

Adsorption of Coliphages T1 and T7 to Clay Minerals

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Coliphages T1 and T7 of *Escherichia coli* were absorbed by kaolinite (K) and montmorillonite (M). Maximum adsorption of T7 (96%) to M was greater than that of T1 (84%), but the adsorption of both coliphages to K was the same (99%). Positively charged sites (i.e., anion exchange sites) on the clays appeared to be primarily responsible for the adsorption of T1 to K but only partially responsible for the adsorption of T1 to M; equilibrium adsorption isotherms of T1 to K and M did not show a correlation between adsorption and the cation exchange capacity of the clays, and the reduction in adsorption caused by sodium metaphosphate (a polyanion that interacts with positively charged sites on clay) was more pronounced with K than with M. The equilibrium adsorption isotherms of T7 to K and M suggested a correlation between adsorption and the cation exchange capacity of the clays. However, studies with sodium metaphosphate indicated that T7 also adsorbed to positively charged sites on the clays, especially on K. Adsorption of the coliphages to positively charged sites was greater with K than with M, probably because the ratio of positively charged sites to negatively charged sites was greater on K than on M.

Clay minerals appear to affect the survival of viruses in natural habitats (1, 5, 13). The adsorption of viruses to clay minerals may protect the viruses against biological and abiological inactivation or degradation, thereby enabling them to persist in soils, sediments, and waters in the absence of their hosts. The adsorbing capability of clays for viruses is one of the bases for using soil as a "living filter" for the presumed removal of viruses from municipal and industrial sewage effluents and sludges (2, 9, 21, 33). Soils with a high clay content appear to have a high adsorptive capacity for viruses (2, 7). For example, Drewry and Eliassen (4) showed a direct correlation between the clay content of nine soils from Arkansas and California and their retention of bacterial viruses.

The association of viruses with clay minerals has been attributed to the large surface area and ion exchange capacity of clays (1, 6, 7, 9, 27, 30). Mechanisms involved in the adsorption of viruses to clay minerals have been suggested, but the exact mechanisms have not been defined. Carlson et al. (3) proposed that polyvalent cations in the environment serve as bridges between anionic groups on the virus and negatively charged sites on the clay. Theng (33) suggested that "water bridging" (a type of cation bridging in which the anionic groups of the clay and virus particles form hydrogen bonds with the water molecules in the primary hydration shell around

charge-compensating cations) affects virus adsorption to clay. Schaub et al. (20) suggested that adsorption between viral particles and clay minerals occurs as a result of secondary bonding (e.g., physical adsorption resulting from van der Waals forces and hydrogen bonding). Lipson and Stotzky (Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, N51, p. 188) and Stotzky et al. (30) showed that the adsorption of reovirus type 3 to clays was correlated with the cation exchange capacity (CEC) of the clays, whereas the adsorption of *Herpesvirus hominis* type 1 was not correlated with the CEC of the clays (30; Yu and Stotzky, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, N53, p. 188).

In the present paper, quantitative data on the adsorption of coliphages (bacterial viruses infecting *Escherichia coli*) T1 and T7 to the hydrous aluminosilicate clay minerals kaolinite (K) and montmorillonite (M) are presented, and possible physicochemical factors involved in the process are discussed.

MATERIALS AND METHODS

Clays. K (kaolin, Fisher Scientific Co.), a two-layer (1:1, silica-alumina) nonexpanding clay, had a CEC of 5.8 meq/100 g of oven-dried clay (29), a particle size distribution of approximately 98% < 8 μm , 52% < 2 μm , and 3% < 0.5 μm (29), and a surface area of approximately 10 m²/g (26). M (bentonite, Fisher Scientific Co.), a three-layer (2:1) expanding clay, had a CEC of 97.7 meq/100 g of oven-dried clay (29), a particle size distribution of approximately 100% < 8 μm , 34% < 2 μm , and 3% < 1 μm (29), and a surface

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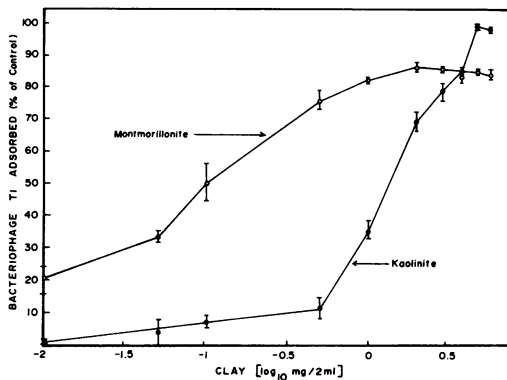


FIG. 1. Adsorption of coliphage T1 (10^6 to 10^7 PFU) to various concentrations of kaolinite or montmorillonite. (\log_{10} mg/2 ml: 0.01 mg = -2.0; 0.05 mg = -1.3; 0.1 mg = -1.0; 0.5 mg = -0.3; 1 mg = -0.0; 2 mg = 0.3; 3 mg = 0.48; 4 mg = 0.6; 5 mg = 0.7; and 6 mg = 0.78.)

area of approximately 800 m²/g (26). Although the anion exchange capacity (AEC) of these clays was not determined, Grim (11) has indicated that the average ratio of AEC to CEC is about 2 for K and 0.15 for M.

Stock suspensions of K and M, consisting of 33 mg of clay per ml of distilled water, were autoclaved and stored at 4°C. Autoclaving does not alter the X-ray characteristics of the clays (28). The clay concentrations used were obtained by dilution of the stock clay suspensions with distilled water (pH 7.1), followed by centrifugation at $7,710 \times g$ for 10 min (Sorvall RC2-B, SS34 rotor). The clay pellets were then suspended in 1 ml of distilled water or in 1 ml of a sodium metaphosphate (SMP; $(\text{NaPO}_3)_{13}$, Fisher Scientific Co.) solution containing from 0.5 to 10% SMP.

Bacteriophages. Coliphage T1, which infects *E. coli* B, was isolated from the sewage treatment plant at Randall's Island, New York, by a standard enrichment technique (25) and identified as coliphage T1 on the bases of morphology (i.e., electron microscopy and size of plaques) and ability to lyse *E. coli* B but not *E. coli* B/1,5. Coliphage T7, which infects both *E. coli* B and B/1,5, was obtained from Martha Baylor (State University of New York at Stony Brook).

Polyethylene glycol (molecular weight = 6,000) was used to concentrate the coliphages from 2-liter volumes of bacterial lysates (35, 37). The stocks of concentrated coliphages (T1 = 10^{11} PFU/ml; T7 = 10^{10} PFU/ml) were stored in Tris buffer (0.001 M Tris [Sigma Chemical Co.], 0.001 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.001 M NaCl; pH 7.4) at 4°C for several months without appreciable loss in titer.

A standard soft agar overlay plaquing technique (25) was used to assay the coliphage titers.

Experimental procedures. (i) **Equilibrium adsorption.** A 1-ml suspension of coliphage (10^6 to 10^7 PFU) in Tris buffer was added to 1-ml volumes of distilled water containing various concentrations of K or M. The clay-coliphage mixtures were mixed (Genie Jr.) for 5 s, placed in a rotating wheel (34 rpm; Kraft Apparatus, Inc.) for 10 min at 30°C, and centrifuged for 10 min at $7,710 \times g$; the supernatants were then decanted, and

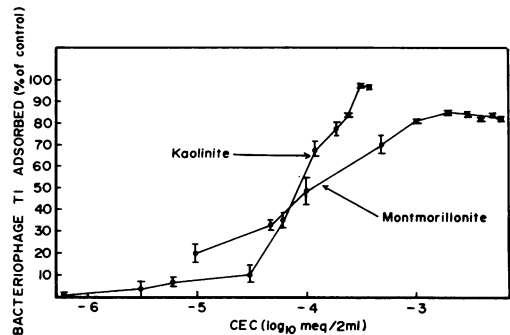


FIG. 2. Adsorption of coliphage T1 (10^6 to 10^7 PFU) to clays as a function of CEC (expressed in \log_{10} milliequivalents) of various concentrations of K and M.

the numbers of coliphages in the supernatants were determined. Preliminary studies showed that maximum adsorption of T1 and T7 to the clays occurred after 10 min of mixing. As a control, 1 ml of the coliphage suspension in Tris buffer was mixed with 1 ml of distilled water and given the same treatment as the clay-coliphage mixtures.

The difference between the number of infective coliphages recovered in the control tubes and the number in the initial supernatants of the clay-coliphage mixtures was designated as the amount of coliphage adsorbed at equilibrium to the clays. Inasmuch as various amounts of the coliphages (10^6 to 10^7 PFU) were used in repeated experiments, the amounts of coliphages adsorbed to the clays were expressed as a percentage of the amounts of coliphages recovered in the control tubes. The loss (<20%) in coliphage titers resulting from both adsorption of the coliphages to the glass tubes and their destruction during the experimental procedure was normalized by expressing the data on the basis of the coliphage titers recovered in the control tubes rather than on the basis of the coliphage titers added initially.

Experiments were repeated 3 to 10 times, and there were at least three replicates per experiment. All coliphage titrations were performed in duplicate.

(ii) **Effect of SMP on adsorption of coliphages to clays.** The desired SMP concentrations were obtained by diluting an autoclaved stock solution, containing 10 g of SMP/100 ml of distilled water, with distilled water. A 2- or 5-mg amount of K or M was suspended in 1 ml of an SMP solution, mixed for 5 s, placed in a rotating wheel (34 rpm) for 5 min at 30°C, and centrifuged at $7,710 \times g$ for 10 min; the supernatant (SMP) was then discarded, and the clay pellet was suspended in 1 ml of distilled water. When washing of the SMP-treated clay was desired, the resuspended clay was washed twice with 1 ml of distilled water (with centrifugation at $7,710 \times g$ for 10 min after each wash). For the control, 1 ml of each SMP solution was added to test tubes without clay that were subjected to the same mixing and centrifugation procedures as tubes containing SMP-treated clays; the SMP supernatant was discarded, and 1 ml of distilled water was added to the tubes. A 1-ml suspension of coliphage (10^6 to 10^7 PFU) in Tris buffer was added to washed or unwashed SMP-pre-

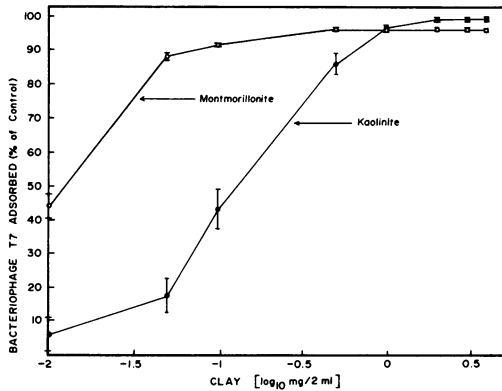


FIG. 3. Adsorption of coliphage T7 (10^6 to 10^7 PFU) to various concentrations of K or M.

treated clay suspensions and to the control tubes. The mixtures were treated, and the amount of adsorption was determined as described above. The percentage of adsorption of the coliphages to SMP-pretreated clays was compared with the percentage of adsorption of equal titers of coliphages to equal concentrations of clays not pretreated with SMP. The studies with SMP-treated clays were repeated two to four times, with three replicates per experiment. All coliphage titrations were performed in duplicate.

The data were expressed as the mean \pm standard error of the mean ($\bar{X} \pm$ SEM), and control and experimental values were compared by Student's *t*-test; $P < 0.05$ was assumed to be statistically significant. Statistical computations were performed with a Tektronic 31 programmable calculator.

RESULTS

Equilibrium adsorption. (i) Coliphage T1. At clay concentrations ranging from 0.01 to 3 mg/2 ml, M adsorbed more T1 than did K (Fig. 1). Maximum adsorption (84%) of T1 to M occurred at a concentration of 2 mg/2 ml; comparable adsorption to K required 4 mg/2 ml. At higher clay concentrations (4 to 6 mg/2 ml), however, K adsorbed more T1. The slope of the adsorption isotherm with M was relatively constant between 0.05 and 2 mg/2 ml, and when all of the adsorbable T1 was adsorbed, the slope decreased and was followed by a plateau. Increasing the M concentration from 2 to 6 mg/2 ml resulted in no further adsorption of T1.

The adsorption of T1 to K was most pronounced above 0.5 mg/2 ml. Maximum adsorption (99%) occurred at 5 mg/2 ml, and further increases in the concentration of K did not result in any additional adsorption.

The adsorption of T1 to K and M was not correlated with the CEC of the clays (Fig. 2). When the concentrations of the clays were converted to CEC (CEC of the clays \times concentration of the clays/2 ml), two distinct equilibrium

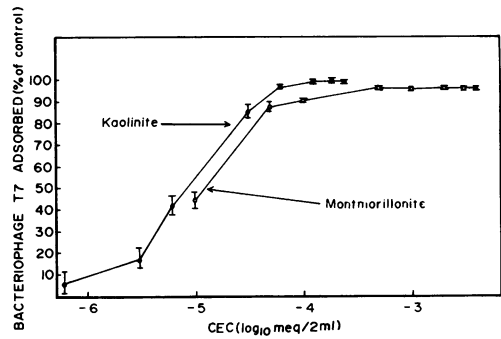


FIG. 4. Adsorption of coliphage T7 (10^6 to 10^7 PFU) to clays as a function of CEC (expressed in log₁₀ milliequivalents) of various concentrations of K and M.

adsorption isotherms were obtained. If the adsorption of T1 correlated with the CEC, a single adsorption isotherm should have been obtained.

(ii) Coliphage T7. At clay concentrations of 0.01 to 0.5 mg/2 ml, more T7 was adsorbed to M than to K (Fig. 3). Maximum adsorption (96%) of T7 to M occurred at a concentration of 0.5 mg/2 ml; comparable adsorption to K required 1 mg/2 ml. At higher clay concentrations (1 to 4 mg/2 ml), slightly more, but statistically significant, T7 was adsorbed to K. As the concentration of M was increased from 0.01 to 0.5 mg/2 ml, the increase in adsorption of T7 to M was greater than that of T1. The adsorption isotherm with M, especially between 0.01 and 0.05 mg/2 ml, reflected the increase in adsorption of T7 as the concentration of M was increased. Above 0.5 mg/2 ml, a plateau in the isotherm occurred, indicating that all of the adsorbable coliphage had been adsorbed.

As the concentration of K was increased from 0.01 to 2 mg/2 ml, the increase in adsorption of T7 was greater than that of T1 (Fig. 3). The adsorption of T7 to K was most pronounced above a 0.05 mg/2 ml-concentration of K. Maximum adsorption (99%) occurred at 2 mg/2 ml, and further increases in K did not result in additional adsorption.

The adsorption of T7 to K and M showed some relation to the CEC of the clays (Fig. 4). When the concentrations of the clays were converted to CEC, the two equilibrium isotherms, although not overlapping and resulting in a single isotherm, had a similar pattern and were very close.

Effect of SMP on adsorption of coliphages to clays. (i) Coliphage T1. Pretreating 2 or 5 mg of K or M with SMP reduced the adsorption of T1. The effect of SMP was dependent on its concentration and was more pronounced with K (Fig. 5); e.g., 2 mg of untreated K or M adsorbed 69%

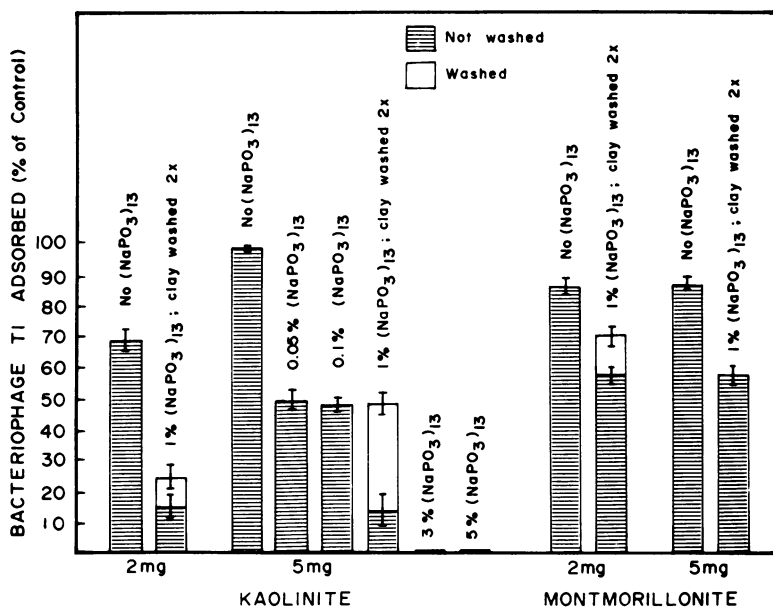


FIG. 5. Adsorption of coliphage T1 (10^6 to 10^7 PFU) to 2 or 5 mg of K or M treated with SMP [(NaPO₃)₁₃].

or 86%, respectively, whereas 2 mg of K or M pretreated with 1% SMP adsorbed 15% or 58%, respectively. Hence, SMP reduced the adsorption of T1 to 2 mg of K by 54% and to 2 mg of M by 28%. Pretreating 5 mg of K with various concentrations of SMP also reduced the adsorption of T1: 0.05% SMP to 49%, 0.1% SMP to 48%, 1.0% SMP to 13%, and 3.0% and 5.0% SMP to 0%; 5 mg of untreated K adsorbed 99%. The adsorption of T1 to 5 mg of M was reduced by pretreatment with 1.0% SMP to only 58% from 87% with 5 mg of untreated M; hence, 1.0% SMP was more effective in reducing the adsorption of T1 to 5 mg of K (reduced by 86%) than to 5 mg of M (reduced by 29%). Washing the clays pretreated with 1.0% SMP twice with distilled water increased the subsequent adsorption of T1, with the exception of 5 mg of pretreated M. Washing the clays apparently removed some of the clay-associated SMP.

(ii) **Coliphage T7.** Pretreating 2 mg of K or M with various concentrations of SMP reduced the adsorption of T7, with the effect being dependent on the SMP concentration and more pronounced with K (Fig. 6). Adsorption to K was reduced from 99% as follows: 0.05% SMP to 87%, 0.1% SMP to 81%, 1.0% and 3.0% SMP to 61%, 5.0% SMP to 53%, and 10.0% SMP to 45%. Adsorption to M was reduced from 96% as follows: 0.1% SMP to 79%, 1.0% SMP to 78%, and 5.0% SMP to 72%. As with T1, SMP was more effective in reducing the adsorption of T7 to K than to M. Washing the clays pretreated

with 1.0, 3.0, and 5.0% SMP twice with distilled water removed some of the inhibitory effect of the polyanion on the subsequent adsorption of T7.

Although SMP inhibited the adsorption of both coliphages to both clays (more so to K than to M), it reduced the adsorption of T1 significantly more than that of T7.

DISCUSSION

At the pH at which these studies were conducted (pH 6.8), K and M (18, 20, 27) and coliphages T1 and T7 (17, 34) had a net negative charge, although they also had some positively charged sites on their surfaces. Consequently, little or no adsorption of the coliphages to the clays would be expected as a result of the electrostatic repulsion between the particles. However, the coliphages showed appreciable adsorption to K and M, probably to AEC sites.

Adsorption of coliphage T1. There was no correlation between the amount of adsorption of T1 and the CEC of the clays (Fig. 2). At clay concentrations of 4 to 6 mg/2 ml, K, with approximately 1/17 the CEC of M, adsorbed more T1 than did equal weights of M, indicating that sites on K and M other than cation exchange sites were involved in the adsorption of T1. Inasmuch as both K and M exhibit anion exchange reactions (11), T1 probably adsorbed to positively charged sites on the clays. This was consistent with other reports that indicated that some proteins (12, 27), polysaccharides (19), and

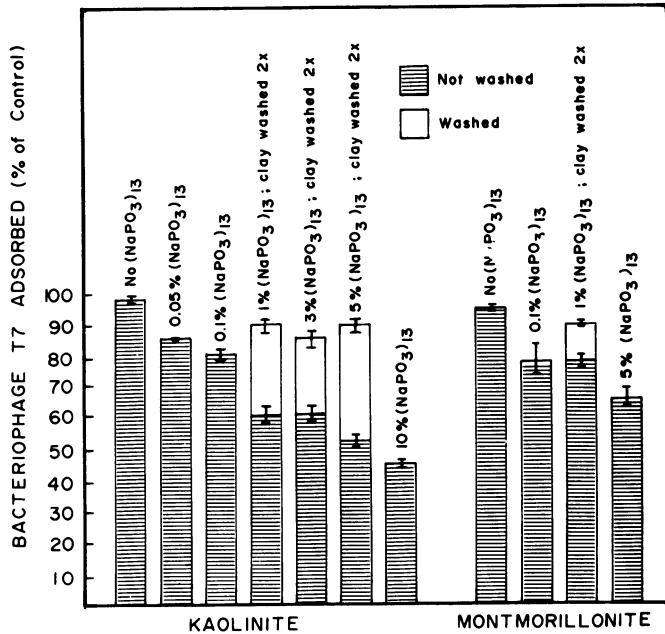


FIG. 6. Adsorption of coliphage T7 (10^6 to 10^7 PFU) to 2 mg of K or M treated with SMP [(NaPO₃)₁₃].

microbial cells (14, 15, 16) adsorb to clays via anion exchange reactions.

The greater adsorption of T1 to K than to M, both of which have an approximately equal AEC (11), at the higher concentrations of clay (4 to 6 mg/2 ml) may have been related to the distribution of charge on K and M; because K has a larger AEC/CEC ratio than M (ca. 2 versus 0.15 [11]), the negatively charged T1 particles were able to approach positively charged sites on K more easily than on M, which was more negative. It is unclear, however, why T1 did not show this greater adsorption to K also at lower concentrations of the clays. One possibility is that more AEC sites were available on M at lower concentrations, when the M was more disperse, than at higher concentrations, when increased interactions between faces (negatively charged) and edges (positively charged) reduced the number of available AEC sites and rendered the M aggregates more relatively negative, therefore increasing the electrostatic repulsion between M and T1 (36). Comparable face-to-edge interactions at higher concentrations of K would not have had the same effect, as the CEC of K was only 1/17 that of M, and the AEC/CEC ratio of K was about 13 times higher than that of M. Sykes and Williams (31) also suggested that positively charged sites on clay were responsible for the greater adsorption of an actinophage that infect *Streptomyces* spp. to K than to equal weights of M.

Pretreatment of K and M with the polyanionic salt SMP, which binds to positively charged sites on clays (14, 16, 22, 32), reduced the adsorption of T1, further indicating that T1 was adsorbed primarily to anion exchange sites. Inasmuch as the reductions were dependent on the concentration of SMP and were more pronounced with K, positively charged sites were apparently the major sites on K to which T1 adsorbed and were only partially responsible for adsorption of T1 to M. The adsorption of some T1 particles even in the presence of SMP (more so to M than to K) may have resulted from mechanisms other than anion exchange, such as cation exchange, hydrogen bonding, and van der Waals forces. Citrate, which also binds to positively charged sites on clay, reduces the adsorption of yeast RNA, which is presumably adsorbed via its negatively charged orthophosphate groups to K almost completely, but only 55% to M (8).

Adsorption of coliphage T7. The amounts of T7 adsorbed showed some correlation with the CEC of the clays (Fig. 4). However, the statistically significant greater adsorption to K than to M at clay concentrations of 1 to 4 mg/2 ml and the reduction in adsorption after pretreatment of the clays with SMP suggested that positively charged sites on the clays were also involved. As with T1, the reduction in the adsorption of T7 by SMP was greater with K, presumably because the ratio of positively to negatively charged sites was greater on K than on M, and

the adsorption of T7 may have also involved hydrogen bonding and van der Waals forces. However, pretreatment of the clays with SMP did not reduce the adsorption of T7 to the same extent as that of T1, which showed no correlation between CEC and adsorption.

Positively charged sites on clay also appear to be involved in the adsorption of bacteriophage R17 and reovirus type 3 to the amorphous clay mineral allophane at the pH of many fresh waters and soils (pH 5 to 7) (32). The adsorption of viruses to positively charged sites on solid surfaces has also been applied in the development of positively charged microporous filters for the concentration of poliovirus type 1 strain LSc and coliphages MS-2, ϕ -174, T2, and T4 from large volumes of sewage, tap water, and lake water at ambient pH levels (7.0 to 7.5) (10, 23, 24).

The results of these studies indicated that the mechanism and sites of adsorption differ for coliphages T1 and T7 and for M and K and that adsorption is influenced by such characteristics of the clays as AEC, CEC, and AEC/CEC ratios. The differences in adsorption between the coliphages may have been the result of differences in the amino acid composition of their capsomer proteins (6, 9) since T1 and T7 have been shown to differ in amino acid composition (25).

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