The Seasonal Distribution of Some Bacteriophages in the Akron Sewage Treatment Plant[1]

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ABSTRACT. **Bacteriophages are present in all human and animal sewage. However, environmental factors, such as the change in seasons, may affect the composition and viability of phages in sewage. The consequence of seasonal change (fall, winter, summer) on the isolation from raw sewage of bacteriophages specific for *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* were studied. The total number of phages isolated in each season varied little. However, the bacteriophage populations did vary concurrently with the change of seasons, with some phages isolated only in one season of the year (i.e., seasonal phage strains). There were many seasonal strains of *E. coli* phages (17 of the 43 isolated) and *Enterococcus* phages in the sewage (12 of the 15 isolated), but only a few seasonal *Pseudomonas* phages (3 of the 15 isolated). While the time of year that the seasonal phages were isolated varied, no season had the majority of the phage isolates. The present study indicates a seasonal distribution in the isolation of bacteriophages from sewage in Northeastern Ohio.**

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INTRODUCTION

Sewage is the most abundant source of bacteriophages in the environment. Many factors, such as geographic area, local climate, and contamination sources can affect the number and types of phages found in sewage (Britton 1987). Seasonal variation appears to have little effect on the total number of phages in sewage (Knott et al. 1974, Bell 1976), however there is no published study reporting the differences in the types or identity of the phages in different seasons. The goal of the present study was to address this deficiency by describing the seasonal distribution of some bacteriophages in the Akron Sewage Treatment Plant.

MATERIALS AND METHODS

Bacterial cultures used included 11 *E. coli*, nine *Enterococcus faecalis*, and five *Pseudomonas aeruginosa* clinical isolates obtained from the Akron Children's Hospital (Akron, OH). *Staphylococcus saprophyticus* (seven isolates) were isolated from rats. All of the cultures were maintained on trypticase soy agar slants.

Primary effluent samples in two gallon volumes were obtained from the Akron, OH, Sewage Treatment Plant on 3 September 1988 (fall), 12 January 1989 (winter), and 2 July 1989 (summer). The sewage samples were clarified by filtration through 15 layers of cheese cloth and microporous paper filters. To amplify the phages in the sewage, 100 ml of clarified sewage was added to a 250 ml flask containing 3 g of trypticase soy broth and 5 ml of an overnight bacterial culture. A separate flask was prepared for each bacterial isolate. The flasks were incubated overnight at 37° C. Phages in the flasks were detected by spotting 20 µl samples onto agar overlays (Adams 1959) using trypticase soy agar as bottom agar and trypticase soy broth with 0.7% agar as the soft agar overlay. The plates were incubated overnight at 37° C. The concentration of each amplified phage was determined by the agar overlay method using serial dilutions. After incubation, only plates with between 30 to 300 plaques where used and the plaque forming units (PFU)/ml determinations were rounded to the closest whole number. The mean PFU/ml for all of the phages specific for one bacterial genus in each season were determined. Phages isolated from each flask were purified by the single plaque pick procedure from agar overlay plates. The single plaque pick procedure involved removing one isolated plaque (by stabbing the plaque with the tip of a sterile Pasteur pipette) and placing the agar plug in fresh medium containing the appropriate host cells. The procedure was done three times and resulted in a purified phage isolate.

The host range of each purified phage isolate was determined by spotting 20 µl of the purified phage preparation onto separate agar overlays seeded with each bacterial strain corresponding to the genus of the phage group. The host range procedure was done in three independent trials. To assign a host range to each phage isolate, the most stringent criteria were used. If clearing of the host cells was seen in any of the three trials, the bacterium was considered a phage host. The host range results were used to establish the phage strains (i.e., phages with different host range results were considered unique strains). Therefore, each seasonal phage strain (isolated in only one season) had a unique host range.

RESULTS

Bacteriophages for *E. coli*, *P. aeruginosa*, and *Enterococcus faecalis* were isolated in all seasons. Phages specific for each isolate of *E. coli* and *P. aeruginosa* were present in all amplified sewage samples for each of the three seasons (Table 1). However, only four

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TABLE 1

Total number of phages isolated in each season.

Number of host strains	Number of phage isolates*		
	Fall	Winter	Summer
11 *Escherichia coli*	14	15	14
5 *Pseudomonas*	5	5	5
9 *Enterococcus*	4	7	4

*For some bacterial hosts, two phage types were recovered.

Enterococcus phages were isolated in the fall and summer, while seven *Enterococcus* phages were isolated in the winter (Table 1).

The mean amplified PFU/ml of the phages in the sewage was between 1 x 10^9 and 10 x 10^9 PFU/ml and varied little from season to season (Table 2). It must be stressed that the concentrations reflect the number of phages in the amplified sewage. These numbers presumably, but not absolutely, reflect the relative numbers of the phages in the original sewage samples. No phages active against *S. saprophyticus* were found in any sample for any of the seasons.

Phages isolated in one season only were of particular interest. The host range studies (Table 3) indicated that for each group of phages and for each season there were seasonal phage strains (i.e., phages isolated in one season only) except for the *Pseudomonas* phages in the summer. More seasonal phage strains were isolated in the winter (13 strains) than in the fall or summer (nine or ten, respectively). For each group of phages the percentage of seasonal phage strains in each season ranged from none (for the *Pseudomonas* phages in the summer) to 86% (for the *Enterococcus* phages in the winter) (Table 3).

DISCUSSION

The study of phage ecology, the interaction of phages with their environment, is in its infancy (Goyal 1987). One proposed consequence of phages in the environment is that the phages may affect the bacterial populations; i.e., when the phage numbers are high the bacterial numbers are low. This idea is supported by Wommack et al. (1992) in a study of the viruses in the Chesapeake Bay and is disputed by Hantula et al. (1991) investigating the phages in activated sludge of a sewage treatment plant. Obviously, the concentration of bacteria, and consequently of phages, in these two environments is drastically different, which may explain the conflicting results. Interestingly, a seasonal variation in the isolation of viruses was seen in the Chesapeake Bay study (Wommack et al. 1992) where more viruses were isolated in August and October.

In the present study, phages were isolated for *E. coli*, *Enterococcus*, and *Pseudomonas* in every season (Table 1). No *S. saprophyticus* phages were isolated. The failure to isolate these phages was probably the result of the host bacteria being isolated from rats, rather than the absence of the phages in the sewage. The PFU/ml of the phages isolated (Table 2) indicate very little difference in the total number of phages in different seasons, and agree with previous studies (Knott et al. 1974, Bell 1976). However, differences in the particular phage strains isolated between seasons were apparent (Table 3). Indeed, 80% (12 of the 15 isolates) of the *Enterococcus* phages, 39.5% (17 of the 43 isolates) of the coliphages, and 20% (3 of the 15 isolates) of the *Pseudomonas* phages were isolated in only one season. The isolation of a phage strain in only one season could be caused by temperature susceptibility of the phage, by a variation in the number of host bacteria in the sewage, or by a dilution effect of the sewage with rainfall. Regardless of the cause (which could vary for each phage type), results of the

TABLE 2

PFU/ml of phages in amplified sewage in each season.

	Coliphages	*Pseudomonas* Phages	*Enterococcus* Phages
Fall			
number*	14	5	4
PFU/ml range	4 x 10^7 - 2 x 10^{10}	1 x 10^7 - 3 x 10^{10}	1 x 10^8 - 2 x 10^{10}
PFU/ml mean	4 x 10^9	6 x 10^9	5 x 10^9
Winter			
number	15	5	7
PFU/ml range	4 x 10^2 - 3 x 10^{10}	1 x 10^5 - 2 x 10^{10}	1 x 10^4 - 5 x 10^{10}
PFU/ml mean	6 x 10^9	4 x 10^9	1 x 10^{10}
Summer			
number	14	5	4
PFU/ml range	2 x 10^7 - 8 x 10^9	2 x 10^6 - 5 x 10^9	1 x 10^9 - 1 x 10^{10}
PFU/ml mean	2 x 10^9	1 x 10^9	8 x 10^9

*Number of phage isolates in each season.

TABLE 3

Number of common and seasonal phages in each season.[a]

	Fall		Winter		Summer	
	Common[b]	Seasonal[c]	Common	Seasonal	Common	Seasonal
Coliphages	9	5 (36)[d]	10	5 (33)	7	7 (50)
Pseudomonas phages	4	1 (20)	3	2 (40)	5	0 (0)
Enterococcus phages	1	3 (75)	1	6 (86)	1	3 (75)

[a]Determined from host range studies done in triplicate.
[b]Phages isolated in one or more season; i.e, common strains.
[c]Phages isolated in only one season; i.e., seasonal strains.
[d]Percentage of the total phages isolated per season that are seasonal phages.

present study indicate there are seasonal distributions in the particular phage strains, but not in total number of phages, isolated from raw sewage in Northeastern Ohio. To determine whether or not these phages effect the phage ecology of the sewage treatment plant in different seasons, further studies must be done. These studies would require the isolation of the phages and their bacterial hosts from the sewage and a comparison of their numbers in multiple samples in various seasons.

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