

2-1-1988

Prevalence of Broad-Host-Range Lytic Bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa*

Ellen C. Jensen

College of Saint Benedict, Saint John's University, Collegeville, Minnesota, ejensen@csbsju.edu

Holly S. Schrader

University of Nebraska-Lincoln

Brenda Rieland

College of Saint Benedict, Saint John's University, Collegeville, Minnesota

Thomas L. Thompson

University of Nebraska-Lincoln

Kit W. Lee

University of Nebraska-Lincoln

See next page for additional authors

Follow this and additional works at: <http://digitalcommons.unl.edu/bioscimicro>

 Part of the [Microbiology Commons](#)

Jensen, Ellen C.; Schrader, Holly S.; Rieland, Brenda; Thompson, Thomas L.; Lee, Kit W.; Nickerson, Kenneth W.; and Kokjohn, Tyler A., "Prevalence of Broad-Host-Range Lytic Bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa*" (1988). *Papers in Microbiology*. 33.

<http://digitalcommons.unl.edu/bioscimicro/33>

This Article is brought to you for free and open access by the Papers in the Biological Sciences at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Papers in Microbiology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

Ellen C. Jensen, Holly S. Schrader, Brenda Rieland, Thomas L. Thompson, Kit W. Lee, Kenneth W. Nickerson, and Tyler A. Kokjohn

Prevalence of Broad-Host-Range Lytic Bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa*

ELLEN C. JENSEN,¹ HOLLY S. SCHRADER,² BRENDA RIELAND,¹ THOMAS L. THOMPSON,^{2†}
KIT W. LEE,² KENNETH W. NICKERSON,² AND TYLER A. KOKJOHN^{2*}

*Department of Biology, College of Saint Benedict, Saint John's University, Collegeville, Minnesota 56321,¹
and School of Biological Sciences, University of Nebraska—Lincoln, Lincoln, Nebraska 68588-0666²*

Received 27 June 1997/Accepted 17 November 1997

Two bacteriophage collections were examined with regard to their ability to form plaques on multiple bacterial host species. Nine of 10 phages studied were found to be broad-host-range bacteriophages. These phages fell into two groups. Group 1, the SN series, was isolated from sewage treatment plant samples with *Sphaerotilus natans* ATCC 13338 as a host. The DNAs of these bacteriophages contained modified bases and were insensitive to cleavage by type I and II restriction endonucleases. The efficiency of plating of these bacteriophages was changed only slightly on the alternate host. Group 2, the BHR series, was isolated by a two-host enrichment protocol. These bacteriophages were sensitive to restriction, and their efficiency of plating was dramatically reduced on the alternate host. Our results suggest that a multiple-host enrichment protocol may be more effective for the isolation of broad-host-range bacteriophages by avoiding the selection bias inherent in single-host methods. At least two of the broad-host-range bacteriophages mediated generalized transduction. We suggest that broad-host-range bacteriophages play a key role in phage ecology and gene transfer in nature.

Bacteriophages are viral parasites of bacteria first recognized early in the 20th century (8, 13). While virion particles are capable of independent existence outside the host, all bacteriophages are obligate intracellular parasites and must enter a host bacterium to replicate. Studies of bacteriophage infection have revealed that the process is initiated when the virion interacts with host cell surface receptor molecules (14). Many bacteriophages are known to be highly specific for their receptors and show little or no interaction with receptors with an even slightly different structure. This specificity forms the basis of numerous phage typing methods for the identification of bacterial species or subspecies (37).

In contrast, it is clear that some bacteriophages do productively infect a range of bacterial species. Of these broad-host-range phages, P1 and Mu are the best studied. Bacteriophage P1 is a generalized transducing virus capable of plaque formation on several enteric species in addition to *Escherichia coli* (40), while bacteriophage Mu produces progeny virions capable of adsorption to and plaque production on different bacterial species (12) due to the differential orientation of the invertible viral G segment region (12, 36). Bacteriophages able to interact with a wide range of host species would be significant in the control of the composition and genetic diversity of microbial communities as well as the processes of transductional gene exchange and the transfer of antibiotic resistance genes through those communities. The prevalence of broad-host-range phages relates to the origin of virus particles which compose such a large percentage of the dissolved organic car-

bon in marine ecosystems (3, 9, 35) and which are present in very large numbers in other ecosystems as well (15, 22, 26).

Surprisingly, systematic studies of broad-host-range interactions of bacteriophages and of the relative frequencies with which such bacteriophages may be isolated are lacking. Two important questions concern the relative frequencies with which bacteriophages exhibiting a wide host range may be isolated from nature and the percentages of broad-host-range phages that can be identified in existing virus collections.

To address the question of the distribution of broad-host-range bacteriophages, we examined several independent phage collections. The first was obtained in the 1970s from sewage treatment plants with *Sphaerotilus natans* as a host, while the others were recently obtained from a freshwater pond and sewage with *Pseudomonas aeruginosa* and *E. coli* as hosts. The experiments demonstrated that broad-host-range bacteriophages are readily isolated from aquatic environments and that some of these bacteriophages are capable of generalized transduction. During the course of this study, we observed that the frequency with which broad-host-range bacteriophages are isolated is increased by the use of isolation protocols that include two bacterial host species for enrichments rather than one.

MATERIALS AND METHODS

Bacteria and bacteriophages. Bacterial strains used for bacteriophage propagation and titration were *E. coli* AB1157 (5), *P. aeruginosa* PAO1, PAO303, and OT684 (32), and *S. natans* ATCC 13338 from the American Type Culture Collection (Rockville, Md.). Bacteriophages used in this study are described in Table 1. Following their isolation 18 to 26 years ago, bacteriophages SN-1, SN-2, SN-X, and SN-T were stored undisturbed in sealed containers until their use in this study. Bacteriophage lysates were produced by modifications of the soft-agar overlay method (1).

Media. Medium components were obtained from Difco (Detroit, Mich.). Luria broth contained 1% (wt/vol) tryptone, 1% (wt/vol) NaCl, and 0.5% (wt/vol) yeast extract. Luria agar contained Luria broth with the addition of 1.3% (wt/vol) agar. Lambda top agar contained 1% (wt/vol) tryptone, 0.5% (wt/vol) NaCl, and 0.65% (wt/vol) agar. TGY medium, containing 1% tryptone, 1% glycerol, and 0.2% yeast extract (39), was used for the propagation of all *S. natans* hosts

* Corresponding author. Present address: Department of Microbiology, Arizona College of Osteopathic Medicine, Midwestern University, 19555 N. 59th Ave., Glendale, AZ 85308. Phone: (602) 572-3225. Fax: (602) 572-3226. E-mail: tkokjo@arizona.midwestern.edu.

† Retired January 1988.

TABLE 1. Bacteriophages used in this study

Bacteriophage	Isolation location and date	Isolation host(s)	Source or reference
SN-1	Lincoln, Nebr., sewage; 1976	<i>S. natans</i> ATCC 13338	39
SN-2	Lincoln, Nebr., activated sludge sewage treatment plant; 1980	<i>S. natans</i> ATCC 13338	38
SN-X	Lincoln, Nebr., activated sludge sewage treatment plant; 1980	<i>S. natans</i> ATCC 13338	38
SN-T	Lincoln, Nebr., sewage; 1972	<i>S. natans</i> LL1	T. L. Thompson
BHR1	Pioneer's Park Pond, Lincoln, Nebr.; 1996	<i>E. coli</i> AB1157 and <i>P. aeruginosa</i> PAO303	This study
BHR2	Pioneer's Park Pond, Lincoln, Nebr.; 1996	<i>E. coli</i> AB1157 and <i>P. aeruginosa</i> PAO303	This study
BHR3	Lincoln, Nebr., sewage; 1996	<i>E. coli</i> AB1157 and <i>S. natans</i> ATCC 13338	This study
BHR4	Lincoln, Nebr., sewage; 1996	<i>E. coli</i> AB1157 and <i>S. natans</i> ATCC 13338	This study
BHR5	Lincoln, Nebr., sewage; 1996	<i>E. coli</i> AB1157 and <i>S. natans</i> ATCC 13338	This study
D3	Australia	<i>P. aeruginosa</i> PAO1	16, 20
F116	Australia	<i>P. aeruginosa</i> PAO1	16, 20

and bacteriophages. TGY agar contained TGY medium and 1.3% (wt/vol) agar, and TGY top agar contained TGY medium and 0.65% (wt/vol) agar. TMN buffer contained 10 mM Tris-HCl (pH 7.4), 10 mM MgSO₄, and 150 mM NaCl. Because *S. natans* is sensitive to inhibition by sodium ions (39), a 0.5% (wt/vol) tryptone solution was used as a diluent for all *S. natans* bacteriophages. Chemicals were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Bacteriophage isolation procedures. Bacteriophages were isolated by an enrichment procedure in which environmental water samples were amended with an equal volume of growth medium containing a 1:100 (vol/vol) dilution of an overnight culture of one or two bacterial host species. When enrichments were performed with the simultaneous addition of two bacterial host species, the bacteriophages obtained are indicated in Table 1 as having multiple isolation hosts. Enrichment cultures were incubated overnight, whereupon the samples were treated with a 1/20 volume of chloroform and centrifuged to eliminate all viable bacterial cells. Bacteriophages were detected by a plaque assay with lawns of the original bacterial host(s). The plaques were then screened for the ability to lyse both originally added indicator hosts. Those observed to cause the lysis of both hosts were used in additional experiments.

Plaque assay and bacteriophage purification procedures. Bacteriophage lysates were produced by a modification of the just-confluent-lysis protocol of Arber et al. (1). Appropriately diluted samples containing bacteriophages were mixed with 0.1 ml of overnight bacterial host culture and plated on 2.5 ml of top agar. Bacteriophage preparations were quantified by plating appropriately diluted samples on lawns of bacterial cells (19). All bacteriophages used in this study, including the *S. natans* bacteriophages, were purified by performing a minimum of three rounds of single-plaque isolation by a modification of the protocols of Arber et al. (1). Well-isolated plaques were cut from agar plates, placed in sterile diluent, and used to produce new bacteriophage lysates. This procedure was repeated through three cycles or until lysates produced only a single-plaque morphology.

The ability of bacteriophage SN-T to infect and lyse bacterial hosts was determined by a spot lysis assay. Lawns of potential host bacterial species were formed by plating samples of overnight cultures mixed with top agar onto appropriate agar plates. After the top agar solidified, samples of bacteriophage lysates were dropped onto the agar and the plates were incubated overnight. Productive interaction of the bacteriophage with the host bacterium was revealed as a zone of lawn killing at the site of virus addition.

Electron microscopy of bacteriophage lysates. The morphology of each of the broad-host-range bacteriophages was examined by electron microscopy. Virions produced in each pair of heterologous bacterial hosts (18 total samples) were compared to confirm that the bacteriophage morphologies were identical in the heterologous hosts. The bacteriophages were negatively stained with 4% (wt/vol) phosphotungstic acid (pH 7.2) and observed with a Philips model 201 transmission electron microscope operated at 60 kV.

EOP. Efficiency of plating (EOP) was quantified by calculating the ratio of the bacteriophage plaque titers obtained with the heterologous host to those obtained with the homologous host, i.e., the same species of bacterium as that used to produce the bacteriophage lysate. Data (see Table 3) are the means of three independent experiments.

Restriction endonuclease digestion of DNA. DNA was isolated from three bacteriophages, SN-1, SN-2, and SN-X, by a modification of the protocol of Thomas and Abelson (34). DNA samples were restricted with type II endonucleases (4) obtained from GibcoBRL Life Technologies (Gaithersburg, Md.) and with the reaction conditions and buffers recommended by the manufacturer. SN-2 DNA was digested with *Bam*HI, *Eco*RI, *Hind*III, and *Hpa*I (4), while SN-1 and SN-X DNAs were digested with *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RI* activity, *Hind*II, *Hinf*I, and *Hpa*II (4). Restriction digests were analyzed by agarose gel electrophoresis (30).

Transduction protocol. The ability of bacteriophages to act as generalized transducing viruses was determined by quantifying phenotypic reversion of *P. aeruginosa* PAO303 arginine auxotrophy after bacteriophage infection. Infected *P. aeruginosa* PAO303 cells were plated on pseudomonas minimal medium (23)

containing 0.4% glucose and incubated at 37°C for 3 to 4 days. Under these conditions, only clones that have received DNA via transduction from an Arg⁺ donor or revertants of the Arg⁻ phenotype will grow to form detectable colonies. Uninfected-cell control experiments were conducted in parallel for each transduction experiment. Under the conditions used, the rate of reversion to arginine prototrophy for *P. aeruginosa* PAO303 was observed to be approximately 10⁻⁸. Experiments were considered positive for transduction only if the infected plates produced a minimum of 10-fold more colonies than the uninfected control plates, i.e., a frequency of Arg⁺ colonies of 10⁻⁷ or higher. Transduction frequency was calculated as the number of Arg⁺ colonies obtained per input PFU. *P. aeruginosa* PAO303 was infected at bacteriophage multiplicities ranging from 10 to 0.01 in order to maximize the probability of detecting transduction. Results presented are the averages of two or three transduction experiments.

RESULTS

Isolation of bacteriophages capable of intergeneric replication. Reexamination of four independently isolated *S. natans* bacteriophages, SN-1, SN-2, SN-T, and SN-X, isolated from sewage treatment plant samples (39) revealed that all four viruses could produce plaques on both the original *S. natans* host and the heterologous hosts *P. aeruginosa* PAO1, PAO303, and OT684 (Table 2). Similarly, we found that bacteriophages active against *P. aeruginosa* PAO303 (D3 and F116) productively interacted with *S. natans* ATCC 13338 as well as with the bacterial species serving as the original isolation host.

Isolation of bacteriophages by use of samples amended with two bacterial hosts. Using a different approach to bacteriophage isolation, we performed enrichments in which two bacterial host species were present. With this procedure, two bacteriophages, BHR1 and BHR2, capable of lysing both *P. aeruginosa* PAO303 and *E. coli* AB1157 as well as three bacteriophages lytic for both *S. natans* ATCC 13338 and *E. coli* AB1157, BHR3, BHR4, and BHR5, were obtained (Table 2). Thus, of six plaque-purified viruses obtained by two-host enrichment, five could produce plaques on both hosts. However, not all of the multiple-host enrichments yielded bacteriophages active on both host species. Multiple-host enrichments that included the gram-positive bacterium *Staphylococcus aureus* along with either *E. coli* or *P. aeruginosa* yielded bacteriophages which were only able to form plaques on *E. coli* or *P. aeruginosa*; no bacteriophages were capable of lysing *S. aureus* as well as *E. coli* or *P. aeruginosa*.

Examination of bacteriophage morphological homogeneity. In order to confirm that the same bacteriophage was actually obtained from both heterologous hosts and to rule out the presence of contaminating bacteriophages in virus preparations that exhibited broad-host-range activity, each of the SN series and BHR series lysates (Table 2) was examined by electron microscopy. In every case, the virions obtained from the homologous and alternate hosts were found to be morphologically indistinguishable. In addition, the virion morphology of

TABLE 2. Bacteriophage host ranges

Bacteriophage	Isolation host(s)	Infective range ^a for:			
		<i>S. natans</i> ATCC 13338	<i>P. aeruginosa</i> PAO303	<i>P. aeruginosa</i> OT684	<i>E. coli</i> AB1157
SN-1	<i>S. natans</i> ATCC 13338	+	+	+	-
SN-2	<i>S. natans</i> ATCC 13338	+	+	+	-
SN-X	<i>S. natans</i> ATCC 13338	+	+	+	-
SN-T	<i>S. natans</i> ATCC 13338	+	+	+	-
D3	<i>P. aeruginosa</i> PAO303	+	+	+	-
F116	<i>P. aeruginosa</i> PAO303	+	+	+	-
BHR1	<i>E. coli</i> AB1157 and <i>P. aeruginosa</i> PAO303	-	+	ND	+
BHR2	<i>E. coli</i> AB1157 and <i>P. aeruginosa</i> PAO303	-	+	ND	+
BHR3	<i>S. natans</i> ATCC 13338 and <i>E. coli</i> AB1157	+	-	ND	+
BHR4	<i>S. natans</i> ATCC 13338 and <i>E. coli</i> AB1157	+	-	ND	+
BHR5	<i>S. natans</i> ATCC 13338 and <i>E. coli</i> AB1157	+	-	ND	+

^a +, plaque formation detected; -, no plaque formation or killing effect detected; ND, not determined.

all of the SN series bacteriophage lysates was identical to that of the prototypical SN-1 virion originally described by Winston and Thompson (39).

EOP. The bacteriophages listed in Table 1 fell into two categories with respect to EOPs on their alternate hosts (Table 3). All of the bacteriophages originally isolated from *E. coli* or *P. aeruginosa* showed a classic response to heterologous restriction systems in that their EOPs on the alternate hosts were 10⁵- to 10⁹-fold lower than those on the host species originally used to produce the lysates (Table 3). In contrast, for the four bacteriophages isolated from *S. natans*, the EOPs were relatively unchanged on the alternate hosts. Indeed, in all cases the phages originally isolated from *S. natans* had a 2- to 35-fold higher EOP on *P. aeruginosa* than on *S. natans* (Table 3).

***S. natans* bacteriophages are insensitive to restriction endonuclease digestion.** In order for a bacteriophage to replicate in heterologous bacterial hosts, it must be able to evade or inactivate a wide variety of restriction-modification systems. Accordingly, purified DNA from phages SN-1, SN-2, and SN-X (4) was tested for sensitivity to cleavage by several type II endonucleases. The three DNA preparations were completely resistant to all restriction endonucleases tested, as judged by agarose gel electrophoresis. Each of the enzymes used produced fragments of the predicted sizes when mixed with phage lambda DNA and, for SN-1 DNA, the restriction enzymes to which SN-1 was insensitive were each able to cleave lambda DNA in the presence of an equal amount of SN-1 DNA. In addition, the *S. natans* bacteriophages seemed unaffected by the activity of heterologous host type I restriction systems, since the plaque-forming ability of these bacteriophages was not changed by infection of a restrictionless derivative of *P. aeruginosa* OT684 (Table 2) or by infection of *P. aeruginosa* cells incubated at 43°C (data not shown). Growth at 43°C inactivates restriction in this species (28).

Generalized transduction competence of bacteriophages. The ability of selected bacteriophages to transduce host chromosome DNA was determined by quantifying the reversion of *P. aeruginosa* PAO303 arginine auxotrophy after infection with bacteriophages. The bacteriophage lysates used were produced on the prototroph *P. aeruginosa* PAO1. Bacteriophage SN-T produced transductants at a frequency of 3×10^{-9} per input PFU, while bacteriophage SN-1 produced transductants at a frequency of 1.5×10^{-10} per input PFU. Both of these viruses can mediate generalized transduction. Uninfected-cell controls were used in parallel for each transduction experiment. Experiments were considered positive for transduction only if the increase in the number of Arg⁺ colonies over the num-

ber obtained with the uninfected-cell control was 10-fold or greater. Transductants were not detected (<1 transduced colony/10¹¹ infecting PFU, i.e., no increase in the number of Arg⁺ colonies above the spontaneous reversion rate) in experiments with bacteriophages BHR1 and BHR2.

Examination of the host range of *S. natans* bacteriophage SN-T. The observations that *S. natans* bacteriophages possess a striking capacity to infect and lyse multiple bacterial hosts and that bacteriophage SN-T acts as a generalized transducing virus led to additional experiments to determine the lytic potential of bacteriophage SN-T for other bacterial hosts. The ability of bacteriophage SN-T to infect and lyse a range of bacterial species was assessed with a spot titration assay (Table 4). As judged by this assay, the lytic range of bacteriophage SN-T was quite extensive and included *Pseudomonas fluorescens*, *Shigella flexneri*, *Proteus vulgaris*, and *Rhodospirillum rubrum* (Table 4). None of the gram-positive bacteria tested was sensitive to phage SN-T.

DISCUSSION

The major theme of this paper is that broad-host-range bacteriophages are more common than had been thought previously. For two bacteriophage collections, 9 of the 10 total isolated phages were found to have a broad host range. This number includes all four of the SN series phages isolated from sewage with *S. natans* hosts (Table 1) and the five BHR series phages identified from the six total phages isolated by the multiple-host procedure. These nine broad-host-range bacteriophages fell into two distinct groups based on their method of isolation and response to restriction enzymes (Table 3). The first group included the four bacteriophages active against *S. natans*, i.e., SN-1, SN-2, SN-T, and SN-X. They were isolated from sewage treatment plant samples by the standard single-host bacteriophage enrichment procedure, and analysis of their DNAs showed that they were insensitive to restriction by all type II restriction endonucleases tested. The most likely explanation for this general insensitivity is a modification of the viral DNA that makes it refractory to endonuclease cleavage, rather than a unique and general inhibition of restriction endonuclease activity, as has been observed for other bacteriophages (21). This idea is supported by the fact that SN-1 DNA did not prevent the cleavage of bacteriophage lambda DNA by any of the restriction enzymes tested. Winston and Thompson (39) demonstrated that SN-1 DNA contained a modified guanosine which comprised ca. 35% of its guanosine residues, while SN-2 DNA is also known to contain two unidentified modified bases

TABLE 3. Influence of bacterial host on plating efficiency

Bacteriophage	Phage production host	Indicator host	Titer ^a	EOP
Group 1				
SN-1	<i>S. natans</i> ATCC 13338	<i>S. natans</i> ATCC 13338	2.65×10^{10}	1.00
	<i>S. natans</i> ATCC 13338	<i>P. aeruginosa</i> PAO303	1.15×10^{11}	4.3
	<i>P. aeruginosa</i> PAO303	<i>P. aeruginosa</i> PAO303	7.5×10^{11}	1.00
	<i>P. aeruginosa</i> PAO303	<i>S. natans</i> ATCC 13338	1.25×10^{11}	0.17
SN-2	<i>S. natans</i> ATCC 13338	<i>S. natans</i> ATCC 13338	4.6×10^9	1.00
	<i>S. natans</i> ATCC 13338	<i>P. aeruginosa</i> PAO303	1.6×10^{11}	34.7
	<i>P. aeruginosa</i> PAO303	<i>P. aeruginosa</i> PAO303	5.25×10^{11}	1.00
	<i>P. aeruginosa</i> PAO303	<i>S. natans</i> ATCC 13338	4×10^{10}	0.076
SN-X	<i>S. natans</i> ATCC 13338	<i>S. natans</i> ATCC 13338	2.8×10^{10}	1.00
	<i>S. natans</i> ATCC 13338	<i>P. aeruginosa</i> PAO303	1×10^{11}	3.6
	<i>P. aeruginosa</i> PAO303	<i>P. aeruginosa</i> PAO303	2.3×10^{11}	1.00
	<i>P. aeruginosa</i> PAO303	<i>S. natans</i> ATCC 13338	1.35×10^{11}	0.59
SN-T	<i>S. natans</i> ATCC 13338	<i>S. natans</i> ATCC 13338	5.55×10^9	1.00
	<i>S. natans</i> ATCC 13338	<i>P. aeruginosa</i> PAO303	8.45×10^9	1.52
	<i>P. aeruginosa</i> PAO303	<i>P. aeruginosa</i> PAO303	3.45×10^{11}	1.00
	<i>P. aeruginosa</i> PAO303	<i>S. natans</i> ATCC 13338	1.7×10^{11}	0.49
Group 2				
BHR1	<i>P. aeruginosa</i> PAO303	<i>P. aeruginosa</i> PAO303	8.85×10^{10}	1.00
	<i>P. aeruginosa</i> PAO303	<i>E. coli</i> AB1157	1.7×10^4	1.9×10^{-7}
	<i>E. coli</i> AB1157	<i>E. coli</i> AB1157	2.57×10^{10}	1.00
	<i>E. coli</i> AB1157	<i>P. aeruginosa</i> PAO303	2.44×10^5	9.5×10^{-6}
BHR2	<i>P. aeruginosa</i> PAO303	<i>P. aeruginosa</i> PAO303	5.95×10^{10}	1.00
	<i>P. aeruginosa</i> PAO303	<i>E. coli</i> AB1157	2.3×10^5	3.86×10^{-6}
	<i>E. coli</i> AB1157	<i>E. coli</i> AB1157	2.76×10^{10}	1.00
	<i>E. coli</i> AB1157	<i>P. aeruginosa</i> PAO303	1.75×10^2	6.35×10^{-9}
BHR3	<i>E. coli</i> AB1157	<i>E. coli</i> AB1157	4.6×10^{10}	1.00
	<i>E. coli</i> AB1157	<i>S. natans</i> ATCC 13338	4.5×10^1	9.8×10^{-9}
	<i>S. natans</i> ATCC 13338	<i>S. natans</i> ATCC 13338	2×10^{11}	1.00
	<i>S. natans</i> ATCC 13338	<i>E. coli</i> AB1157	1.3×10^3	6.4×10^{-9}
BHR4	<i>E. coli</i> AB1157	<i>E. coli</i> AB1157	6.8×10^7	1.00
	<i>E. coli</i> AB1157	<i>S. natans</i> ATCC 13338	1×10^2	1.5×10^{-6}
	<i>S. natans</i> ATCC 13338	<i>S. natans</i> ATCC 13338	1.4×10^9	1.00
	<i>S. natans</i> ATCC 13338	<i>E. coli</i> AB1157	1.6×10^4	1.1×10^{-5}
BHR5	<i>E. coli</i> AB1157	<i>E. coli</i> AB1157	2.3×10^8	1.00
	<i>E. coli</i> AB1157	<i>S. natans</i> ATCC 13338	1.2×10^2	5×10^{-7}
	<i>S. natans</i> ATCC 13338	<i>S. natans</i> ATCC 13338	1.1×10^9	1.00
	<i>S. natans</i> ATCC 13338	<i>E. coli</i> AB1157	4.8×10^3	4.4×10^{-6}

^a Data shown are the means of triplicate independent experiments.

(38). DNAs from bacteriophages SN-T and SN-X have not yet been studied in this regard.

Unlike that with other, previously described broad-host-range bacteriophages (24), infection with the broad-host-range bacteriophages characterized in this study is not dependent on the presence of a specific plasmid. *S. natans* ATCC 13338 has been screened for the presence of plasmids by several methods (38). These experiments failed to reveal the presence of plasmids in *S. natans* ATCC 13338 but did allow for the detection of plasmid RP1, introduced into this strain by conjugation (38). In addition, experiments with *P. aeruginosa* PAO303 and *E. coli* AB1157 failed to reveal the presence of plasmids in these hosts as well (19a).

The second group of broad-host-range bacteriophages was obtained by a two-host isolation method. This alternative method included in the sample amendments two distinct po-

tential bacterial host species in high numbers and then ascertained the ability of isolated viruses to infect alternate hosts before the lysates had undergone repeated passages on a single host species. This approach was used to isolate bacteriophages BHR1, BHR2, BHR3, BHR4, and BHR5 (Table 1). These bacteriophages, however, did not share the trait of being insensitive to restriction, and they showed greatly reduced titers (10^5 - to 10^9 -fold lower) when plated on the alternate host species. These experiments revealed that viruses capable of interactions with several heterologous hosts are readily isolated from natural microbial communities and suggest that standard methods for virus enrichment may favor bacteriophages possessing a more limited host range.

The rationale for our use of a two-host enrichment system was as follows. The standard method of bacteriophage enrichment is to amend environmental samples with nutrients and

TABLE 4. Host range of bacteriophage SN-T produced by *S. natans* ATCC 13338

Bacteria	Effect on bacterial lawn ^a
Gram-negative eubacteria	
Sheathed bacteria (<i>Sphaerotilus natans</i>)	
Sn-2.....	+
TLT1.....	-
<i>Pseudomonadaceae</i>	
<i>Pseudomonas aeruginosa</i>	
PAO1.....	++
PAO127.....	++
PAO303.....	++
<i>Pseudomonas fluorescens</i>	+
<i>Enterobacteriaceae</i>	
<i>Escherichia coli</i>	
ATCC 23744.....	+
B.....	-
DH1.....	-
W3110.....	-
<i>Citrobacter freundii</i> ATCC 8090.....	-
<i>Salmonella typhimurium</i> ATCC 14028.....	-
<i>Shigella flexneri</i> ATCC 12022.....	++
<i>Klebsiella pneumoniae</i> ATCC 13883.....	-
<i>Enterobacter cloacae</i> ATCC 23355.....	-
<i>Enterobacter aerogenes</i>	-
<i>Serratia marcescens</i> KWN.....	-
<i>Proteus vulgaris</i> ATCC 13315.....	++
<i>Vibrionaceae</i> (<i>Aeromonas hydrophila</i> B2865).....	-
<i>Neisseriaceae</i> (<i>Acinetobacter calcoaceticus</i> ATCC 19606).....	-
Phototrophic bacteria (<i>Rhodospirillum rubrum</i>).....	++
Uncertain affiliation (<i>Flavobacterium capsulatum</i>).....	-
Gram-positive eubacteria	
<i>Micrococcaceae</i>	
<i>Micrococcus luteus</i>	-
<i>Staphylococcus aureus</i>	-
<i>Staphylococcus epidermidis</i>	-
<i>Streptococcaceae</i>	
<i>Streptococcus pyogenes</i>	-
<i>Streptococcus faecalis</i>	-
<i>Bacillaceae</i> (<i>Bacillus subtilis</i>).....	-
Yeasts	
<i>Candida albicans</i> A-72.....	-
<i>Saccharomyces cerevisiae</i>	-

^a +, decreased growth of bacterial lawn; -, no change in bacterial lawn; ++, clearing of bacterial lawn.

high numbers of a single bacterial host. After overnight incubation, bacteriophages are detected by a plaque assay with lawns of the same, originally added host cells (2). This was the approach taken to isolate the SN series viruses with *S. natans*. The host range of bacteriophages obtained by such a method is subsequently assessed by screening their lytic activity for additional bacterial host species. Thus, even though the isolation initially might select for all bacteriophages capable of interaction with a host, continued rounds of phage growth on the same host ultimately result in the unintentional selection of virions capable of the most avid adsorption to host cells, resulting in phage lysates that have become more specific for the host. If true, this scenario suggests that standard bacteriophage enrichments are unavoidably biased against the development of viruses with a broad host range, and this bias may partially explain the general view that bacteriophages are restricted in their interactive range (16).

We examined bacteriophage collections obtained from a

range of geographically dispersed and diverse habitats over a very long time scale. In this limited pool, 9 of 10 total viruses isolated were capable of broad-host-range interactions. Our experiments support the hypothesis that bacteriophages with a broad host range, as judged by plaque-forming ability, are frequently and readily isolated from complex natural microbial communities. This prevalence has profound ecological significance, especially with regard to natural mechanisms for gene transfer. Numerous studies have revealed the presence of large numbers of virus particles in aquatic and other ecosystems (3, 9, 15, 22, 26, 35), and the existence of broad-host-range bacteriophages may partially explain these observations. It is certain that many natural microbial communities will contain complex and rich assemblages of many bacterial species existing in close proximity (25). For such communities, it has been suggested (i) that virus replication would be most effective in ecosystems characterized by a low overall prey diversity (25) and (ii) that dominant community members attaining a high population density would be constantly eliminated by virus-mediated lysis (33). If these ideas are correct, their combined action suggests that for bacteriophages with a relaxed host receptor specificity, selection would allow for productive interaction with several prey species, i.e., a broad host range. The potential to infect a variety of alternate bacterial host species would maximize opportunities for effective viral reproduction in complex natural communities and biofilms, since bacteriophages able to capitalize on a wider variety of potential hosts would be more likely to encounter suitable prey and replicate under the conditions prevalent in situ.

Broad-host-range bacteriophages may also promote genetic diversity and genetic exchange in microbial communities. Two of the phages that we examined, SN-1 and SN-T, are capable of generalized transduction and thus may contribute to gene exchange between diverse host species. The potential for inter-specific gene transfer has been noted by other investigators to depend more on ecological proximity than on evolutionary relatedness (27). The concept of ecological proximity (27) has been used to explain the observed bacteriophage P1-mediated transduction of genes between *E. coli* and mycobacteria (17).

To date, a very small pool of transducing bacteriophages has been intensively examined with regard to their host range. Intrageneric bacteriophage-host interactions have been observed, and several transducing bacteriophages producing plaques on species of the same genus have been characterized (6, 11, 18, 29). While intrageneric infection and transduction have been observed, true intergeneric infection by and replication of bacteriophages are much less frequently reported (17, 40). Experiments with bacteriophage P1 containing a transposable element encoding a selectable antibiotic resistance have shown that high-titer lysates include mutant virion forms that adsorb to and inject their DNA into a large array of bacterial hosts (10). The ability of P1 to interact with heterologous hosts was revealed by the growth of P1-infected cells on antibiotic-containing medium; however, whether the P1 mutants actually replicated in these hosts or the precise frequency with which mutants having an altered host specificity were present in P1 lysates was not determined (10). Clearly, while the host range of phage P1 may be described as extensive (40), productive P1 infection is limited primarily to members of the family *Enterobacteriaceae*. In contrast, our results suggest that broad-host-range bacteriophages capable of true intergeneric infection are widely distributed.

One of the bacteriophages examined in this study, SN-T, is a generalized transducing virus and was demonstrated to be clearly capable of productive interactions with a wide variety of bacterial hosts. *S. natans* is widely distributed and commonly

isolated from streams, sewage treatment plants, waste streams of potato plants and paper mills, compost piles, and cooling tower water supplies (7). This wide distribution suggests that *S. natans* bacteriophages, including generalized transducing varieties possessing very broad host ranges, exist in those sites as well. Some of these sites probably include additional bacterial species with antibiotic resistance genes. This juxtaposition creates the potential for the dissemination of these antibiotic resistance genes through the microbial community by broad-host-range transducing bacteriophages. Few studies have been undertaken to quantify transducing bacteriophages in environments of any kind (31), and no data exist to enable meaningful estimates of the general prevalence of broad-host-range bacteriophages in nature and their capacity to influence host ecology. The lack of information regarding broad-host-range transducing bacteriophages complicates the risk assessment for recombinant DNA release associated with human modification of plants, animals, or microbes. The results of our study suggest that bacteriophages possessing a broad host range are prevalent and can be readily isolated from many ecosystems.

ACKNOWLEDGMENTS

This work was supported in part by Cooperative Agreement CR822163 with the Gulf Breeze Environmental Research Laboratory of the U.S. Environmental Protection Agency, Cooperative Agreement 9255225 from the NSF-EPSCoR program, and grants from the University of Nebraska Water Center, the University of Nebraska Research Council, the University of Nebraska Biotechnology Center, and the Consortium for Plant Biotechnology Research (593-0009-04).

We thank S. Hattman for suggesting the control in which restriction enzymes were provided with equal amounts of lambda and SN viral DNAs.

REFERENCES

- Arber, W., L. Enquist, B. Hohn, N. E. Murray, and K. Murray. 1983. Experimental methods for use with lambda, p. 433-466. In R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), *Lambda II*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Benson, H. J. 1990. Microbiological applications. A laboratory manual in general microbiology. W. C. Brown, Dubuque, Iowa.
- Bergh, Ø., G. Børshheim, G. Bratbak, and M. Haldal. 1989. High abundance of viruses found in aquatic environments. *Nature* **340**:467-468.
- Brenton, B. L. 1985. M.S. thesis. University of Nebraska—Lincoln, Lincoln.
- Clark, A. J., and A. D. Margulies. 1965. Isolation and characterization of recombination deficient mutants of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **53**:451-459.
- Colón, A. E., R. M. Cole, and C. G. Leonard. 1972. Intergroup lysis and transduction by streptococcal bacteriophages. *J. Virol.* **9**:551-553.
- Dondero, N. C. 1975. The *Sphaerotilus-Leptothrix* group. *Annu. Rev. Microbiol.* **29**:407-428.
- Duckworth, D. H. 1976. Who discovered bacteriophage? *Bacteriol. Rev.* **40**:793-802.
- Fuhrman, J. A., and C. A. Suttle. 1993. Viruses in marine planktonic systems. *Oceanography* **6**:51-63.
- Goldberg, R. B., R. A. Bender, and S. L. Streicher. 1974. Direct selection for P1-sensitive mutants of enteric bacteria. *J. Bacteriol.* **118**:810-814.
- Green, J., and R. B. Goldberg. 1985. Isolation and preliminary characterization of lytic and lysogenic phages with wide host range within the *Streptomyces*. *J. Gen. Microbiol.* **131**:2459-2465.
- Harshey, R. M. 1988. Phage Mu, p. 193-234. In R. Callendar (ed.), *The bacteriophages*, vol. 1. Plenum Press, New York, N.Y.
- Hauduroy, P. 1925. Le bacteriophage de D'Hérelle. *Librarie le François*, Paris, France.
- Hayes, W. 1968. *The genetics of bacteria and their viruses*, 2nd ed. Blackwell Scientific Publications Ltd., London, United Kingdom.
- Hennes, K. P., and M. Simon. 1995. Significance of bacteriophages for controlling bacterioplankton growth in a mesotrophic lake. *Appl. Environ. Microbiol.* **61**:333-340.
- Holloway, B. W., and V. Krishnapillai. 1975. Bacteriophages and bacteriocins of *Pseudomonas aeruginosa*, p. 99-132. In P. H. Clarke and M. H. Richmond (ed.), *Genetics and biochemistry of Pseudomonas*. John Wiley & Sons, Inc., New York, N.Y.
- Kaiser, D., and M. Dworkin. 1975. Gene transfer to a mycobacterium by *E. coli* phage P1. *Science* **187**:653-654.
- Kelln, R. A., and R. A. J. Warren. 1971. Isolation and properties of a bacteriophage lytic for a wide variety of pseudomonads. *Can. J. Microbiol.* **17**:677-682.
- Kokjohn, T. A., G. S. Saylor, and R. V. Miller. 1991. Attachment and replication of *Pseudomonas aeruginosa* bacteriophages under conditions simulating aquatic environments. *J. Gen. Microbiol.* **137**:661-666.
- Kokjohn, T. A. Unpublished results.
- Krishnapillai, V. 1971. A novel transducing phage. Its role in recognition of a possible new host controlled modification system in *Pseudomonas aeruginosa*. *Mol. Gen. Genet.* **114**:134-143.
- Krüger, D. H., and T. A. Bickle. 1983. Bacteriophage survival: multiple mechanisms for avoiding the deoxyribonucleic acid restriction systems of the hosts. *Microbiol. Rev.* **47**:345-360.
- Marsh, P., and E. M. H. Wellington. 1994. Phage-host interactions in soil. *FEMS Microbiol. Ecol.* **15**:99-108.
- Miller, R. V., and C.-M. C. Ku. 1978. Characterization of *Pseudomonas aeruginosa* mutants deficient in the establishment of lysogeny. *J. Bacteriol.* **134**:875-883.
- Olsen, R. H., J. Siak, and R. H. Gray. 1974. Characteristics of PRD1, a plasmid-dependent broad-host-range DNA bacteriophage. *J. Virol.* **14**:689-699.
- Pedrés-Alió, C. 1993. Diversity of bacterioplankton. *Trends Ecol. Evol.* **8**:86-90.
- Reanny, D. C. 1976. Extrachromosomal elements as possible agents of adaptation and development. *Bacteriol. Rev.* **40**:552-590.
- Reanny, D. C., W. P. Roberts, and W. J. Kelly. 1982. Genetic interactions among microbial communities, p. 287-321. In A. T. Bull and J. H. Slater (ed.), *Microbial interactions and communities*. Academic Press, Inc., New York, N.Y.
- Rolfe, B., and B. W. Holloway. 1966. Alterations in host specificity of bacterial deoxyribonucleic acid after an increase in growth temperature of *Pseudomonas aeruginosa*. *J. Bacteriol.* **92**:43-48.
- Ruhfel, R. E., N. J. Robillard, and C. B. Thorne. 1984. Interspecies transduction of plasmids among *Bacillus anthracis*, *B. cereus*, and *B. thuringiensis*. *J. Bacteriol.* **157**:708-711.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Schicklmaier, P., and H. Schmieger. 1995. Frequency of generalized transducing phages in natural isolates of the *Salmonella typhimurium* complex. *Appl. Environ. Microbiol.* **61**:1637-1640.
- Simonson, C. S., T. A. Kokjohn, and R. V. Miller. 1990. Inducible UV repair potential of *Pseudomonas aeruginosa* PAO. *J. Gen. Microbiol.* **136**:1241-1249.
- Thingstad, T. F., M. Haldal, G. Bratbak, and I. Dundas. 1993. Are viruses important partners in pelagic food webs? *Trends Ecol. Evol.* **8**:209-213.
- Thomas, C. A., and J. Abelson. 1966. The isolation and characterization of DNA from bacteriophage, p. 553-561. In G. L. Cantoni and D. R. Davies (ed.), *Procedures in nucleic acid research*, vol. 1. Harper & Row, Publishers, Inc., New York, N.Y.
- Torrella, F., and R. Y. Morita. 1979. Evidence by electron micrographs for a high incidence of bacteriophage particles in the waters of Yaquina Bay, Oregon: ecological and taxonomical implications. *Appl. Environ. Microbiol.* **37**:774-778.
- Van de Putte, P., S. Cramer, and M. Giphart-Gassler. 1980. Invertible DNA determines host specificity of bacteriophage Mu. *Nature* **286**:218.
- Welkos, S., M. Schreiber, and H. Baer. 1974. Identification of *Salmonella* with the O-1 bacteriophage. *Appl. Microbiol.* **28**:618-622.
- Williams, R. L., Jr. 1984. Ph.D. dissertation. University of Nebraska—Lincoln, Lincoln.
- Winston, V., and T. L. Thompson. 1979. Isolation and characterization of a bacteriophage specific for *Sphaerotilus natans* which contains an unusual base in its deoxyribonucleic acid. *Appl. Environ. Microbiol.* **37**:1025-1030.
- Yarmolinsky, M. B., and N. Sternberg. 1988. Bacteriophage P1, p. 291-438. In R. Callendar (ed.), *The bacteriophages*, vol. 1. Plenum Press, New York, N.Y.