers in the environmental chambers. Free bacteriophage in all chambers were <100 PFU/ml (detection limit) at time zero. In chambers II through V, phage titers reached levels of 10^4 PFU/ml by 24 h of incubation. The highest titers were obtained between 24 and 48 h in the various chambers and remained at these levels through 72 h. They began to decrease by day 5 of incubation.

When the natural community was present, free phage were detected only in chamber X. The concentrations of phage in the other chambers remained below the limit of detection. The titers in chamber X reached 5×10^2 PFU/ml at 24 h and remained at this level.

Survival of introduced organisms in the test chambers. When the natural microbial community was present, it was found to reduce the number of viable cells of the introduced strains significantly during the in situ incubation period (Fig. 5). Chamber XI, which contained only fresh uninoculated Fort Loudoun Lake water, was screened at each sampling to determine naturally occurring background organisms. The organisms introduced into each chamber began to decrease rapidly after 12 h of incubation, and by 48 h the number of viable cells was reduced to a level equivalent to that of the uninoculated chamber.

Figures 6 and 7 show the changes in donor and recipient cell numbers in each chamber over the in situ incubation period. In all cases, recipient cell numbers dropped off to $<10^2$ CFU/ml by 72 h of incubation. Donor cells also decreased to 10^2 CFU/ml except in chamber X, where 10^3 CFU/ml was present throughout the 7 days of incubation.

DISCUSSION

The occurrence of phage-mediated transduction of genetic material in nature must by necessity not only involve interaction between the components of the transduction system themselves but also deal with their interaction with the environment. We chose to test the following three models for plasmid transfer in situ: (i) transduction by free bacteriophage in the form of lysates which were grown on the donor strain and a nonlysogenic recipient, (ii) a lysogenic plasmid donor and a nonlysogenic recipient strain, and (iii) a nonlysogenic plasmid donor strain and a lysogenic recipient. We reasoned that all three systems could be representative of those which function in nature, and preliminary laboratory data indicated that plasmid transduction was plausible in these systems. Evidence for chromosomal markers being transferred by the first two systems has been described by Morrison et al. (20), who used phage F116 as a transducing vector.

In our first field trials, transductants were detected only in model system iii, which contained a nonlysogenic plasmid donor strain and a lysogenic recipient strain. The cell density in this chamber remained equivalent to environmental *Pseudomonas* concentrations, indicating that a potential exists

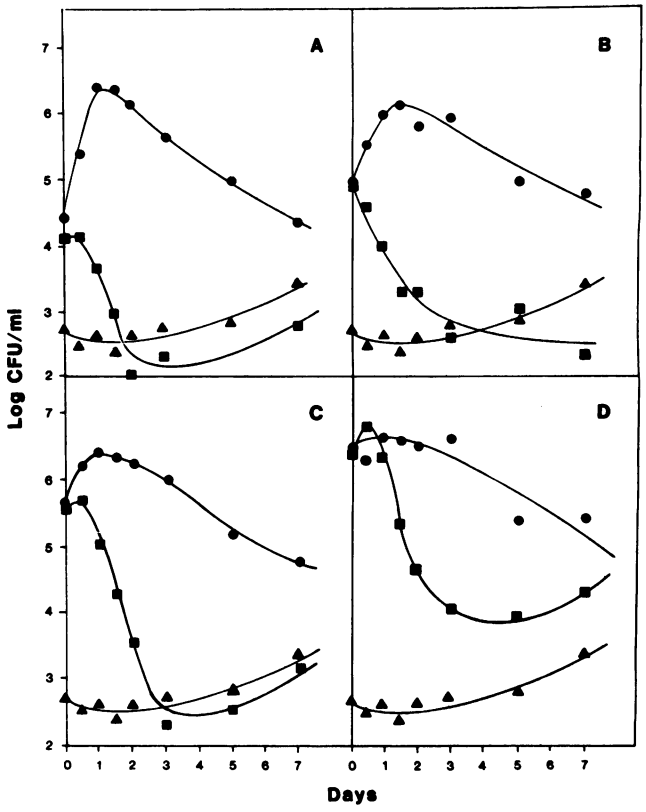

FIG. 5. Effect of natural community on survival of introduced organisms in environmental test chambers. Chambers were inoculated, incubated, and sampled as described in the text. Viable cell counts (enumerated on L agar) in the absence (●) or presence (■) of the natural community are shown for each donor/recipient ratio. The viable count in uninoculated (chamber XI) Fort Loudoun Lake water (▲) is shown in each panel to represent the contribution of the natural microbial community to the total viable cell counts. Panels: A, chambers II (●) and VII (■); B, chambers III (●) and VIII (■); C, chambers IV (●) and IX (■); D, chambers V (●) and X (■).

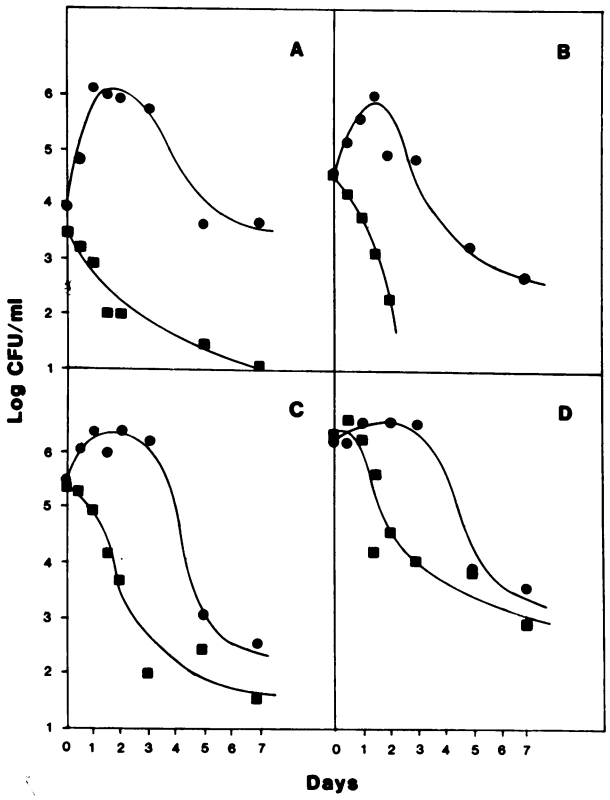

FIG. 6. Survival of introduced plasmid donor cells in test chambers. Viable counts of RM2140 in the presence (●) or absence (■) of the natural community were enumerated as described in the text. Panels: A, chambers II (●) and VII (■); B, chambers III (●) and VIII (■); C, chambers IV (●) and IX (■); D, chambers V (●) and X (■).

for transduction of plasmid DNA in a natural freshwater environment.

For transduction of plasmid DNA to occur in system iii, the following sequence of events must occur. (i) Spontaneous induction of phage from the lysogen must take place. (ii) These viral particles must infect, propagate, and lyse the plasmid-containing donor. (iii) The bacteriophage particles produced (which include transducing particles containing host DNA) must be adsorbed and transfer DNA to the original lysogen. This series of events allows transfer of the donor DNA (either plasmid or chromosomal) to the lysogenic recipient strain.

Genetic analysis of the environmental transductants indicates the direction of gene transfer. The *nalA5* and *amiE200* markers are not cotransducible (6) and would therefore require two transduction events to generate the observed phenotype in the strain used as a donor, whereas a single transduction event, transferring the plasmid, would generate the observed phenotype if transfer proceeded from RM2140 to RM272. Therefore the spontaneously released phage from the lysogenic recipient are more likely to have resulted in a primary lytic infection of the donor releasing transducing particles which were then able to transfer the plasmid to the recipient.

Benedik et al. found that the frequency of transduction by F116L was 10- to 100-fold greater when F116L lysogens were used as recipients (3). Adsorption of F116L to lysogens and nonlysogens has been shown to be equally efficient (11). We suggest that the same effects occur in situ. The recipient can still be transduced, and the immunity of the lysogen to superinfection by infectious particles enhances its ability to persist in the environment.

In our first field trial, no transductants were observed in the presence of the natural community. Although the natural community may inhibit plasmid transduction, the recovery of transductants in chamber VIII suggests that transduction may have occurred but at levels below the sensitivity of our screening procedure.

To test this possibility, a second field trial was conducted in which the number of plasmid-containing cells (potential donors) was varied while the concentration of lysogenic recipient cells was held constant at an environmentally appropriate level. Parallel chambers with and without the natural microbial community were examined. Again, the introduction of the natural microbial community into the test model significantly reduced the numbers of transductants which could be recovered. However, transductants were isolated from two chambers. Transductants detected in the presence of the natural community were isolated from chambers which corresponded to those which showed the greatest numbers of detectable transductants in the absence of the natural community. This supports our hypothesis that transduction is reduced but not eliminated by the presence of the indigenous microbial community.

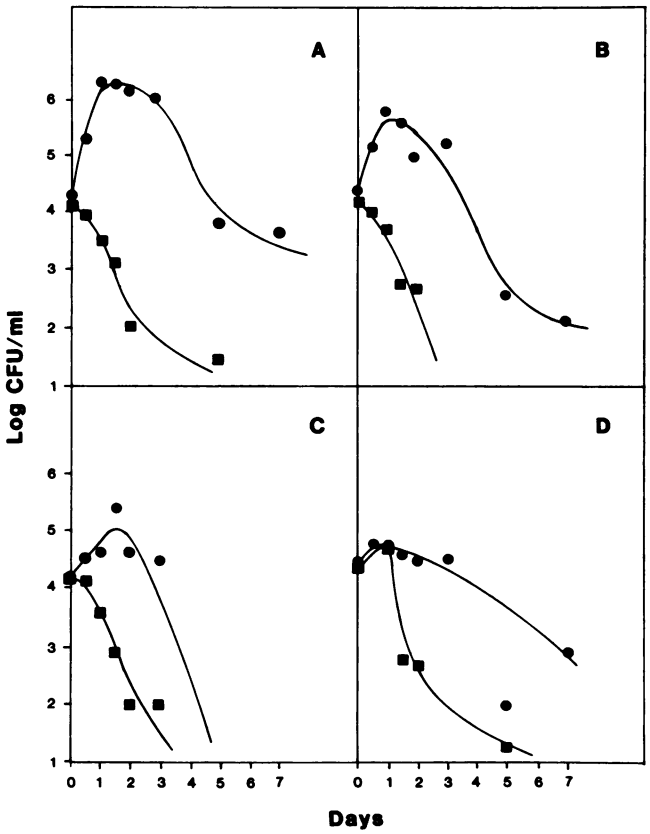

FIG. 7. Survival of introduced recipient cells in test chambers. Viable counts of RM272 recipient cells in the presence (●) or absence (■) of the natural microbial community were enumerated as described in the text. Panels: A, chambers II (●) and VII (■); B, chambers III (●) and VIII (■); C, chambers IV (●) and IX (■); D, chambers V (●) and X (■).

TABLE 6. Frequencies of transduction at various donor/recipient ratios during in situ incubation

Donor/recipient ratio	No. of transductants/10^6 recipients at an incubation time (h) of[a]:						No. of transductants/10^6 donors at an incubation time (h) of[a]:					
	0	12	24	36	48	72	0	12	24	36	48	72
1/5	—	0.05	0.27	0.51	0.86	1.80	—	0.14	0.40	0.84	1.58	3.20
2/1	—	—	0.06	1.26	2.80	1.53	—	—	0.09	0.44	0.30	0.12
20/1	—	2.29	38.00	3.96	17.70	15.30	—	0.05	0.54	0.76	0.25	0.25
200/1	—	2.00	4.84	20.00	16.20	12.00	—	0.07	0.07	1.95	0.12	0.13

[a] The number of transductants recovered at the indicated incubation time was normalized to the number of viable donor or recipient cells recovered at that sampling time. —, No transductants were detected.

In chambers which did not contain the natural community, the frequency of transduction was dependent on the donor/recipient ratio. When plasmid-containing, nonlysogenic donors were in excess, transduction was maximized at the intermediate donor/recipient ratio of 20:1 (Fig. 3). This relationship may result from a difference in the MOIs in the various test chambers. An optimum MOI may be achieved when donors and recipients interact at a specific ratio and concentration. When the donors/recipient ratio is altered, the MOI and hence the potential for transduction may also be altered.

P. aeruginosa bacteriophage have previously been shown to occur naturally in aquatic habitats in concentrations as high as 2×10^3 PFU/ml (23). The presence of naturally occurring strains of *P. aeruginosa* which carry one or several prophage has been demonstrated as well (10). It seems reasonable that these naturally occurring lysogenic strains could serve as a rich source of generalized transducing phage in nature.

The results of laboratory studies on phage longevity in nonsterile Fort Loudoun Lake water indicate that survival of infectious phage particles is decreased in the presence of the natural community. Therefore, it seems reasonable to assume that the effective in situ reservoir of temperate phage capable of mediating gene transfer is from the spontaneous induction of prophage from environmental lysogens. Subsequent infection and lysis of the introduced plasmid-containing strain would result in a locally high titer of fresh transducing particles which may be able to overcome the immense dilution potential of a freshwater lake.

The greatest numbers of transductants were detected in our test chambers at times when the concentrations of recipient and donor cells were highest. Decreased recovery of transductants after 36 h may simply reflect decreasing recovery of viable cells in general. When the number of transductants observed at a given time was normalized to the number of viable recipient or donor cells in the chambers at that time, the number of transductants remained relatively constant after the peak values were obtained (Table 6). Whether the transductants have either a selective advantage or a disadvantage over either of the parental strains introduced into the environmental chambers could not be determined from these data. It is important to point out that no artificial selective pressure was imposed on these populations during the course of these studies.

We have demonstrated in vitro transduction of both high- and low-molecular-weight plasmids. Transduction of Rms149 was also demonstrated in situ with lysogenic strains as phage reservoirs and recipients. Therefore, it seems reasonable to conclude that the potential for transduction of genes carried on plasmid vectors exists in natural freshwater environments. Our data also demonstrate that the concentration of plasmid-bearing donor cells significantly influences the yield of detectable transductants. Since the number of recipients in each chamber was held constant at a level which could reasonably be found in freshwater habitats, these findings imply that an important parameter in assessing the potential for genetic transfer in the environment is the load of plasmid-containing organisms released into an environmental system.

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