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Elution and Inactivation of Bacteriophages on Soil and Cation-Exchange Resin

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A variety of elution schemes was tested to determine the most effective procedure for eluting *Arthrobacter* bacteriophages from soil. A buffer solution of pH 8.0 was found to be the most satisfactory eluent. Bacteriophages were adsorbed to cation-exchange sites on soils, clays, and Dowex-50 resin and eluted. Eluted bacteriophages were detected by passive hemagglutination and plaque assay. Although bacteriophage antigen was successfully eluted, most recovered bacteriophages were noninfective. Inactivation was greatest in bacteriophages of Bradley group B and was associated with the elution process. Inactivation is believed to be caused by physical damage to bacteriophage tails upon elution. The significance of this inactivation relative to bacteriophage models for vertebrate viruses is discussed.

Bacteriophages are normally found in most soils where their cells are present. Interest in the occurrence of bacteriophages in soils has resulted from the use of host organisms as crop symbionts, insect control agents, and models for ecological studies on vertebrate viruses in soil. Soil can also serve as a source of bacteriophages for the genetic manipulation of agronomically important bacteria. However, little is known about the survival and host interaction of phages in soil. Bacteriophages for many soil bacteria have proven difficult to isolate in quantity from soils even when large numbers of host cells are known to be present.

The genus *Arthrobacter* has been shown to be a significant portion of the bacterial population of many soils (16, 20). However, it has also been noted that attempts to isolate bacteriophages for *Arthrobacter* from soils have been only marginally successful unless enrichment procedures are used (4, 8). Recent attempts to classify the genus *Arthrobacter* by bacteriophage typing have been hampered by the difficulty of isolating phages of *Arthrobacter* from soil (4).

Several studies attempting to utilize phages as models for the movement of vertebrate viruses through soils have been reported, but these met with little success due to the differences in the behavior of bacteriophages and vertebrate viruses in the test systems (2, 9). Interaction between soils and viruses and sorptive phenomena were reviewed by Bitton (2), and a number of studies utilizing bacteriophages as models for vertebrate and entomogenous viruses were discussed.

The effect of bacteriophage adsorption to clay surfaces is the subject of much controversy. It has been reported that coliphage adsorption on bentonite does not affect coliphage infectivity (5), and desorption of bacteriophage from clays in an infective state has been widely reported (6). However, in several studies, the majority of the bacteriophages added to clays and soils could not be eluted in an infective state only a short time after adsorption (8, 16; W. Jakubowski, Bacteriol. Proc., p. 179, 1969). The adsorption of actinophage to clays in an infective state has recently been reported, although desorption of infective actinophage could not be accomplished on a large scale (22). The comparisons of these studies are difficult, due to the large number of bacteriophage types utilized and the different soils involved. A rapid and large-scale inactivation of bacteriophages adsorbed to an artificial cation-exchange resin was noted by Puck and Sagik (18), although the inactive phage could be eluted easily. Although many soils are known to have significant cation-exchange capacities, the possibility of a related inactivation of bacteriophages on soil cation-exchange sites has not been widely considered (2).

Due to the difficulty encountered in isolating bacteriophages from soil without enrichment and the frequency of attempts to utilize bacteriophages as model systems for the movement of vertebrate viruses in soil, we decided to determine the optimal conditions for the elution of bacteriophages for *Arthrobacter* spp. from soil and soil components and to examine the possibility of bacteriophage inactivation during adsorption or elution. A variety of elution media was employed, and the relative efficiencies of

several are reported here. Further, the occurrence and mechanisms of bacteriophage inactivation during these elution schemes are discussed, and the ability of several types of bacteriophages to survive adsorption and elution under standard conditions is examined.

MATERIALS AND METHODS

Bacteria. The bacterial cultures utilized in this project are listed in Table 1. The soil isolates were examined microscopically for typical *Arthrobacter* morphology (15). The *Arthrobacter* soil isolates were originally isolated by Hagedorn and Holt (14) from the same soils used for the bacteriophage elution studies in this project.

Bacteriophages. The bacteriophages utilized in this study are listed in Table 2. The bacteriophages were originally isolated by Brown et al. (4) from sewage and river water for use in a phage-typing scheme for *Arthrobacter*. Bacteriophages were stored at 4°C in the bacteriophage suspension medium of Weigle et al. (24).

Media. Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.), supplemented with 0.2% yeast extract (Difco Laboratories, Detroit, Mich.) (TSYE), was used as a growth medium for bacterial cultures and as a basal layer for bacteriophage plaque assays and reproduction. Trypticase soy broth (BBL) supplemented with 0.2% yeast extract was used as a diluent for bacteriophage and host cells, and when supplemented with 0.9% agar (Difco, Laboratories), it was used as an overlay for plaque assay and bacteriophage propagation. Cystine Trypticase agar (BBL) was used for the maintenance of bacterial stock cultures. Nutrient broth (Difco) was used as a propagation medium for *Escherichia coli* host cells. The various elution media and their sources are as follows: 0.3% beef extract (BBL) plus 0.5% glucose (Fisher Scientific Co., Pittsburgh, Pa.), brain heart infusion (Difco), Loeffler blood serum (Difco), nutrient broth (Difco), 0.5% neopeptone, rehydrated beef blood (Difco), TSYE, Wort broth (General Biochemicals Div. [Mogul Corp.], Chagrin Falls, Ohio), 1% hen egg albumen (Difco) plus 0.1 M NaCl (Fisher) plus 0.001 M $MgCl_2$ (J. T. Baker Chemical Co., Phillipsburg, N.J.) plus 0.2% gelatin (Difco), phage suspension medium of Weigle et al. (24), ammonium chloride (Baker)-ammonium acetate (Baker) (NH_4Cl-NH_4 Ac), and deionized water. All systems were tested at pH 5.0 to 8.0, at 0.5 pH unit intervals, and at 4, 24, and 30°C, and the pH was altered with 1.0 and 0.1 N HCl or 1.0 and 0.2 N NaOH.

TABLE 1. *Bacterial cultures utilized*

Culture name	Strain designation[a]	Remarks
Arthrobacter globiformis	ATCC 8010	Type strain
A. polychromogenes	ATCC 15216	
A. citreus	ATCC 11624	
A. crystallopoietes	ATCC 15841	Type strain
Arthrobacter spp.	AC 21	Soil isolate[b]
Arthrobacter spp.	AC 24	Soil isolate[b]
Arthrobacter spp.	AN 25	Soil isolate[b]
Arthrobacter spp.	AN 31	Soil isolate[b]
Arthrobacter spp.	AN 39	Soil isolate[b]
E. coli K-12 (Hfr^+)	C3000	

[a] ATCC, American Type Culture Collection, Rockville, Md.; AN, *Arthrobacter*, Nicollet soil isolate; AC, *Arthrobacter*, Clarion soil isolate.

[b] Originally isolated by Hagedorn and Holt (14).

TABLE 2. *Bacteriophage strains utilized*

Strain designation[a]	Host cell[b]	Bradley group (3)
AN25s-1[c]	AN 25	C
AN31s-1[c]	AN 31	B
AN31n-1	AN 31	Unknown
AN31n-2	AN 32	Unknown
AN31n-3	AN 31	Unknown
8010n-1	ATCC 8010	Unknown
8010n-2	ATCC 8010	Unknown
8010c-1	ATCC 8010	Unknown
15216n-1	ATCC 15216	Unknown
15216c-1	ATCC 15216	Unknown
11624n-1	ATCC 11624	Unknown
15841n-1	ATCC 15841	Unknown
15841n-2	ATCC 15841	Unknown
15841n-3	ATCC 15841	Unknown
MS2[d]	*E. coli* K-12 (Hfr^+)	E

[a] s-1, First isolate to the host cell from sewage; n-1, first isolate to the host cell from Nicollet soil; c-1, first isolate to the host cell from Clarion soil.

[b] Abbreviations as in Table 1, footnote *a*.

[c] From D. Brown, Department of Biological Sciences, Michigan Technological University.

[d] From L. Quinn, Department of Bacteriology, Iowa State University.

Bacteriophage propagation. High-titer lysates for bacteriophage elution studies were prepared by a modification of the method of Rogerson and Rushizky (19) and Davis and Sinsheimer (11). Fifty-milliliter amounts of TSYE in liquid form at 45°C were inoculated with 2.0-ml portions of heavy 24-h host cell suspensions (ca. 10^{11} colony-forming units per ml), prepared by the method of Eisenstark (13) and with 1.0 ml of bacteriophage suspension (ca. 10^{10} plaque-forming units [PFU]/ml). Each inoculated amount was poured over a TSYE basal layer in a Pyrex baking dish (34 by 22 by 4 cm), covered with aluminum foil, and incubated at 30°C for 12 h. After incubation, each soft agar layer was harvested in 100 ml of TSYE, pooled, and centrifuged at 9,150 × *g* for 30 min at 4°C (Sorvall RC2B, GSA rotor, Corex glass centrifuge bottles). The supernatant was filtered through a 0.45-μm membrane filter (type HA, Millipore Corp., Bedford, Mass.) to remove cellular debris.

Ammonium sulfate (Fisher) was added to the lysate in a ratio of 300 g of $(NH_4)_2SO_4$ per liter of phage suspension and mixed with a magnetic stirrer at 4°C for 30 min. After agitation, the solution was allowed to sit overnight at 4°C. The solution was then centrifuged at 9,150 × *g* for 30 min (as before). The pellet was suspended in phage suspension medium (24). The precipitation was repeated three times, and the final

pellet was resuspended in 250 ml of phage suspension medium for every liter of bacteriophage-TSYE suspension originally treated. The suspension was filtered by passage through a 0.22-μm Millipore filter (type GS) and stored at 4°C.

Determination of elution efficiency. Bacteriophages were isolated and eluted from soil by a modification of the method of Casida and Liu (8). Soil samples (5 g) were amended with host cells from 24-h cultures to a concentration of approximately 10^8 colony-forming units per 5 g and with bacteriophages to a concentration of approximately 10^6 PFU/5 g. The samples were then adjusted to 60% moisture holding capacity with TSYE or deionized water when necessary and incubated for periods of 2 to 4 days in a moist environment to prevent desiccation. After incubation, each soil sample was suspended in 100 ml of the elution medium to be tested (see above) and shaken for 10 min. The suspension was then centrifuged at 9,150 × *g* at 4°C for 15 min (Sorvall RC2B, GSA rotor, Corex glass centrifuge bottles). The supernatant was filtered through 02 Selas filters (average pore diameter, 0.4 μm; Selas Flowtronics, Spring House, Pa.). Bacteriophage concentration was determined by plaque assay with incubation at 30°C for 24 h. The efficiency coefficient was determined by arbitrarily assigning the treatment isolating the most bacteriophage a coefficient of 100 and ranking the remaining treatments as percentages of the maximum number of bacteriophages eluted by the top-ranked treatment.

Detection of inactivated bacteriophages. Bacteriophages which were eluted in a noninfective state were detected by passive hemagglutination (PHA) of the eluted bacteriophage antigen. Antisera to the *Arthrobacter* and *E. coli* bacteriophages employed were prepared in domestic Leghorn chickens by intramuscular injection of bacteriophage suspensions with Freund complete adjuvant (Difco) at 1-week intervals. The collected antiserum was pooled and stored at 4°C. The antiserum was attached to chicken erythrocytes by glutaraldehyde fixation (1% glutaraldehyde, vol/vol; Matheson, Coleman, and Bell, Norwood, Ohio) (21; J. H. Bush, M. S. thesis, Iowa State University, Ames, 1977).

Soil or cation-exchange resin columns were prepared in elution columns with a packed volume of 28 ml (4-cm height), and 5-ml amounts of bacteriophage suspension (about 10^{10} PFU/ml) were added to the top of the column and allowed to react for approximately 1 min. The phages were then eluted with 45 ml of NH_4Cl-NH_4Ac buffer, pH 8.0 (Baker), under mild suction. The amounts of eluted infective phages were determined by plaque assay on TSYE with 24-h host cells, as previously described. Nonviable eluted bacteriophages were determined by PHA.

The Dowex-50 cation-exchange resin was recharged before each use with 10% NaCl (Fisher) and washed with deionized water until the wash no longer produced a precipitate when treated with acidified $AgNO_3$ (D. F. Goldsmith Chemical and Metal Corp., Chicago, Ill.). Soil and soil component-organic matter columns were discarded after each use. All columns were saturated with deionized water before use.

To determine the number of bacteriophages adsorbed before elution, a modification of the method of Moore et al. (17) was used. Five-milliliter portions of each substance used in the elution columns, saturated with deionized water, were mixed with 50-ml amounts of bacteriophage suspension (ca. 10^{10} PFU/ml). Immediately after bacteriophage addition, 1.5-ml samples were taken, diluted 1:100 in TSYE, and stored at 4°C until assayed (time zero count). Samples (0.5-ml) were taken at 1-min intervals for 20 min, diluted, and stored as above. Each diluted sample was centrifuged at high speed in a tabletop centrifuge for 20 min at room temperature to remove the suspended solids. The supernatant was then assayed for infective phages by plaque assay. Bacteriophages not recovered in the test series and unaccounted for by loss in the controls (minimal) were considered physically adsorbed.

The presence of infective bacteriophages adsorbed to the solid phase was demonstrated by plating dilutions of the solid phase as if for a plaque assay. This technique was considered too inaccurate and subject to error for an actual plaque assay to be meaningful, so actual numbers of phages adsorbed in an infective condition were not determined.

PHA. The PHA procedure was a modification of the method employed by Bush (M.S. thesis, Iowa State University, Ames, 1977).

Approximately 1.0 ml of fresh chicken erythrocytes diluted 1:1 in Alsever solution (1) was washed three times in 0.01 M phosphate-buffered saline (PBS), pH 7.2, and fixed by mixing with 0.05 ml of 1% glutaraldehyde (Matheson, Coleman, and Bell) and 0.5 ml of antiserum per 0.1 ml of washed, packed cells. The contents were mixed by inversion and incubated at room temperature for 30 min with occasional mixing. The sensitized cells were washed three times (as before) with PBS containing 1% complement-inactivated (erythrocyte adsorbed) newborn calf serum (PBS-1% NCS). Control cells were prepared as above, but with PBS in place of antiserum. The sensitized erythrocytes were reconstituted to a 1% suspension in PBS-1% NCS after the final wash.

PHA assay of the infective plus noninfective bacteriophages eluted from the test columns was performed with 50-μl portions of antigen (eluate) diluted 1:2 per well in a Cooke microtiter plate (Cooke Engineering Co. [Dynatech Corp.], Alexandria, Va.) with PBS-1% NCS as the diluent. After dilution, 50 μl of 1% sensitized erythrocytes was added to each well. A normal serum control was provided by substituting normal serum (chicken) for antigen in one row of wells. To provide a basis for correlating the observed titers to actual phage concentration in PFU per milliliter, stock solutions of 10^{10} through 10^4 PFU/ml were titrated as for the antigen solution. These stock solutions were diluted in elution buffer to account for any effects of the buffer on the assay. The assay plates were sealed and incubated at room temperature for 18 h. The assay was scored, as described by Carpenter (7), at 3 and 18 h.

Electron microscopy. Electron microscopy was performed with a Hitachi model 11C transmission electron microscope at × 27,200. All preparations were negatively stained with 1% phosphotungstic acid, pH 7.2. Preparations were of stock solutions (ca. 10^{10} PFU/ml or ca. 10^9 PFU equivalents/ml in eluted samples) diluted with NH_4Cl-NH_4Ac elution buffer, pH 8.0.

Samples of phages eluted from clay and resin were eluted by the procedure described previously.

RESULTS AND DISCUSSION

Elution of bacteriophages. The elution media and conditions employed are listed in order of their efficiency in Table 3. Temperature and protein type were unrelated to elution efficiency. Ionic strength appeared to have some effect on elution, but a great number of phages were still eluted with deionized water as well as with solutions of higher ionic strength. It is possible that deionized water picks up soluble ions from the soil during the elution process, thereby increasing its ionic strength. The pH of the elution solution has a considerable effect, however, with solutions of a high pH eluting the greatest number of bacteriophages (Table 4). Attempts to elute with solutions of a pH higher than those presented in Tables 3 and 4 were of a preliminary nature, and no significant increase in efficiency was noted above the pH range of 7.8 to 8.2. Since the possibility of damaging phages at a higher pH was recognized, NH_4Cl-NH_4Ac buffer at pH 8.0 was adopted as the standard elution buffer. The increase in efficiency of the elution mixtures at higher pH values is consistent with adsorption of bacteriophages to cation-exchange sites in the soil. The low elution efficiencies at low pH regimes may indicate either that bacteriophage adsorption to anion-exchange sites did not occur or that the anion-exchange capacity of the soil used was extremely low. It must also be noted that, even with the most efficient elution media and conditions, only a small fraction (ca. 0.25% or less) of the added phages could be eluted in an infective condition and that this eluted viable fraction remained low regardless of the host cell-bacteriophage system employed as long as the bacteriophages were of Bradley group B.

Although most bacteriophages have a net negative charge at neutral pH, their adsorption to cation-exchange sites is not unknown. Puck and Sagik (18) reported the adsorption of coliphages to cation-exchange resin in the presence of sufficient amounts of soluble cations. The phages may be adsorbed to cation-exchange sites by

TABLE 3. *Efficiency of elution media in the recovery of bacteriophage AN31s-1 added to Nicollet loam*

Ranking by relative efficiency (high to low)	Recovery coefficient[a]	Efficiency of recovery (%)[b]	Medium[c]
	100	0.174	Ammonium acetate-ammonium chloride buffer, pH 8.0, 4°C and 28°C
1	100	0.173	Deionized H_2O, pH 8.0, 4 and 28°C
	100	0.173	Albumen (1%) plus salts, pH 8.0, 4°C
2	93	0.161	Albumen (1%) plus salts, pH 8.0, 28°C
3	80	0.140	BEG,[d] pH 8.0, 4°C
	80	0.138	BEG, pH 8.0, 28°C
	75	0.131	LBS,[e] pH 8.0, 28°C
4	75	0.131	Neopeptone, pH 8.0, 4 and 28°C
5	72	0.125	BHI,[f] pH 8.0, 4°C
6	71	0.123	Wort broth, pH 8.0, 4°C
7	70	0.122	Rehydrated beef blood, pH 8.0, 4°C
	70	0.121	BHI, pH 8.0, 28°C
8	68	0.118	LBS, pH 8.0, 4°C
9	67	0.116	Wort broth, pH 8.0, 4°C
10	65	0.113	Phage suspension medium, pH 8.0, 4 and 28°C
11	48	0.084	Albumen (1%) plus salts, pH 7.0, 4°C
12	45	0.078	Albumen (1%) + salts, pH 7.0, 28°C

[a] Recovery coefficient calculated as a percentage of maximum number of bacteriophages recovered by the most efficient elution medium tested and rounded to the nearest whole number.

[b] (PFU recovered/PFU added) × 100, rounded to three places.

[c] Media listed are a representative selection of the various combinations employed.

[d] BEG, Beef extract plus glucose.

[e] LBS, Loeffler blood serum.

[f] BHI, Brain heart infusion.

TABLE 4. *Effect of direct pH variation of single elution media on the recovery of bacteriophage AN31s-1 added to Nicollet loam*[a]

pH	Recovery in following medium	
	Deionized water	Beef extract-glucose
5.0	6	12
5.4	7	7
5.8	7	8
6.0	7	8
6.4	8	9
6.8	9	9
7.0	10	12
7.2	26	35
7.4	45	33
7.6	78	58
7.8	95	78
8.0	100	80
8.2	100	81

[a] Recovery presented as percent efficiency calculated as for Table 3. Data presented are the averages of three replicates.

their tails in a direct manner, although the evidence for this is not conclusive (5). This postulated direct adsorption may be analogous to the initial, reversible adsorption of phages to host cells. It is important to note that the pH under which bacteriophage adsorption is studied will determine the type of adsorption occurring. Little or no adsorption to cation-exchange sites will occur if the system is buffered at a high pH. Since few agronomically important soils are alkaline in nature, the practical significance of adsorption studies in which the adsorption occurs at high pH would appear to be limited.

To determine the significance of the cation-exchange capacity to bacteriophage adsorption under field conditions, additions of bacteriophages to soil samples at 60% moisture holding capacity were made without further amendment. The pH of the soil-phage mixture was determined and found to be equivalent to the pH of untreated soil. The majority of bacteriophages added to solid phases of a high cation-exchange capacity could not be eluted in an infective state (Table 5), and bacteriophages added to solid phases of lower cation-exchange capacity were affected considerably less. Surface area was apparently not of primary importance as a great deal of inactivation was noted with Dowex-50 cation-exchange resin, which has a surface area much lower than that of bentonite.

Inactivation of bacteriophages. Because of the failure of even the most efficient elution techniques previously discussed to recover a majority of the infective phages added to soil, the possibility of bacteriophage inactivation was considered. Noninfective bacteriophages eluted from the solid phases employed was detected by PHA. Since the bacteriophage nucleic acid is a sequestered antigen and poorly antigenic when released, the bacteriophage capsid was probably the main source of antigen. It is necessary to correlate the titer observed with eluted bacteriophage to that observed with standardized quantities of bacteriophage to determine the quantity of bacteriophage eluted. This correlation must be made for every PHA assay performed as the separate batches of erythrocyte-adsorbed antibody will vary in potency.

TABLE 5. *Elution of bacteriophages from various soil-resin systems*

Solid phase[a]	Cation-exchange capacity (meq/100 g)[b]	Bacteriophage recovered in an infective state (%)[c]
Dowex-50	4.8×10^5	0.023
Bentonite	82.4	0.09
Nicollet loam	68.5	0.17
Florida river sand	20.5	52.6
Sea sand (washed and ignited)	8.2	88.5

[a] Dowex-50 cation-exchange resin from Dow Chemical Co., Midland, Mich.; bentonite clay from J. G. Holt, Iowa State University. A 2:1 clay. Nicollet loam from Iowa State University Agronomy Research Farm, Ames; approximately 38.5% sand, 34% silt, 24% clay, and 3.5% organic matter, by weight (oven dry). Florida river sand from the Little Econolahatchee River, Seminole county, Fla.; approximately 85% sand, 10% silt, 3% clay, and 2% organic matter, by weight (oven dry).

[b] Calculated by the method of Troeh and Frederick (23).

[c] Calculated as PFU per gram recovered divided by PFU per gram added, times 100.

The bacteriophages (AN31s-1, AN25s-1, and MS2) were eluted through columns of soil, soil components, and Dowex-50 cation-exchange resin, and the percent antigen eluted was determined by PHA assay. The elution of AN31s-1 and its assay by PHA are shown in Table 6. The vast majority of the bacteriophages adsorbed to Nicollet loam, but only a small fraction was eluted in a viable state. However, between 75 and 90% of the bacteriophage antigen was recovered, indicating that the bacteriophage was eluted with a reasonable efficiency but was being inactivated either by the adsorption process or by the elution procedure.

The high infectivity of bacteriophage eluates from the sands may be partially due to the low adsorption of bacteriophages to these solid phases. In both cases, more bacteriophage was inactivated than was believed to be adsorbed. This may be explained as due to the adsorption reaction attaining an equilbrium state, with some bacteriophage being adsorbed and desorbed in a noninfective state, to be replaced with other, infective bacteriophage, which may, in turn, be inactivated. This adsorption-inactivation-desorption cycle does not, however, indicate whether inactivation is caused by adsorption or desorption.

Moore et al. (17) studied the adsorption of several coliphages to soil particles and noted differences in the adsorption and recovery rates of the various coliphages in clay suspensions. To determine whether similar differences exist in the soil-resin systems studied here and whether these differences corresponded to morphological differences in the bacteriophages, two *Arthrobacter* bacteriophages and a coliphage, representing Bradley groups B, C, and E, were eluted through soil and resin columns, and the adsorption, inactivation, and infective recovery rates were compared (Table 7).

TABLE 6. *Elution of AN31s-1 from soil-resin systems and the effect of these systems on infectivity*

Solid phase	Cation-exchange capacity (meq/100 g)	Bacteriophage adsorbed (%)[a]	Bacteriophage adsorbed (antigen) (%)	Bacteriophage antigen eluted (%)	Infectivity (%)
Dowex-50	4.8×10^5	96	>90	>90	0.019
Bentonite	82.4	95	>90	>75 <90	0.05
Nicollet loam	68.5	93	>90	>75 <90	0.13
Florida river sand	20.5	12	>25 < 50	>90	44.3
Sea sand	8.2	9.5	>12.5 <25	>90	75.8

[a] PFU/g.

TABLE 7. *Elution of various bacteriophages from a Dowex-50 resin column*

Bacteriophage	Bradley group	Tail length (nm)	Bacteriophage PFU adsorbed (%)	Bacteriophage PFU eluted (%)
AN31s-1	B	124	96	0.019
AN25s-1	C	29	95	25.8
MS2	E	0[a]	92	46.0

[a] None observed (10).

The amount of bacteriophage adsorbed decreased slightly as the tail length of the bacteriophage decreased, although only the B versus E difference is statistically significant ($\lambda = 0.90$). The number of infective phages eluted from Dowex-50 and Nicollet loam was much higher for the group E (short or no tail) bacteriophage than for the long-tailed AN31s-1 bacteriophage. The correlation between tail length and bacteriophage inactivation was positive but did not appear to be linear in this case. The low number of bacteriophage types used and the variation in host cells, however, may have disguised any linear relationships.

The apparent association of long tails with bacteriophage inactivation inevitably leads to speculation as to the possibility of physical damage to the tail structures as the cause of inactivation, which appears all the more likely due to our inability to produce an inactivation-resistant strain of AN31s-1, despite our repeated attempts to select for this characteristic.

In an attempt to determine the effect of elution on bacteriophage tail structures, electron microscopy was used to visualize type B phages before, during, and after adsorption to clay and Dowex-50 resin. Approximately 50 bacteriophages were observed, and damage to tail structures was apparent in about 90% of the eluted phages. The observed damage consisted of breakage of tail structures, removal of terminal knobs from the tail, and a loss of phage deoxyribonucleic acid. The bacteriophages appeared to be intact while adsorbed to clays. Loss of nucleic acid from bacteriophage heads appears to accompany damage to tails upon elution, but the exact nature of the damage is uncertain.

It must be noted that the *Arthrobacter* bacteriophages isolated by Brown et al. (4) were tailed phages of Bradley groups B and C, whereas tailless bacteriophages were not encountered. Thus, it must be assumed that a tail structure confers a selective advantage to a bacteriophage, which outweighs the possibility of damage to this structure. Another, but more unlikely, possibility is that bacteriophage isolation techniques somehow select for tailed bacteriophages.

The nature of the observed damage to the bacteriophage tails indicates that actual physical damage is occurring as opposed to biochemical or conformational changes. Since positively charged amino acids at the bacteriophage tail may result in adsorption of the bacteriophage to the clay by its tail, shear forces caused by water and buffer movement through the bacteriophage-clay complex during elution would cause strain to the tail as the head moved in response to the current and the end of the tail remained stationary on the clay surface (unless instantly desorbed). Although the magnitude of these forces remains unknown and would, in any case, vary with the soil type, climate, and topography, it is apparently sufficient to cause damage to bacteriophage tails during laboratory elutions in columns and agitated soil-buffer suspensions.

Whether the shear forces present in laboratory elutions are equivalent to those found in the field is, of course, open to debate. However, the elution procedures used in laboratory elutions were as gentle as possible, and minimal agitation was used to suspend soil-buffer mixtures. Further, the flow of groundwater and surface runoff through the upper layers of soil during rainstorms is significant, as demonstrated by the large quantities of water involved and the volume of runoff from tile lines, particularly in well-cultivated areas. Since any attempt to isolate bacteriophages from soil must involve desorption of bacteriophage bound to clays by

their tails, the shear forces postulated resulting in activation of bacteriophage are invariably present in these schemes.

The efforts of many researchers to develop bacteriophage model systems to mimic the behavior of vertebrate viruses thus appear futile. Since no vertebrate viruses possess a tail structure and since vertebrate viruses have been demonstrated to survive for extended periods in soils, it is unlikely that the relatively short-lived bacteriophages could serve as effective models. The search for bacteriophage models is all the more difficult as the majority of the bacteriophages studied appear to be from Bradley groups A, B, and C, possessing tails of various lengths. If efforts to develop such model systems continue, it would seem only prudent to concentrate screening efforts on the tailless bacteriophages.

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