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Application of DNA Probes to Analysis of Bacteriophage Distribution Patterns in the Environment

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Radiolabeled bacteriophage DNA probes have been used in this study to determine the distribution of *Pseudomonas aeruginosa*-infecting bacteriophages in natural samples of lake water, sediment, soil, and sewage. The sensitivity of detection of bacteriophage with the DNA probes was between 10^3 and 10^4 PFU and 10^6 to 10^7 CFU of lysogenized bacteria detectable with a homologous phage DNA probe. Analyses of environmental samples suggest that up to 40% of *P. aeruginosa* in natural ecosystems contain DNA sequences homologous to phage genomes. By using different bacteriophage DNA probes, the diversity of the bacteriophage population in sewage was estimated to be higher than that in other natural samples. The indication that transducing phages and prophages are widely distributed in the *Pseudomonas* populations investigated has considerable implications for the frequency of natural gene transfer by transduction and of lysogenic conversion of host bacteria in natural ecosystems.

Very few techniques are available for studying bacteriophage population dynamics in environmental samples because of the limitations posed by high dilution in aquatic systems and sorption to particulate materials in terrestrial and other coarse ecosystems (24, 29, 33). The traditional technique for isolating phages from environmental samples is enrichment with a specific host bacterium, followed by plaque assay (5). The interpretation of data gathered through enrichment procedures is limited by inadequate quantification. Furthermore, the small amount of environmental inoculum that is often used renders the detection of sparsely distributed phage particles difficult (29). Another limitation of the enrichment procedure is the tendency to select for the most vigorously virulent particles in a heterogeneous phage population, thereby masking the detection of temperate phages and phages with small burst sizes (8). Alternative methods for detecting phages in environmental samples are (i) the filtration of large volumes of water, sometimes up to 200 liters (23, 29), and (ii) direct electron microscopy, a technique useful only for samples containing a large population of phage particles (1, 22, 25). All of these techniques aim to detect the presence of phage particles, but not enough importance is attributed to the fate of host bacteria inhabiting the same environment. The occurrence of temperate and facultative virulent phages in natural ecosystems suggests that a large proportion of phage populations remains undetected through the use of techniques that depend entirely on the plaque-forming ability of isolated bacteriophages.

In this paper, we describe the use of DNA probes to investigate the dynamics and potential distribution of phage particles in natural ecosystems. The application of DNA probes in environmental microbiology has been credited with several improvements on traditional techniques for analyzing natural microbial communities (6). By directly analyzing concentrates of bacteriophage particles and isolated bacteria from the same environment, DNA probes enhance the investigation of specific phage-host interactions in nature. The application of DNA probes to characterize heterogeneous phage populations may also simplify phylogenetic investigation because different phages from similar habitats can be classified according to their level of DNA sequence similarity. In addition, DNA probes potentially allow the determination of the distribution of lysogens and phage-sensitive host bacteria within natural bacterial communities. Such determinations may significantly improve our ability to predict the invasiveness of a particular gene through exchange mechanisms, such as transduction, in natural microbial ecosystems (20, 22, 27). *Pseudomonas aeruginosa* was used as a model organism because of its ubiquitous distribution in the environment and extensive background information available on the genetics of some of its phages.

MATERIALS AND METHODS

Environmental samples. Fresh water was collected from Fort Loudon Lake, Knoxville, Tenn. Salient biological and physical-chemical characteristics of the site have been previously published (20, 22). For the determination of bacterial population density and bacterial-host enrichment studies, 2.5 liters of water was collected from a depth of 0.3 m into sterile Whirl-Pak bag samplers (Nasco, Ft. Atkinson, Wis.). For the recovery of bacteriophages through filtration, 25 liters of water was pumped into sterile carboys, kept at 4°C, and processed within 24 h. Sediment was collected from a depth of 5 m at the Little River embayment of Fort Loudon Lake with a Bottom Grab-Sampler (LaMotte Chemical, Chestertown, Md.). The sediment samples (approximately 1.5 g/ml) were collected into Whirl-Pak bags and kept on ice until analyzed.

Soil was collected from the Plant Science Field Laboratory of the University of Tennessee, Knoxville. Several soil samples were taken from within 5 to 15 cm of the surface layer, sieved to remove stones and twigs, and kept at 4°C until analyzed.

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Sewage was collected from the port of domestic waste entry into the municipal wastewater treatment plant of Knoxville, Tenn. Raw sewage was collected from homogenization wells and stored in sterile 2.5-liter polypropylene tanks at 4°C until analyzed.

Enumeration of bacteria in environmental samples. To determine the population densities of bacteria, serial dilutions of environmental samples were done in phosphate-buffered saline and plated on Luria-Bertani (LB) agar (16) (Difco Laboratories, Detroit, Mich.), yeast extract-peptone-glucose (10% YEPG) agar (28), or *Pseudomonas* Isolation (PI) agar (Difco). The LB agar plates were incubated at 37°C for 24 to 48 h to select for enterobacteria that may be present in fecally contaminated samples. Similarly, PI agar plates were incubated at 37 or 42°C (for selecting *P. aeruginosa*) for 24 to 48 h before counting. YEPG agar plates were incubated at 22°C for 10 days before counting.

Direct isolation of bacteriophages from environmental samples. The water filtration procedure of Primrose and Day (23) was used to determine the occurrence of free phage particles in the lake water. Water (25 liters) was filtered through a 20-ml column of hydroxyapatite (Bethesda Research Laboratories, Gaithersburg, Md.). Phage particles were specifically eluted from the hydroxyapatite column with 10 ml of 0.8 M sodium phosphate, pH 7.2. The eluate was assayed for PFU on *P. aeruginosa* RM273 (nalidixic acid-resistant PAO1 derivative) or the lake water isolate *P. aeruginosa* LL10 (22) by the soft-agar overlay technique (30). By this procedure, a strain of a pseudotemperate phage was isolated from the lake water, purified, identified as belonging to the family *Myoviridae*, and named UT1. Important properties of this phage and the nature of its interaction with *P. aeruginosa* have been previously described (21, 22).

A host enrichment procedure (9) was also used to isolate phages from fresh water, using *P. aeruginosa* LL10 as the target host strain. By this procedure, a heterogeneous population of phages containing at least four morphologically distinct particle types was isolated and designated M1. The characteristics of this mixed phage population have been described elsewhere (22).

Soil, sediment, and sewage samples were investigated for the occurrence of free phage particles by assaying supernatants of low-speed-centrifuge-clarified samples for PFU on *P. aeruginosa* LL10 and RM273. The soft-agar overlay technique was used to isolate phage particles, and purification of selected plaques was done by transfer onto fresh bacterial lawns.

High concentrations of phage particles were obtained from purified plaques by infecting 2.5-ml broth cultures of the host bacteria. Phage lysates were clarified by centrifugation (3,000 × *g* for 10 min) and filtration through 0.45-μm-pore-size sterile polycarbonate membranes (Nuclepore Corp., Pleasanton, Calif.). Further purification of phage preparations was achieved by ultracentrifugation at 35,000 rpm for 2 h in a 5 to 40% glycerol gradient at 4°C in a TL 100 rotor (Beckman Instruments, Inc., Palo Alto, Calif.). Bacteriophage pellets were suspended in phage dilution (PD) buffer for molecular analysis or 1% ammonium acetate for electron microscope visualization (22).

Extraction, purification, restriction, and labeling of phage DNA. DNA was extracted and purified from six different *P. aeruginosa* bacteriophage preparations, UT1 (22), M1 (22), F116L (11, 17, 20), DS1 (27), D3 (4, 11, 17), and E79 (11, 17, 22), according to the method of Silhavy et al. (30). Phage DNAs were purified by cesium chloride ultracentrifugation and dialyzed against Tris-EDTA-sodium acetate buffer, pH 7.5 (30). To evaluate the intrinsic diversity among bacteriophage genomes, restriction digestions of the purified phage DNAs were conducted with *Sal*I (Bethesda Research Laboratories) according to the method of Silhavy et al. (30). The restriction fragments were resolved on a 0.5% agarose gel containing 0.5 μg of ethidium bromide per ml. Southern hybridization analysis of restricted DNA was done according to the method of Silhavy et al. (30).

To generate radiolabeled phage DNA probes, between 0.5 and 1 μg of restriction fragments from the *Sal*I digest was nick translated, with ^{32}P-dCTP (Bethesda Research Laboratories) as the source of radioactive isotopes. The specific activity of radiolabeled DNA was always greater than or equal to 10^8 dpm/μg of DNA.

Use of phage genomic probes to detect viral particles and lysogenic bacteria in lake water. To evaluate the sensitivity of DNA probes in detecting the presence of phage particles in filter-concentrated water or other aqueous media, intact virions, purified DNA, or lysogenized bacterial cells were seeded into lake water at various concentrations. The lake water suspensions of phage particles, DNA, and bacteria were then immobilized on Biotrans nylon membranes (ICN Biomedicals, Irvine, Calif.) in a dot blot filtration manifold (Schleicher & Schuell, Keene, N.H.). The DNA from the immobilized samples was denatured, neutralized, and permanently fixed according to the method of Silhavy et al. (30). The membranes were then hybridized to radiolabeled DNA from phage DS1 or UT1. Hybridization was conducted at 65°C without formamide (Biotrans Protocols; ICN).

Use of phage genomic probes to determine the occurrence of prophages in bacteria isolated from environmental samples. To determine the occurrence of phage DNA-related sequences in bacterial colonies (putative lysogens) isolated from environmental samples, colonies from plates inoculated with dilutions of freshwater, sediment, soil, or sewage samples were replica plated, lifted onto Biotrans nylon membrane discs, and hybridized to radiolabeled DNA from phage UT1, M1, F116L, or D3. Signals from colonies containing sequences complementary to the labeled probe were detected by autoradiography on X-ray films (X-Omat AR; Eastman Kodak Co., Rochester, N.Y.) (22). To determine whether colonies that contained phage DNA sequences were true lysogens and could be induced to produce free phage particles, the colonies were restreaked and inoculated into LB broth containing 1 μg of mitomycin per ml. The resulting culture filtrates were assayed for phage particles by plaque formation on *P. aeruginosa* LL10 or RM273.

Plaque hybridizations to determine the distribution of specific bacteriophage particles in the environment. To determine the abundance of different *P. aeruginosa* phages in the bacteriophage community of sewage and fresh water, 33 plaques isolated from sewage and 33 plaques isolated from fresh water were purified and replica plated on *P. aeruginosa* LL10, transferred to Biotrans nylon membranes, and probed with radiolabeled phage DNA from UT1, M1, F116L, or D3. Hybridizations were conducted at 65°C (Biotrans) for at least 18 h. Hybridization membranes were washed in a 0.5% sodium dodecyl sulfate–10 mM salt solution at 68°C. Autoradiographic signals from plaques that hybridized to the phage DNA probes were detected by exposure of membranes to Kodak X-ray films.

RESULTS AND DISCUSSION

Bacteriophages may be involved in either virulent or lysogenic associations with their hosts in natural ecosystems

TABLE 1. Population density of bacteria in environmental samples

Isolation medium, temp (°C)	CFU[a] per:			
	ml of lake water	g of sediment	g of soil	ml of sewage
LB, 37	$(2.45 \pm 0.25) \times 10^2$	$(2.86 \pm 0.41) \times 10^4$	$(5.45 \pm 0.41) \times 10^6$	$(1.89 \pm 0.16) \times 10^6$
YEPG, 22	$(2.49 \pm 0.10) \times 10^4$	$(3.75 \pm 0.65) \times 10^7$	$(1.10 \pm 0.06) \times 10^6$	$(3.16 \pm 0.12) \times 10^6$
PI agar, 22	$(7.00 \pm 1.99) \times 10^1$	$(1.30 \pm 0.89) \times 10^3$	$(1.76 \pm 0.11) \times 10^4$	$(5.29 \pm 0.01) \times 10^4$

[a] Values recorded are means of four estimates representing duplicate samples from each environment.

(15, 25), but current methods of detecting viable phages rely on a lytic reaction against a test strain in vitro and do not provide direct evidence of the state of phages in nature. Because bacteriophage particles have a finite half-life in natural habitats (22, 31, 34), we can assume that the abundance and, more importantly, the metabolic state of host bacteria determine whether phage populations are maintained in a specific ecosystem (15) without eliminating the bacterial hosts. Thus, the distribution and frequency of occurrence of specific, actively metabolizing hosts within the bacterial community are of major significance in the analysis of natural phage distribution patterns.

Availability of active hosts for phage replication in the environment. Table 1 shows the population densities of culturable bacteria isolated on different media in the samples investigated. Bacteria capable of growing at 37°C on nutrient-rich medium (LB) represented 0.98, 0.08, 100, and 59.8% of the total aerobic heterotrophs (selected on YEPG) in lake water, sediment, soil, and sewage, respectively. The fluorescent pseudomonads represented 0.03, 0.003, 1.6, and 1.7% of the heterotrophs and 28.6, 4.5, 0.32, and 2.8% of the copiotrophs in lake water, sediment, soil, and sewage, respectively. Previous workers have attempted to distinguish between copiotrophic and oligotrophic environmental bacteria on the basis of differences in maximum growth rate and substrate saturation constant (K_s) in culture (10, 32). The proportionate abundance of bacteria in the samples investigated in this study indicates that in lake water and sediment, where a relatively high proportion of fast-growing bacteria (copiotrophs) are *Pseudomonas* species, the frequency of successful host recognition (through adsorption) by specific *Pseudomonas* bacteriophages may be higher than in soil and sewage, where there are several other fast-growing bacteria competing with the *Pseudomonas* species. Alternatively, if the abundance of specific hosts is sporadic, as may be the case in soil or sewage, bacteriophages may emulate hosts by rapid replication in the restricted growth periods to maintain sufficient numbers of potentially infective particles in the environment.

Sensitivity of DNA probes in detecting free and lysogenized bacteriophage. Figure 1 shows the autoradiograph of signals from bacteriophages and bacteria seeded into PD buffer or lake water and hybridized to radiolabeled DNA from phage DS1 (closely related to phage F116L) (19). The limit of detection of phage particles that are homologous to the DNA probe was 10^4 PFU (for DS1 and F116L) in buffer and 10^7 PFU in natural lake water. When lake water was autoclaved or filtered before seeding with phage particles, the limit of detection by DNA hybridization was increased relative to that in natural lake water to about 10^6 PFU. This result indicates that particulate matter, probably nonhost bacteria present in lake water, may mask the detection of bacteriophages by inactivation of particulate phages and their DNA through nonproductive adsorption.

The detection limit was also dependent on the extent of DNA sequence similarity (13) to the DNA probe. For phage D3, the limit of detection with essentially heterologous DNA from phage DS1 was 10^6 PFU in dilution buffer, compared with 10^4 PFU for DS1-related phage F116L (Fig. 1).

Approximately 10^6 CFU of lysogenized bacteria (*P. aeruginosa* RM272 containing phage DS1) was detected in lake water with a radiolabeled phage DS1 probe (Fig. 1). A weak, probably nonspecific, signal was detected from 10^8 CFU of a bacteriophage-free, DS1-sensitive strain (*P. aeruginosa* RM273). These results demonstrate the potential of using DNA probes derived from phage genomes to quantitatively detect the presence of both free phage particles and lysogenized bacteria in aquatic systems, but interference from viable and nonviable particulate matter exists in natural environments.

Detection of lake water bacteriophage UT1 with radiolabeled DNA probe. Figure 2 shows autoradiographic signals from hybridization experiments with a DNA probe specific for bacteriophage UT1 that was originally isolated from Fort Loudon Lake. About 10^4 PFU was the limit of detection of phage UT1 particles in PD buffer. Phage particles (10^5 PFU) seeded into lake water and incubated for 24 h at either 25 or 37°C were no longer detectable by the DNA probe (Fig. 2). However, the incubation of phage in LB broth, distilled

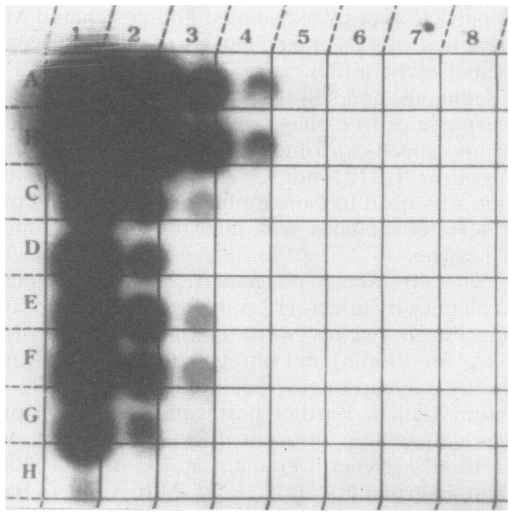


FIG. 1. Autoradiograph signals identifying the occurrence of DNA sequences homologous to the radiolabeled bacteriophage DS1 genomic probe. Columns 1 to 8 represent serial dilutions from 10^8 PFU or CFU to 10^1 PFU or CFU per well. Rows: A, bacteriophage F116L diluted in PD buffer; B, phage DS1 in PD buffer; C, phage D3 in PD buffer; D, phage DS1 diluted in natural lake water; E, phage DS1 in filtered lake water; F, phage DS1 in autoclaved lake water; G, *P. aeruginosa* RM272 (DS1 lysogen) diluted in natural lake water; H, *P. aeruginosa* RM273 (DS1 sensitive) in natural lake water.

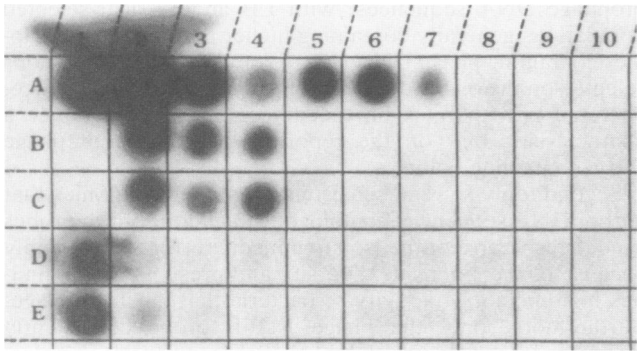


FIG. 2. Autoradiograph signals identifying the occurrence of bacteriophage UT1. Row A, PD buffer used to dilute phage from 10^9 PFU (column 1) to 1 PFU (column 10); rows B and C, 10^5 PFU of phage UT1 incubated at 25°C (row B) or 37°C (row C) for 24 h in natural lake water (column 1), LB broth (column 2), distilled water (column 3), and PD buffer (column 4); row D, *P. aeruginosa* LL6 (naturally occurring phage UT1-resistant strain isolated from lake water) diluted at 10^8 CFU per well (column 1) to 1 CFU per well (column 8); row E, *P. aeruginosa* LL5 (phage UT1 lysogen) spotted at 10^8 CFU per well (column 1) to 1 CFU per well (column 8).

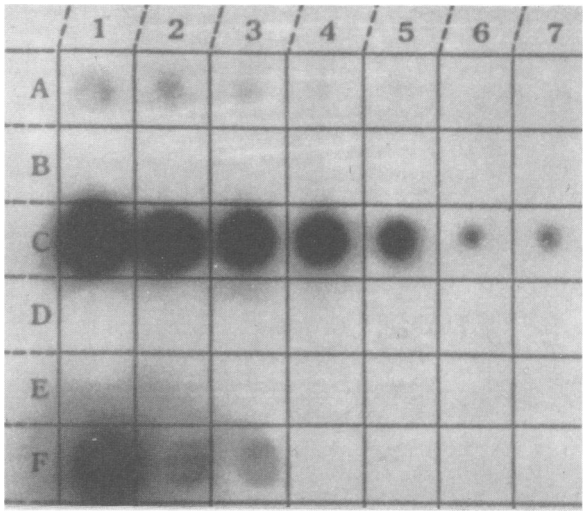


FIG. 3. Autoradiograph signals identifying phage UT1-related DNA. Row A, purified DNA extracted from a heterogeneous population of phages (M1) isolated by enrichment from lake water, spotted at 10, 5, 2, 1, 0.5, 0.1, and 0.05 μg per well in columns 1 to 7; row C, purified DNA from phage UT1, spotted in columns as described for row A; row F, heterogeneous phage particle population (M1) spotted in serial dilutions at 10^{11} PFU per well (column 1) to 10^4 PFU per well (column 7). Rows B, D, and E were not used.

water, or dilution buffer for 24 h did not affect the detection of phage through DNA hybridization (Fig. 2). There was no apparent difference due to phage incubation temperature (25 or 37°C) in the limit of phage detection by DNA hybridization (Fig. 2). The inability to detect phage particles incubated for 24 h in lake water through DNA hybridization was probably due to degradation of phage DNA through nonproductive adsorption to particulate materials in the lake water. If the phage were engaged in infection of susceptible host cells, it is likely that the DNA would still be detectable through hybridization to the phage DNA probe, because 10^6 CFU of a UT1 lysogen was detected by the same phage probe after incubation for 24 h (Fig. 2).

Bacteriophage UT1 could also be detected in a heterogeneous population of bacteriophages (M1) isolated from lake water (Fig. 3). Densitometric analysis of the autoradiographic signals indicate that phage UT1 and related bacteriophages represented about 2% of the total population of *P. aeruginosa*-infecting bacteriophages in the microbial community from this lake water sample.

Genetic relatedness of particulate bacteriophages isolated from fresh water and sewage. The restriction digest patterns (Fig. 4A) and Southern hybridization analysis (Fig. 4B and reference 22) of DNA from the six different bacteriophage populations studied show different levels of homology. A certain degree of DNA sequence similarity exists among the genomes of F116L, DS1 (19), and D3 (13) (Fig. 1) and among the genomes of UT1, E79 (22), and the heterogeneous M1 group (Fig. 2, 3, and 4). Thus, three major groups of bacteriophages, to which DNA probes were made, were involved in this study. Phages F116L and DS1, classified in the family *Siphoviridae*, and phage D3 (*Myoviridae*) are all temperate and share homologous sequences of DNA. Phages UT1 and E79 (predominantly virulent, *Myoviridae*) and fractions of M1 also share regions of DNA sequence similarity. The third group represents the largely uncharacterized fraction of the heterogeneous phage population of M1. The extent to which these genomic homologies are represented in natural populations of particulate bacteriophages is illustrated in Fig. 5. Free phage particles were isolated from lake water (population density of 48 PFU/liter) and from sewage ($1.55 \times 10^2 \pm 0.1 \times 10^2$ PFU/ml). Free bacteriophage particles infective for *P. aeruginosa* RM273 or LL10 could not be isolated from the sediment and soil samples examined. Of 33 independent plaques isolated from lake water, about 8% hybridized to DNA from the lake water isolate UT1, 20% hybridized to F116L, and most of the plaques (48%) hybridized to DNA from the heterogeneous phage population M1 (Fig. 5). The fact that phage UT1-homologous phages are present at a higher proportion (8%) in the heterogeneous population of free PFU (Fig. 5) than is estimated from analysis and bulk phage DNA (2%) extracted from a heterogeneous population of phages isolated by enrichment (M1; Fig. 2, row B) is probably due to the fact that the ability to form plaques is a very specialized process, depending on molecular recognition of host bacterial cell surface components. Therefore, it is likely that the diversity of phages capable of producing plaques on the indicator host is less than the diversity of DNA extracted directly from the heterogeneous phage population.

About 5% of the 33 plaques isolated from sewage hybridized to DNA from phage UT1, while 18% hybridized to DNA extracted from M1. None of the plaques isolated from sewage hybridized to phage F116L or D3 (Fig. 5). The data indicate that a more genetically diverse population of *P. aeruginosa*-infecting bacteriophages exists in sewage than in fresh water, because about 80% of the particulate phage population isolated from sewage are not homologous to the probes used in this study. Alternatively, it is possible that virulent phages of a type different from UT1 or E79 predominate in sewage. Both of these putative explanations are supported by the fact that there is a higher population density of pseudomonads in sewage than in lake water (Table 1).

Distribution of bacteriophage DNA sequences in bacteria isolated from various natural ecosystems. Many naturally occurring bacteriophages are able to alternate between tem-

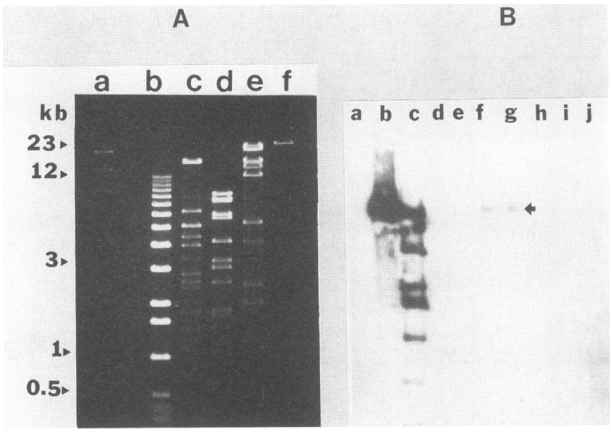


FIG. 4. (A) Ethidium bromide-stained agarose gel (visualized under UV light) used to resolve *Sal*I-restricted DNA extracted from different bacteriophages. Lanes: a, phage UT1; b, 1-kb ladder DNA size marker (Bethesda Research Laboratories); c, phage D3; d, phage DS1; e, phage E79; f, phage population M1 (not restricted). (B) Autoradiograph of Southern hybridization, showing the absence of significant DNA homology between phage UT1 (the radiolabeled probe) (lanes b [unrestricted] and c [restricted]) and *Escherichia coli* phage lambda (lane a), phage D3 (lane d), and phage DS1 (lane e). Some homology is observed in lanes f and g, which contain DNA extracted from heterogeneous phage mixture M1 (arrow). No significant DNA homology exists among phage UT1, the *P. aeruginosa* plasmid RMS149 (lane h), and the genomes of *P. aeruginosa* RM273 (lane i) and PAO381 (lane j). All DNA samples were restricted with *Sal*I, and the resulting fragments were resolved in a 0.5% agarose gel (not shown). A Southern blot of the gel was probed with radiolabeled *Sal*I restriction fragments from phage UT1.

perate and lytic infective life cycles (14). The molecular reason for which the bacteriophage is temperate or initiates a virulent process has not been clearly elucidated. Nonetheless, the metabolic status of host bacteria is hypothesized to contribute significantly to the direction of phage infection (15, 31). In natural nutrient-poor environments, a majority of phage infections may be nonlytic, resulting in the phage genome being established in the host as a prophage (14, 15) and replicating in synchrony with the host chromosome either in an independent state as a plasmid or integrated into the host chromosome. Therefore, it should be possible to determine the distribution of bacteria that contain DNA from a particular temperate phage by hybridization of isolated colonies to phage DNA. The techniques involved in this detection process have been described in a study of dynamic interaction between phage UT1 and a lake water host bacterium (22). In the present study, bacteriophage DNA probes representing four different *P. aeruginosa*-infecting phages were used to screen a large population of bacteria selected from water, sediment, soil, and sewage. The number of colonies probed with the phage DNA reflects the population density of bacteria in the environmental sample that are able to grow on the different isolation media, but steps were taken to represent colonies from all dilutions in the hybridization process. Two major inferences concerning (i) the nature of the environmental sample and (ii) the specific nature of the phage can be drawn from the summary of the results (Fig. 6).

First, the use of different selective media to isolate bacteria from the environmental samples (Table 1) results in different numerical patterns of bacteria containing putative prophage DNA sequences, with LB and PI agar-selected populations generally containing higher proportions of colonies containing phage DNA (Fig. 6A and C) than populations selected on the oligotroph-selecting YEPG (Fig. 6B). Hence, bacterial growth rates on the different media introduce a characteristic bias on the reported distribution of phage DNA-containing colonies.

Second, only sewage consistently contained colonies that harbor DNA sequences homologous to all four phage probes used, irrespective of the isolation medium (Fig. 6), probably because of the extensive diversity and presumably continuous high metabolic activity of bacteria that inhabit sewage. Furthermore, only the phage F116L probe consistently hybridized to colonies from all environmental samples, irrespective of isolation medium (Fig. 6). The latter observation may result from the ability of phage F116L to lysogenize bacteria in a variety of metabolic states or from the fact that F116L has sequence similarity to a large number of phages. Further in vitro investigation of the comparative lysogenization abilities of F116L and other *P. aeruginosa*-infecting phages is warranted to further elucidate the factors leading to the stability of lysogens in natural environments.

Where they are present, a larger proportion of all colonies contain phage UT1-related DNA sequences than contain any of the other four phage probes (Fig. 6). Bacteriophage UT1 contains a genome smaller than that of the virulent phage E79 (Fig. 4), but the two viruses share extensive DNA homology (22). Preliminary data suggest that phage UT1 will maintain a stable nonlytic association with host bacteria (15, 22) and will transduce certain host genes at frequencies higher than that of E79-mediated transduction (18). The results presented in this study further suggest that phage UT1 and related phage particles are widely distributed in pseudomonad communities from all of the environments sampled (Fig. 6). However, the detection of prophage DNA sequences in bacteria is not conclusive proof for lysogeni-

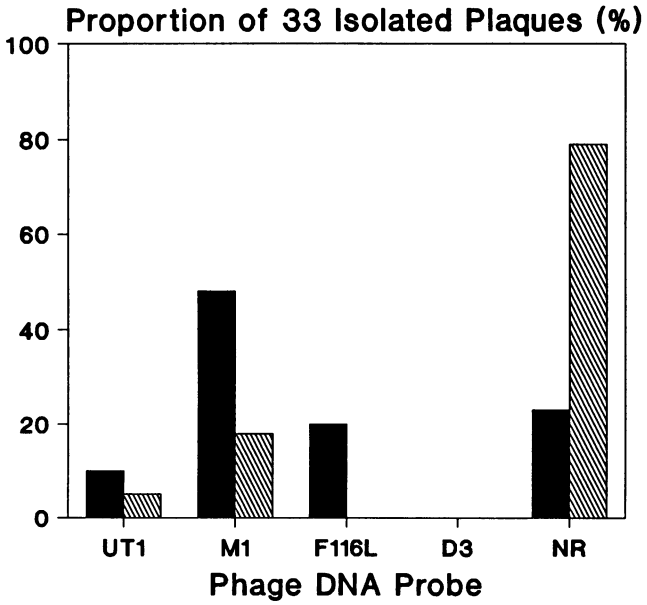


FIG. 5. Graphic plot of the relative abundance of phages UT1, M1, F116L, and D3 among PFU isolated as free particles in lake water (■) and sewage (▨). Radiolabeled DNA from the four characterized phages was used to probe the PFU isolated from the environmental samples. NR, no reaction.

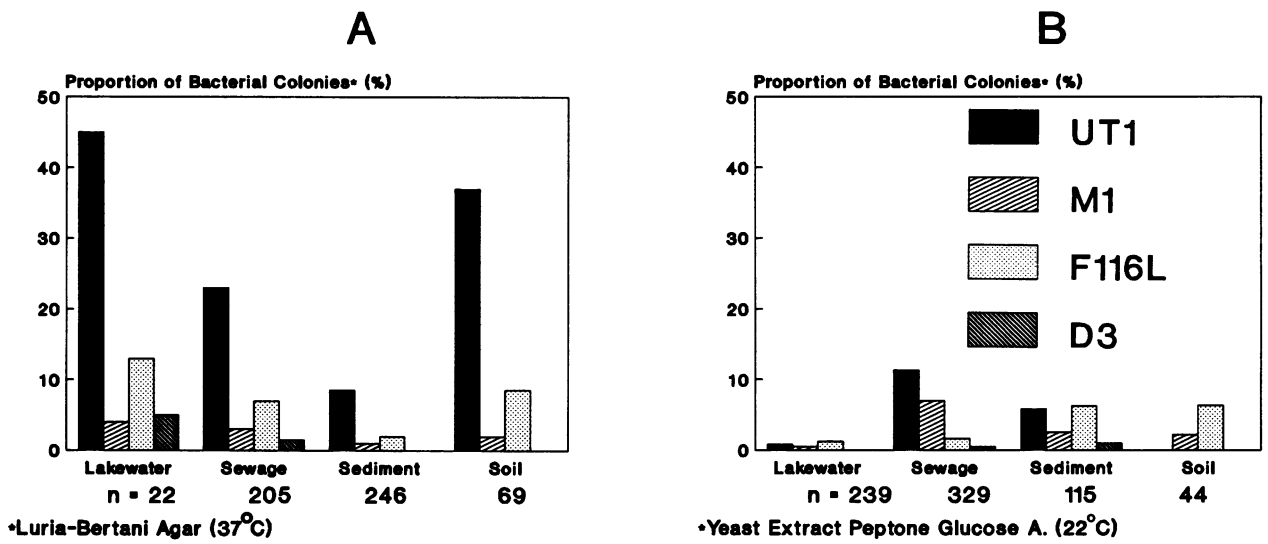
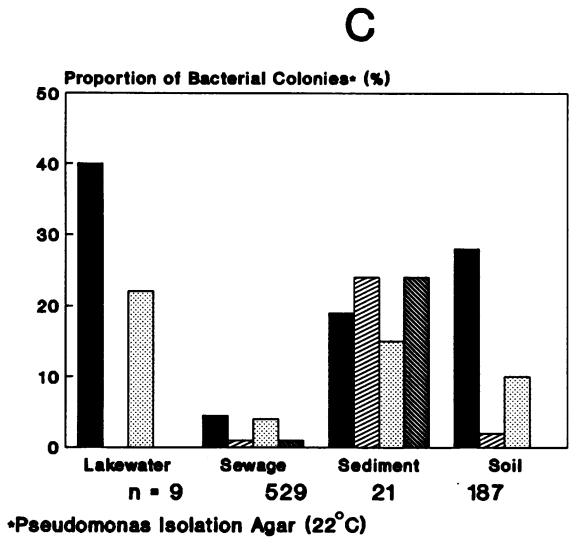


FIG. 6. Graphic plots of the relative abundance of phage DNA-related sequences in bacterial colonies isolated on LB agar (A), YEPG (B), and PI agar (C) from lake water, sewage, sediment, and soil samples. Phage DNA was radiolabeled and used to probe replica-plated colonies by the Biotrans colony hybridization procedure.



zation of the host bacteria by the phage. For example, only 3% of colonies that hybridized to DNA from phage UT1 were inducible for the production of PFU through treatment with mitomycin. Because related phages do not necessarily share similar mechanisms of induction (2, 14), it cannot be concluded that the noninducible colonies are not lysogens. Certain *P. aeruginosa* strains have been shown to carry a phage D3-derived insertion sequence, IS*222*, in their chromosome (7). Bacteriophage D3 infection of *P. aeruginosa* is of considerable medical importance because of a lysogenic conversion of pathogenic consequence (7). While the occurrence of IS*222* in natural populations of *P. aeruginosa* has not been fully characterized, the distribution of phage D3 DNA sequences in the natural bacterial isolates investigated in this study indicates that up to 30% of pseudomonads in the sediment and 5% of pseudomonads in lake water contain DNA sequences related to D3. The public health significance of these findings warrants further investigation.

The occurrence of phage DNA sequences (modules) in bacteria may also have significant phylogenetic implications for the origin and evolution of bacteriophages and bacteriophagelike bacteriocin and pyocin particles (3, 12, 26). The consequences of such wide distribution of bacteriophages and phage-related DNA sequences in bacterial communities are extensive with respect to ecological interactions between bacteria, including antagonistic activities leading to population density control and genetic transfer and exchange through transduction.

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