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Frequency of F116-Mediated Transduction of *Pseudomonas aeruginosa* in a Freshwater Environment

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Transduction of *Pseudomonas aeruginosa* streptomycin resistance by a generalized transducing phage, F116, was shown to occur during a 10-day incubation in a flow-through environmental test chamber suspended in a freshwater reservoir. Mean F116 transduction frequencies ranged from 1.4×10^{-5} to 8.3×10^{-2} transductants per recipient during the in situ incubation. These transduction frequencies were comparable to transduction frequencies determined in preliminary laboratory transduction experiments. The results demonstrate the potential for naturally occurring transduction in aquatic environments and concurrent environmental and ecological ramifications.

Since its discovery by Zinder and Lederberg in 1952 (26), bacteriophage-mediated transfer of genetic material (transduction) has been found to occur in many species of bacteria. It may well be the most common mechanism of genetic exchange in bacteria. Both bacteria capable of carrying out transduction and their phages have been found in soil, freshwater, and marine and estuarine waters and sediment (3, 16, 18, 22). However, little work has been done to estimate the frequency of transduction among natural populations of bacteria in nature. With today's concern over the introduction of artificially produced bacterial genotypes into the environment, it seems timely to investigate the potential occurrence of genetic transfer in the natural environment.

The purpose of this study was to determine whether transduction could occur between a lysogenic donor bacterium and a susceptible recipient bacterium in the presence of the nutritive, thermal, and ionic limitations encountered in a freshwater environment. The bacterium chosen for this study was *Pseudomonas aeruginosa*, a common soil microorganism which has been extensively characterized genetically (5-7, 24). Several transducing phages are known for *P. aeruginosa* (8, 9), including bacteriophage F116, which was used in this study. This phage has been characterized (8, 11, 12) and is a generalized transducing vector (8).

At first thought it seems unlikely that random contact of a phage with a compatible host or of a donor cell with a recipient would occur in situ due to the vast dilution potential of a lake or river. However, bacterial cells and phage particles can accumulate and come into close proximity to one another at high concentrations in certain microenvironments. Primrose and Day (16) found *P. aeruginosa*-specific phage particles in concentrations as high as 2.0×10^3 plaque-forming units per liter of river water downstream from a sewage effluent. In addition, both bacteria and phage particles have been shown to adsorb to particulate matter in water both at high electrolyte concentrations (18) and at concentrations encountered in freshwater (3). It has also been shown that both viruses and human pathogens are concentrated by filter-feeding mollusks (3, 22). It is in these situations, where phage and host may attain high concentrations in relation to the surrounding water column, that phage-mediated gene transfer is most likely to occur.

MATERIALS AND METHODS

Bacteria and bacteriophage. All bacterial strains employed in these studies were derivatives of *P. aeruginosa* PAT and are listed in Table 1. Bacteriophage strains are also listed in Table 1.

Media and cultivation of strains. Bacteria were maintained in Luria broth (10 g of tryptone [Difco], 5 g of yeast extract, 10 g of NaCl, and 80 mg of NaOH per liter of water). Lysates of phage F116 and E79 were prepared by the method of Miller and Ku (10) using strain PAO or the indicated strain as the host cell.

Selection of recombinants. Selection for streptomycin-resistant (*str-910*) recombinants after transduction was made on L-agar (Luria broth solidified with 1.5% [wt/vol] Difco agar) containing 1,000 mg of streptomycin per ml. The plates were overlaid with 5×10^{10} plaque-forming units of phage E79. This phage was used as a contraselecting agent to eliminate any

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TABLE 1. *Bacterial and bacteriophage strains*

Strain designation	Genotype[a] or relevant characteristics	Source or reference
Bacterial strains		
PAO	Prototrophic	6
PAT	Prototrophic	7
RM2051	*str-910*	Spontaneous mutant of PAT
RM2054	*esn-901*	Spontaneous mutant of PAT
RM2060	*str-910* (F116)	F116 lysogen of RM2051
Bacteriophage strains		
F116	Temperate, generalized transducing	8
E79	Virulent	8

[a] Genotypic symbols follow the conventions proposed by Demerec et al. (2). Abbreviations: *esn*, resistant to phage E79 infection; *str*, resistant to the antibiotic streptomycin. When used with a phenotype, r indicates resistance and s sensitivity.

donor strain cells from F116 lysates and to eliminate lysogenic donor cells (RM2060) from selection plates inoculated from test chambers containing both RM2054 and RM2060 (see below). Total cell counts of RM2054 were made on L-agar plates without streptomycin, which were overlaid with E79. Total cell counts of RM2060 were on L-agar plates containing streptomycin but not overlaid with phage E79.

Preparation of bacterial cultures. Cultures of strains to be studied were grown overnight in 100 ml of L-broth incubated in a reciprocating shaker bath at 37°C. Concentrated cell suspensions were prepared by centrifuging 50 ml of each culture for 20 min at 3,000 rpm in an IEC model CL clinical centrifuge, followed by resuspension of the pellet in 10.0 ml of 0.85% sterile saline. A 1:100 dilution of this suspension was made in sterile (autoclaved) lake water obtained from Ft. Loudon resevoir, adjacent to the University of Tennessee campus, and inoculated into the environmental test chambers.

In situ transduction location. A site located on Ft. Loudon reservoir at Knoxville, Tenn. was chosen for the examination of transduction frequencies in a freshwater environment. The site was located approximately 1 km downstream from a secondary sewage treatment outfall in a region that is moderately polluted by industrial and agricultural, point and nonpoint sources of contamination.

Environmental test chambers. Modified Carolina Biomonitors (Carolina Biological Supply) were employed as environmental test chambers for performing in situ transduction experiments (Fig. 1). The chambers used consisted of polycarbonate cylinders 25 cm in length and 7.5 cm in diameter. The open ends of the cylinders were covered with 90-mm Nucleopore membranes (0.2-μm pore size) and sealed with silicone rubber sealant. The cylinders were autoclaved for 20 min and were aseptically filled to the total capacity of 720 ml with autoclaved lake water using a peristaltic pump. Access into the cylinders for filling and introduction and removal of samples was by way of a 1-cm-diameter plastic cannula fitted with a rubber serum stopper in the side of the cylinder. The filled chambers were transported to the test site and inoculated in situ.

Four chambers, designated A, B, C, and D, were used in this study. Chamber A was inoculated with the Str^s strain RM2054 to a final cell concentration of 9.0×10^5 cells per ml. This chamber served as a control to determine the spontaneous mutation rate to streptomycin resistance. Chamber B was inoculated with RM2054 to a final cell concentration of 2.0×10^5 cells per ml and a lysate of phage F116 (prepared on RM2060) at a multiplicity of infection of 1.5 phage per cell. This chamber allowed for an estimation of the rate of transduction in the presence of free phage in a natural environment. Chambers C and D were inoculated with RM2054 and RM2060 (a Str^r, F116 lysogenic strain). The final concentrations of RM2054 and RM2060 were 2.36×10^5 and 1.85×10^4 cells per ml, respectively, in chamber C. Chamber D contained RM2054 at a concentration of 4.3×10^4 cells per ml and RM2060 at 3.35×10^4 cells per ml.

The four chambers were secured with weights, tethered to floats, and suspended at a depth of about 1 m below the surface of the lake. Samples were taken at 1 h, 4 days, and 10 days. A 1.0-ml sample was removed from each chamber at each time point with a sterile syringe and was immediately transported back to the laboratory to be assayed (about 0.5 h).

RESULTS

Laboratory transduction assay. A preliminary transduction experiment was performed in the laboratory prior to the in situ studies. In this experiment RM2054 was infected with a lysate of F116 (prepared on RM2060) at a multiplicity of infection of 3.15. After incubation at 37°C for 20 min to allow for adsorption, 0.1 ml of the suspension was plated on L-agar plates containing streptomycin. In this experiment, *str-910* was transduced to RM2054 at a frequency of 3.3×10^{-5} transductants per recipient.

In situ transduction assay. The physical and chemical characteristics of the field site during the course of the in situ transduction experiment are given in Table 2. These data indicate a relatively constant environment during the time span in which the in situ experiments were performed.

Transduction of strain RM2054 by F116 at this in situ site was comparable to the laboratory frequency after 1 h of incubation in lake water (Fig. 2B). The frequency of transduction was 5×10^{-6}, 1.2×10^{-2}, and 9.5×10^{-1} transductants per recipient for 1 h, 4 days, and 10 days, respectively. These results indicate that there are no physical or chemical limitations inhibiting adsorption of phage F116 or infection of the recipient cell. In addition, the transduced cells were viable, increased in number during the first 4 days of the study, and showed survival characteristics similar to those of strain RM2054 (Fig. 2A).

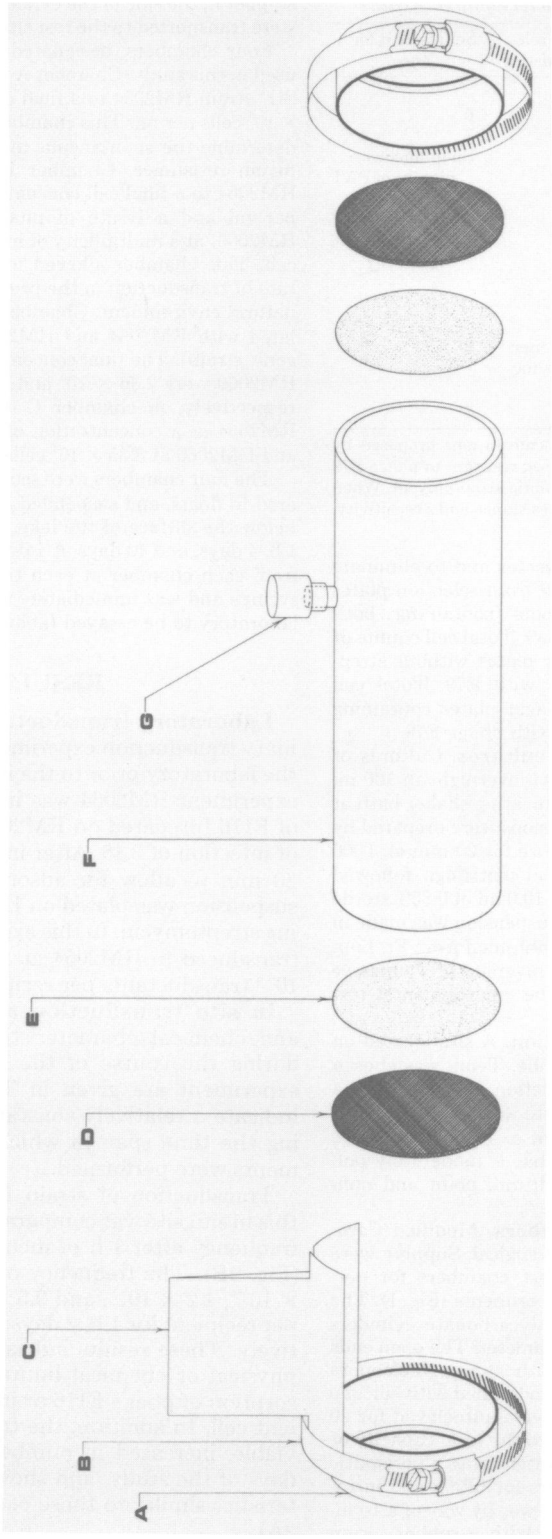

FIG. 1. *Flow-through environmental chamber used for in situ transduction assays. (A) Polyethylene retainer ring; (B) stainless steel band clamps; (C) aluminum sheet metal stabilizer fin; (D) polyurethane foam pad; (E) 0.02-μm Nucleopore polycarbonate filtration membrane; (F) clear polycarbonate cylinder; (G) rubber sampling system. Overall dimensions: length 25 cm, diameter 75 mm.*

TABLE 2. *Selected physical-chemical characteristics of in situ transduction sites on Ft. Loudoun Reservoir*

Date	Air temp (°C)	Water temp (°C)	Dissolved oxygen (mg per liter)	pH	Conductivity (μmhos)
24 July 1977	29	25	8.2	7.0	210
29 July 1977	22	25	7.8	6.1	380
4 August 1977	29	26	8.0	6.2	192

A more exciting result was found in the chambers containing a lysogenic strain (RM2060) capable of donating Str^r through F116-mediated transduction to RM2054 (Fig. 2C and D). The mean transduction frequencies in these chambers were 1.4×10^{-5}, 5.9×10^{-3}, and 8.3×10^{-2} after 1 h, 4 days, and 10 days, respectively. The appearance of transduced cells in these chambers can only be an indication of the lysogenic donor cells' ability to release viable phage and the concurrent infection of the recipient cells in situ. The transduced cells attained population densities comparable to those of the donor.

The rate of appearance of transduced cells in these chambers (Fig. 2C, D) is comparable to the rate of appearance of transduced cells in chamber B, which contained a transducing lysate of phage F116 (Fig. 2B). This indicates that the relative rate of transduction in this aquatic environment is not limited by the release of phage by the lysogenic strain.

DISCUSSION

Bacteriophage-mediated transduction of genetic material has long been used in the laboratory as a method of genetic analysis of bacteria. Even though this method of genetic transfer is known to occur in more bacterial species than any other method of reassortment of genetic material, little information has been gathered concerning its occurrence in nature. This study has attempted to estimate the potential for phage-mediated genetic transfer in an aquatic environment. We found that, under the conditions employed, transduction not only takes place but has the potential to mediate high levels of genetic reassortment.

Realistically, the in situ test chamber incubation can only approximate ambient field conditions. Variables such as re-aeration rates, flow rates, light intensity, dilution, etc. will be severely affected by maintaining a sample within any enclosure. In addition, it is well documented that sample enclosure can greatly enhance the growth of microorganisms through periphytic attachment and/or the adsorption of nutrients to the chamber (27). Such factors may have accounted for some of the population growth observed in this study (Fig. 2). However, the diffusion into the chambers of oxygen, organic and mineral nutrients, and toxic agents does undoubtedly occur. In addition, temperature fluctuations, a very important variable, are closely approximated within the chambers. In essence, the test chambers employed in this study were far superior to laboratory assays in simulating natural field conditions and were immeasurably more ethical and practical than attempting to dose an entire aquatic habitat with phage, donor, and recipient cells.

Transduction frequencies of streptomycin resistance using a cell-free lysate of phage F116 were comparable in laboratory and in situ experiments. More interestingly, phage-mediated transduction of *str-910*, in experiments where transducing particles were produced by spontaneous induction of a lysogenic strain in situ, was as frequent as when a laboratory-produced transducing lysate was present in the test chamber.

Apparent transducing frequencies after 4 and 10 days of incubation in the aquatic environment were quite high. It is likely that this frequency of transduction is artificially elevated due to several factors. First, the decrease in the total viable population after 10 days of incubation may have increased the portion of cells that had undergone transduction. This is probably not a highly significant factor, since there was no apparent selection for streptomycin-resistant organisms (no spontaneous mutants were selected in chamber A). Second, many of the *str-910* [*esn-901*] cells scored after 4 and 10 days were probably not primary transductants but daughter cells which arose as a result of cell division of the primary transductants. The percentage of the total *str-910* [*esn-901*] population due to this in situ growth can be estimated by examining the total populations in chambers B, C, and D. After 4 days of incubation, the viable count of cells in these chambers doubled approximately six times. If all the transductants were produced in the first generation, then 1.5% of the total transductants observed on day 4 represent the number of transductional events. (This is a minimum estimate of the primary transductants, since some will have arisen during subsequent generations and therefore contribute fewer daughter cells to the total population.) Thus, the minimum frequency of primary transductants after 4 days of incubation in situ can be estimated at 8.8×10^{-5}. This corrected figure,

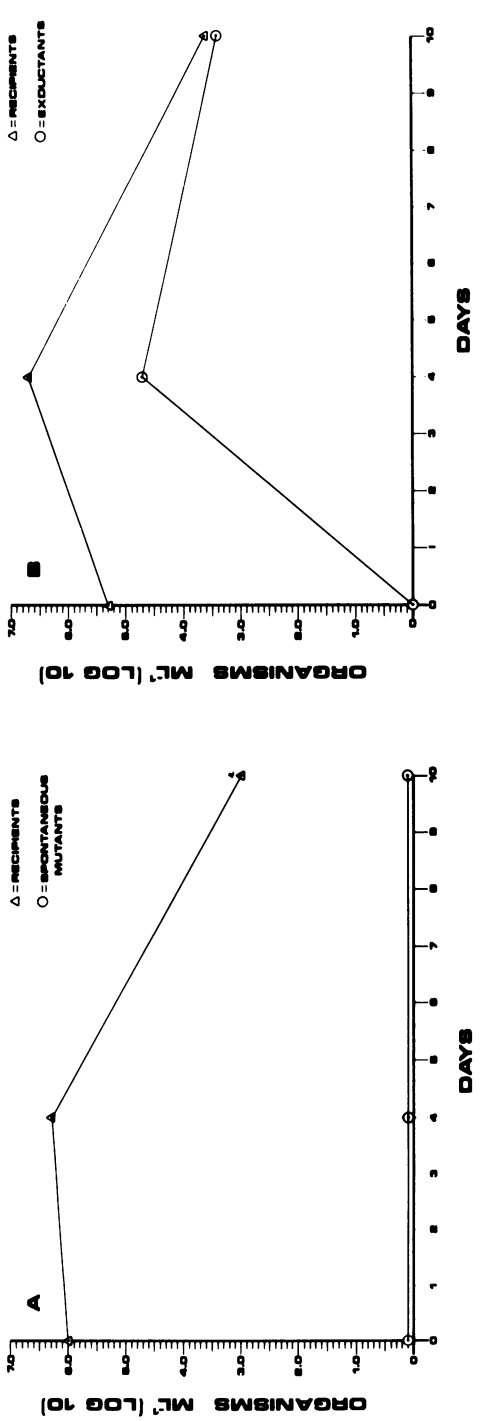

FIG. 2. *Viable count of transductants and parental strains in environmental test chambers. Chambers were inoculated, incubated, and sampled as described in the text. Frequencies of exductants (str-910 [esn-901]) were measured by determining F116-mediated transfer of str-910 from the F116 lysogen, streptomycin-resistant strain RM2060 (donor), to the streptomycin-sensitive, phage E79-resistant strain RM2054 (recipient). Chamber A contained only RM2054. Chamber B contained RM2054 and a cell-free lysate of F116 produced on RM2060. Chambers C and D contained RM2054 and RM2060 (▲, lower limits of detection).*

which is approximately six times greater than the figure obtained for the frequency after 1 h of incubation, indicates that the frequency of transduction remained essentially constant throughout the in situ incubation.

It is exciting to speculate on the significance of phage-mediated gene transfer in natural environments. Reanney (17) has discussed the potential importance of various types of gene transfers and their relationship to microbial evolution. In this respect, the "genospecies" concept (17) can relate directly to phage-mediated gene transfer and the production of physiologically aberrant bacterial strains, the nemesis of the taxonomist studying environmental isolates. Furthermore, genome size conservation may be considered possible since it would not be necessary for every individual within a population to have a constitutive gene for every potential physiological characteristic of that population.

Gene transfer from extra-environmental microorganisms to isolates from natural habitats is documented (15). Such transfers may play a part in the ubiquity of antibiotic-resistant microorganisms in natural environments (1, 15), as well as the perpetuation of antibiotic resistance among natural populations (1). In addition, utilizable substrate transmissibility, observed in the enterobacteria (19), could also operate for other environmental microbial populations. Although these phenomena are related primarily to conjugal plasmid transfer, the transduction of plasmid DNA has also been demonstrated under laboratory conditions (4, 13, 23). This raises interesting questions concerning *Pseudomonas*, which demonstrates plasmid-dependent metabolism of rather exotic hydrocarbon substrates (4, 25) as well as heavy metal resistance (20).

The results of this investigation demonstrate the ability of phage F116 to transduce susceptible *P. aeruginosa* under field conditions. Bacteria and phage densities were optimized to detect transducible phenotypic expression (i.e., antibiotic resistance). This manipulation of population densities was justified by the occurrence of localized high concentrations of susceptible bacteria and phage in aquatic sediments and wastewater (18, 21, 22). Consequently, it can be predicted that transduction, resulting in microbial gene pool diversification, occurs in natural aquatic environments.

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