The Effect of pH on Soil Actinophage

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(Received 28 April 1980; revised 5 September 1980)

Actinophage active against both neutrophilic and acidophilic streptomycetes were isolated from a variety of soils by a specific enrichment method. No phage were detected in soils with a pH below 6.0, despite the presence of acidophilic streptomycetes in these soils. However, some phage isolated from neutral soils were able to lyse acidophilic streptomycetes at pH 5.5. Selected actinophage were shown to be stable between pH 5.5 and 9.0 in sterile soils and broth, but were rapidly inactivated at lower or higher pH values. Survival of these phage was good in neutral soils, but negligible in acidic soils. This suggested that free phage were unable to remain infective in acidic soils, thus at least partially accounting for the failure to isolate them. Acidity was shown to have variable effects on several stages of phage replication including adsorption, penetration and the length of the latent period. The latter effect was directly related to the metabolic activity of the host.

INTRODUCTION

Acidity is an important factor governing the activity and survival of streptomycetes in soil. A survey of the pH requirements of streptomycetes isolated from two acid soils (Williams *et al.*, 1971) revealed that most species could be categorized as neutrophiles or acidophiles. Neutrophilic species grew between pH 5.5 and 8.5 with an optimum around pH 7.0, whilst acidophiles grew between pH 3.5 and 6.5 with optimum growth around pH 4.5. Khan & Williams (1975) demonstrated the presence of acidophilic actinomycetes in 17 acid soils; the majority of these were streptomycetes. It was concluded that acidophilic actinomycetes are widespread and may play important roles in acid soils. Studies on the effect of pH on growth rate and enzyme production in laboratory conditions further underlined the distinction between acidophilic and neutrophilic streptomycetes (Flowers & Williams, 1977; Williams & Flowers, 1978).

Phage can attack a wide range of hosts inhabiting diverse environments. Patel (1969) demonstrated the presence of phage of the thermophile *Thermoactinomyces vulgaris* in composts and showed it to be capable of survival and activity at elevated temperatures. Phage for *Bacillus stearothermophilus* (Saunders & Campbell, 1966) and an extreme thermophile *Thermus thermophilus* (Sakaki & Oshima, 1975) have also been reported. Phage capable of replication on salt-tolerant organisms and halophiles have also been detected (Zachary, 1974; Torsvik & Dundas, 1974). However, acid-tolerant organisms, such as lactobacilli and lactic streptococci, are infected by phage, but always at pH values close to neutrality. The aim of this study was to determine if actinophage in soil are adapted to low pH as are some of their potential hosts.

METHODS

Soils. Details of soils screened for actinophage are given in Table 1. The constituent horizons of the two podzolic soils (Freshfield and Delamere) were analysed separately. The presence of actinomycetes had been demonstrated previously in all soils and studied in detail in the podzolic samples (Davies & Williams, 1970; Khan

& Williams, 1975) and in the salt lake soil (Taber, 1959). Soil samples were stored at 4 °C in polyethylene screw-cap bottles for up to 7 d. with the exception of the salt lake soil, which was stored for 8 years. The pH of soil samples was determined with a glass electrode (Reed & Cummins, 1945).

Streptomyces. Five neutrophilic and three acidophilic streptomycetes were used for phage isolation. The neutrophilic isolates were A29 (from an arable soil, pH 7.2), F6, F13 (Davies & Williams, 1970) and C5 (Watson & Williams, 1974) (from Freshfield pine forest soil, C horizon, pH 7.8) and M8 (Mayfield *et al.*, 1972) (from a deciduous woodland soil, pH 6.0). The acidophilic isolates were H1 (from Freshfield pine forest soil, F₂/H horizon, pH 3.9), SW53 (from coal mine waste, pH 3.9) and SW72 (from coal mine waste, pH 4.5) (Khan & Williams, 1975). Cultures were grown on oatmeal agar (Shirling & Gottlieb, 1966) at 25 °C for 14 d. The medium was adjusted to pH 7.0 for neutrophiles and pH 5.5 for acidophiles. Spore suspensions were prepared by transferring surface growth into 10 ml sterile 20% (v/v) glycerol, agitating for 5 min and filtering through sterile cotton wool to remove mycelium.

Phage isolation and enumeration. The following specific enrichment method for phage isolation was used. Soil samples (25 g) were added to flasks containing 50 ml nutrient broth (CM1, Oxoid), adjusted to pH 5.5 for acidophiles or to pH 7.0 for neutrophiles. Each flask was inoculated with a streptomycete spore suspension to give a final concentration of approximately 1×10^7 colony-forming units (c.f.u.) ml⁻¹ and incubated for 16 h at 25 °C on an orbital shaker operating at 200 rev. min⁻¹. The suspension was then centrifuged at 600 g for 15 min, and the supernatant fluid was passed through 0.45 µm pore size Millipore filters and assayed for phage by the double-layer method (Adams, 1959).

For the phage assay using neutrophilic hosts, the base layer consisted of nutrient agar (CM3, Oxoid), pH 7.0; for acidophilic hosts, it consisted of glycerol/asparagine agar (Pridham & Lyons, 1961), pH 5.5. Basal layers were dried at 65 °C for 20 min and air-cooled. The top layers consisted of nutrient broth or glycerol/asparagine broth with 0.8% (w/v) agar (L13, Oxoid) seeded with the host spore suspension. Neutrophile assay plates were incubated for 16 h and acidophile assay plates for 48 h, both at 25 °C.

Phage purification and storage. Single plaques were removed from the isolation plates with a Pasteur pipette, placed in 5 ml nutrient broth and vigorously shaken for 1 to 2 min. The extract was filter-sterilized and assayed for plaque-forming ability. This purification cycle was repeated twice before high titre stocks were prepared from single plaques.

Stock phage suspensions were prepared by inoculating nutrient agar base layers with 1 ml purified phage [approximately 1×10^4 plaque-forming units (p.f.u.) ml⁻¹], overlaying with 0.6% (w/v) nutrient agar seeded with host spores and incubating at 25 °C for 16 h. The confluent lysed plates were flooded with 5 ml nutrient broth and allowed to stand at room temperature for 2 h. Lysates containing approximately 5×10^9 p.f.u. ml⁻¹ were collected, filtered and stored at 4 °C.

Phage morphology. High titre suspensions of two phage (f6 and f13) in 0.1 M-ammonium acetate were stained with 2% (w/v) phosphotungstic acid and examined with an electron microscope (Corinth 500, A.E.I.) at 60 kV.

Host-range. Phage isolated from soil were tested on a range of neutrophilic and acidophilic streptomycetes. Nutrient broth (CM1, Oxoid) containing 0.6% (w/v) agar was seeded with a host spore suspension, poured over a nutrient agar base layer and allowed to solidify. The pH of the media in both layers was adjusted to pH 7.0 for assay on neutrophiles and pH 5.5 for acidophiles. Spots of phage suspension in nutrient broth (appropriately diluted to yield single plaques) were inoculated on to the surface layer (eight phage isolates per plate) and plates were air-dried in a laminar flow cabinet. Neutrophile assay plates were incubated for 16 h at 25 °C and acidophile assay plates for 48 h at 25 °C, before plaques were noted.

Effect of pH on stability of phage. Addition of phage f6 and f13 to a range of different buffers and media at pH 7.0 for 1 h at 25 °C showed that optimum survival (99 to 100%) was obtained in glucose/Casamino acids/proline (G.C.P.) broth (Flowers & Williams, 1977). This medium was therefore used to test the effect of pH on stability. Flasks containing 20 ml G.C.P. broth were adjusted to the selected pH in the range 4.0 to 11.0 with 0.1 M-HCl or 0.1 M-KOH and allowed to equilibrate for 15 min at room temperature. The pH was readjusted if necessary. Phage suspension was added to each flask to give a final concentration of approximately 5×10^4 p.f.u. ml⁻¹. Flasks were incubated at 25 °C on an orbital shaker operating at 60 rev. min⁻¹ for 24 h. The pH of the suspension was checked before and after the experiment. Samples were withdrawn at intervals and assayed for phage by the double-layer method using nutrient agar basal and top layers at pH 7.0. Death rates were calculated from the slopes obtained by regression of log₁₀ (surviving phage) against time for each pH value.

Soils (Table 1) were also used to determine the effect of pH on the stability of phage f6 and f13. Soils were air-dried at room temperature for 96 h and homogenized to remove large aggregates. The moisture-holding capacity and pH of the dried soils were determined. Samples (30 g) of soil were placed in flasks and a phage suspension of known titre in nutrient broth, pH 7.0, was added to each flask to give a final moisture content of 75% moisture-holding capacity and a phage concentration of approximately 1×10^6 p.f.u. (g soil)⁻¹. Immediately after the addition of phage (*t*₀) and subsequently at 3 min and 200 h, two 1 g samples were transferred to flasks containing 10 ml nutrient broth, pH 7.0. These were placed on an orbital shaker operating at 200 rev. min⁻¹ for 30 min. The pH of the extract was checked frequently and readjusted to neutrality with 0.1 M-KOH (especially with the acid soils). The extract was assayed for phage.

Table 1. *Phage isolated by specific enrichment from soils of different pH values*

Soil	Location	Plant cover	Main features	pH value	No. of streptomycete species yielding phage		
					Neutrophiles (5 species)	Acidophiles (3 species)	
Podzol (F ₁ horizon)	Freshfield, Merseyside	<i>Pinus nigra</i>	Partially decomposed leaf litter	3.7	0	0	
Podzol (F ₂ /H horizon)	Delamere, Cheshire	<i>Pinus sylvestris</i>	Decomposed leaf litter and humus	3.8	0	0	
Podzol (B ₁ /B ₂ horizon)	Delamere, Cheshire	<i>Pinus sylvestris</i>	Sandy with some humus	3.8	0	0	
Podzol (F ₂ /H horizon)	Freshfield, Merseyside	<i>Pinus nigra</i>	Decomposed leaf litter and humus	3.9	0	0	
Podzol (A ₁ /A ₂ horizon)	Delamere, Cheshire	<i>Pinus sylvestris</i>	Sandy with some humus	4.0	0	0	
Podzol (A ₁ horizon)	Freshfield, Merseyside	<i>Pinus nigra</i>	Sandy with high humus content	4.2	0	0	
Clay	Wirral, Merseyside	None	Heavy clay, little organic matter	6.1	5	NT	
Garden soil	Liverpool, Merseyside	<i>Rosa</i> spp.	Cultivated loam	7.0	5	NT	
Riverbed soil	Castletown, Isle of Man	None	Saline silt, with organic matter	7.2	0	NT	
Peat	Parkgate, Cheshire	<i>Scirpus maritimus</i>	Organic soil	7.4	4	NT	
Podzol (C horizon)	Freshfield, Merseyside	<i>Pinus nigra</i>	Sandy with little organic matter	7.8	5	0	
Saline sand	Parkgate, Cheshire	<i>Scirpus maritimus</i>	Saline sand from salt marsh	8.0	NT	NT	
Salt lake soil	Misiki Lake, Saskatchewan, Canada	Grass	Saline loam bordering salt lake	8.3	4	NT	

NT, Not tested.

pH requirements of hosts used in adsorption and infectivity tests. Streptomycetes with different pH requirements for growth were selected (Table 2): F6 and F13 were the propagation hosts of phage f6 and f13, respectively. Host spore suspension (0.5 ml) was added to flasks containing 35 ml G.C.P. broth buffered to a range of pH values. The total number of spores in each flask was counted, using a Thoma counting slide, before incubation at 25 °C on an orbital shaker operating at 100 rev. min⁻¹. When spores had germinated, 3 ml host culture were transferred to a Clarke-type polarographic electrode (Rank Bros., Cambridge) and allowed to equilibrate for 5 min with constant agitation to ensure oxygen saturation. The electrode was sealed and the ambient temperature was maintained at 25 °C. The concentration of germinated spores in the electrode was calculated by comparing the percentage of spores germinated before and after testing. Respiration rates expressed as mV deflection per 10⁶ germinated spores min⁻¹ were plotted against pH values, and the pH optimum and range were calculated for each streptomycete (Table 2).

Effect of pH on adsorption of phage. Flasks containing 20 ml G.C.P. broth were adjusted to the optimum pH for each of the four hosts, inoculated with spores to give a concentration of approximately 5×10^7 c.f.u. ml⁻¹ and incubated at 25 °C. The number of germings was determined using a Thoma counting slide. The suspensions were adjusted to the desired pH with 0.1 M-HCl or 0.1 M-KOH and phage were added (0.01 to 0.05 phage per germling). At intervals, 2 ml of the suspension was removed, membrane-filtered and appropriately diluted. The filtrate was assayed for phage by the double-layer method using nutrient agar basal and top layers adjusted to pH 5.5 or 7.0, and incubated at 25 °C. The minimum possible pH for phage stability was 4.9 (f13) or 4.4 (f6). Ungerminated spores were also tested.

Adsorption was initially linear, as determined by measurement of percentage adsorption against time, and then it declined: 18 min was selected as a suitable period for adsorption for all host/phage combinations except H1/f13, where 10 min was appropriate. Adsorption rates of those host/phage combinations shown to give lysis in host-range tests were studied at the selected period over a range of pH values. Adsorption was expressed as K , the adsorption velocity constant (Adams, 1959):

$$K = \frac{2.3}{Bt} \times \log_{10} \left(\frac{P_0}{P_t} \right)$$

where B is the bacterial concentration (c.f.u. ml⁻¹), P_0 is the initial phage concentration (p.f.u. ml⁻¹), P_t is the phage concentration at t min (p.f.u. ml⁻¹) and t is the period of adsorption (18 min, except 10 min for H1/f13).

Effect of pH on overall replication. Flasks of G.C.P. broth buffered at different pH values were seeded with host spores to give a concentration of approximately 5×10^7 c.f.u. ml⁻¹ and phage to give a concentration of 0.01 to 0.05 phage per spore. Flasks were incubated for 18 h at 25 °C on an orbital shaker operating at 200 rev. min⁻¹. Evidence for replication was obtained from a comparison of p.f.u. at $t = 0$ and 18 h. Control flasks without host were set up at the lowest pH to check phage stability.

Efficiency of infection. Germling suspensions were taken at the end of the adsorption period (see above) and used to determine the number of phage adsorbed in the pH range 4.4 to 6.9. The final adsorption suspensions were filtered (Millipore, pore size 0.45 µm) and 2 to 3 ml fresh broth was passed through to remove free phage trapped on the filter. The filters (carrying adsorbed phage) were resuspended in 10 ml broth, agitated for 30 s and assayed immediately at pH 5.5 or 7.0 for infective centres. The percentage efficiency of infection was calculated as [(no. of infected germings)/(no. of adsorbed phage)] $\times$ 100.

Effect of pH on intracellular development. One-step growth experiments were performed on each phage/host combination at the optimum and a sub-optimum pH of the host (Table 2). Flasks of G.C.P. broth were adjusted to the appropriate pH with 0.1 M-HCl or 0.1 M-KOH. Host spores were added to give a concentration of approximately 5×10^7 c.f.u. ml⁻¹ and flasks were incubated at 25 °C on an orbital shaker operating at 200 rev. min⁻¹. After germination of spores, KCN was added to give a concentration of 10⁻⁴ M and the pH was readjusted to the initial value. Phage were added to each flask to give a concentration of 0.01 to 0.05 phage per germling and adsorption was allowed to proceed for 15 min. A 1 ml sample of adsorption mixture was then removed, filtered as before and the filter was washed to remove free phage. The filter was resuspended in 50 ml KCN-free broth, a 10-fold dilution was prepared and both suspensions were incubated in a shaking water-bath at 25 °C. At intervals, 0.1 ml samples were removed from each flask, diluted and assayed for plaque-forming ability. The time at which the filters were washed in KCN-free broth was designated t_0 .

RESULTS

Phage morphology

Both phage f6 and f13 had hexagonal heads (50 nm diam.) with non-contractile tails (145 and 175 nm long, respectively); they could be placed in Group B of Bradley (1967). Phage f6

Table 2. pH requirements of streptomycete hosts, determined by oxygen uptake

<i>Streptomyces</i> sp.	Optimum pH for growth	Selected sub-optimum pH	pH range
Neutrophiles { F6	7.5	6.4	4.8-9.3
F13	6.6	5.6	5.3-9.5
Acidophiles { SW72	5.7	7.0	4.7-7.6
H1	5.4	6.5	3.5-7.0

Fig. 1. Effect of pH value on death rates of phage f6 (▲) and phage f13 (□) in G.C.P. broth. The death rate was calculated from the slope of the regression line of $\log_{10}$ (surviving phage) plotted against time.

formed large, clear plaques (2.18 mm diam.) and f13 formed small, turbid plaques (1.55 mm diam.).

Distribution of phage

Phage were not isolated from soils with a pH below 6.1 or from the riverbed soil (Table 1). It was not possible to isolate phage from soil by enrichment with acidophilic streptomycetes, even though the latter were known to be present in the acidic soils used (Khan & Williams, 1975). Phage for the five neutrophilic hosts were isolated from soils of neutral pH values. All these phage were polyvalent, lysing more than one of the neutrophilic hosts tested. Acidophilic streptomycetes are not normally isolated from neutral soils and yet several of the phage isolated from such soils also lysed acidophilic hosts grown at pH 5.5. Thus phage host-ranges did not reflect the distribution of phage and hosts in the soils tested.

Effect of pH on stability of phage

Inactivation of both phage f6 and f13 tested in broth followed simple first order kinetics, irrespective of the length of exposure to high or low pH. Phage f6 showed maximum stability between pH 5.0 and 9.0, whereas f13 was most stable between pH 5.5 and 8.5 (Fig. 1).

Both phage f6 and f13 were unstable in soils with $\text{pH} \leq 4.2$, none being detected after only 3 min contact with such soils (Table 3). In soils with $\text{pH} \geq 6.1$, both phage f6 and f13 were detectable after 200 h although numbers declined over this period.

Table 3. *Survival of phage f6 and f13 in soils of different pH values*

Soil	pH value	Time	Phage f6 [p.f.u. (g dry wt soil) ⁻¹]			Phage f13 [p.f.u. (g dry wt soil) ⁻¹]		
			0	3 min	200 h	0	3 min	200 h
Podzol (F ₁)	3.7		3×10^2	0	0	0	0	0
Podzol (B ₁ /B ₂)	3.8		0	0	0	0	0	0
Podzol (F ₂ /H)	3.9		1×10^2	0	0	0	0	0
Podzol (A ₁ /A ₂)	4.0		0	0	0	0	0	0
Podzol (A ₁)	4.2		2×10^2	0	0	1×10^2	0	0
Clay	6.1		1×10^4	NT	2×10^3	3×10^3	NT	3×10^1
Garden soil	7.0		5×10^3	NT	4×10^2	2×10^3	NT	2×10^2
Peat	7.4		2×10^4	NT	2×10^4	2×10^3	NT	1×10^1
Podzol (C)	7.8		2×10^4	NT	3×10^3	5×10^4	NT	2×10^3
Salt lake soil	8.0		2×10^3	NT	3×10^4	6×10^4	NT	3×10^3

NT, Not tested.

Table 4. *Effect of pH on phage adsorption*

Adsorption rates were measured after 10 min for H1/f13 and 18 min for all other host/phage combinations.

	Host	Phage	$10^{10} \times$ Adsorption rate (ml min ⁻¹)					
			pH value					
			4.4	4.8	4.9	5.6	6.5	6.9
Neutrophiles	F6	f6	8.53	NT	34.63	11.90	NT	28.37
	F13	f13	NT	NT	9.50	6.09	NT	10.63
	F13	f6	2.71	NT	8.87	5.97	NT	7.77
Acidophiles	SW72	f13	NT	3.77	NT	8.97	4.94	NT
	H1	f13	NT	7.13	NT	3.63	7.60	NT

NT, Not tested.

pH requirements of hosts

The four streptomycetes studied were active over different pH ranges and had different pH optima (Table 2). They were therefore suitable for the study of the phage cycle using hosts at optimum and sub-optimum pH. They also allowed the study of the same phage on different hosts over a range of pH values.

Effect of pH on adsorption of phage

Phage f6 adsorbed to both neutrophilic hosts (F6 and F13) throughout the pH range tested; adsorption to F6 was generally more efficient than to F13 (Table 4). Phage f13 adsorbed equally efficiently to neutrophilic (F13) and acidophilic (SW72 and H1) hosts at all pH values tested. No adsorption to ungerminated spores occurred.

Effect of pH on infectivity

Overall replication. Acidity had little effect on the replication of phage f13 on all three hosts tested (F13, SW72 and H1) within the limits of stability of free phage. At low pH there was a decrease in the 18 h titres probably due to the inactivation of some free phage. Phage f6, however, failed to replicate on both F6 and F13 when the pH was below 6.0 for the 18 h period. This was despite the normal growth of the host and the stability of free phage at pH values lower than 6.0.

Table 5. *Effect of pH on phage infection*

			Efficiency of phage infection (%)				
			pH value				
	Host	Phage	4.4	4.9	5.6	6.5	6.9
Neutrophiles	F6	f6	8.8	14.0	13.6	NT	17.4
	F13	f13	NT	4.4	6.1	NT	4.4
	F13	f6	51.7	41.7	61.0	NT	77.2
Acidophiles	SW72	f13	4.5	NT	4.1	3.7	NT
	H1	f13	2.4	NT	3.2	2.6	NT

NT, Not tested.

Fig. 2. One-step growth curves of phage f6 on streptomycete F6 under various pH regimes: ■, pH 5.6; ●, pH 7.0; △, pH 5.6, adsorption at pH 7.0; ○, pH 7.0, adsorption at pH 5.6.

Efficiency of infection. Phage f13 showed a low percentage infection (2 to 6%) on both neutrophilic and acidophilic hosts (Table 5). The efficiency of infection of F6 and F13 by phage f6 was considerably higher (17.4 and 77.2%, respectively at pH 6.9). There was still a significant infection of both hosts below pH 6.0 although this was reduced with decreasing pH.

It was therefore necessary to try to account for the failure of phage f6 to replicate when kept below pH 6.0 for 18 h. Since phage f6 appeared to be capable of both adsorption and infection at low pH, one-step growth experiments were performed under various pH regimes. When maintained at pH 7.0 throughout the experiment, phage f6 replicated normally on F6 with a latent period of 73 min and a burst size of about 40 p.f.u. per infected cell (Fig. 2). When maintained at pH 5.6, replication did not occur, thus confirming the previous observation of failure to replicate below pH 6.0. When phage f6 was allowed to adsorb for 15 min at pH 7.0, and then the pH was reduced to 5.6, replication occurred but the latent period was extended to 90 min. When the phage were allowed to adsorb for 15 min at pH 5.6 before increasing the pH to 7.0, there was little replication. Thus, it seems that phage f6 requires a pH above 6.0 for at least part of the infection period and possibly also for the early stages of replication.

Effect of pH on intracellular replication

The properties of the replication cycles of phage f6 and f13 determined at optimum and sub-optimum pH are summarized in Table 6. At sub-optimum pH the latent period was

Table 6. *Effect of pH on intracellular replication of phage*

	Host	Phage	pH value	Latent period (min)	Burst size (p.f.u. per cell)	Rise period (min)
Neutrophiles	F6	f6	7.5	70	15.5	35
	F6	f6	6.4	80	35.8	10
	F13	f13	6.6	76	6.6	15
	F13	f13	5.6	95	9.6	40
Acidophiles	SW72	f13	5.7	78	29.1	22
	SW72	f13	7.0	90	7.9	19
	H1	f13	5.4	75	11.5	26
	H1	f13	6.4	86	11.0	28

extended to varying degrees in all phage/host combinations. The largest burst sizes occurred under acidic conditions irrespective of the pH optimum of the host. The rise period of phage f13 grown on the acidophilic hosts (SW72 and H1) was not significantly different at either pH.

DISCUSSION

The successful isolation of actinophage from soil appears to be largely dependent on soil pH. No phage were isolated from soils with a pH below 6.1 despite the presence of potentially susceptible acidophilic hosts in these soils. Thus phage were abundant in the lower alkaline regions of the Freshfield podzol, but absent from the upper acidic layers (Table 1), although susceptible hosts were present throughout the profile (Davies & Williams, 1970; Khan & Williams, 1975). Williams & Mayfield (1971) demonstrated the presence of neutrophilic streptomycetes in the acidic layers of Freshfield soil, but it was postulated that their activity was confined to microsites with a pH higher than that of the surrounding soil. Such microsites would be limited in space and time. If phage were able to replicate in them, they would be rapidly denatured when the pH returned to the ambient level.

The stability of phage f6 and f13 in broth was maximum in the pH range 5.5 to 9.0. The rapid transition towards instability over a small pH range (0.5 units) is characteristic of the acid denaturation of protein. Similar results have been obtained with various animal, plant and bacterial viruses (Finkelstein *et al.*, 1940; Miller, 1944; Best & Samuel, 1936; Putnam *et al.*, 1949; Riverin *et al.*, 1970). The survival of actinophage in soils of near-neutral pH was good, although some inactivation occurred over a 200 h period. Inactivation in soil occurred at faster rates than those obtained in G.C.P. broth, suggesting that other factors in the soil promoted phage inactivation. It has been shown that the pH stabilities of free phage differ from those adsorbed to clay (Sykes & Williams, 1978). In acid soils (pH < 6.0) phage survival was negligible. Thus the reactions of these phage to pH in both broth and soil indicate that free phage are not likely to be present in acidic soils.

Since acid-tolerant actinophage were not detected in acid soils it seems unlikely that acidophilic streptomycetes are attacked by phage *in vivo*. However, some phage isolated from neutral soils (f13 and a29) were capable of replicating on acidophilic hosts at pH 5.5. This suggests that the absence of phage activity *in vivo* is due to the instability of free phage at low pH rather than to host immunity.

The investigation of the effect of different factors on phage replication usually necessitates subjecting the host to the same factors. Consequently the results apply to a host/phage system in which the host has been altered. Effects on the phage replication cycle can then usually be explained in terms of host reaction. The use of phage capable of attacking both acidophiles and neutrophiles facilitated the study of the effect of pH on phage replication without subjecting the host to pH stress.

Adsorption of phage f6 by neutrophilic hosts and f13 by neutrophilic and acidophilic hosts proceeded initially at a constant rate, after which rates declined. Adsorption rates were similar to those determined for other host/phage systems under optimum conditions.

pH has been shown to influence the adsorption of phage attacking a variety of hosts including *Escherichia coli* (Tolmach & Puck, 1952; Puck & Tolmach, 1954), *Acholeplasma laidlawii* (Fraser & Fleischmann, 1974) and certain *Salmonella* species (Kwiatkowski & Taylor, 1970; Takeda & Uetake, 1973). The adsorption of phage f13 to all hosts appeared to be virtually independent of pH. At low pH the attachment of phage f13 to acidophilic and neutrophilic hosts was equally efficient, indicating that adsorption was independent of the pH requirements of the host and therefore probably a passive process. Thompson & Shafia (1962) suggested that the adsorption of phage ϕ /4 to *Pseudomonas aeruginosa* was an active process which could be totally prevented by the addition of inhibitors such as 2,4-dinitrophenol or cyanide. Some authors recognize the specific adsorption of phage by spores (Mikhailova, 1964; Wilde, 1966). In this study, phage were not adsorbed by ungerminated spores which agrees with the observations of Rice *et al.* (1954) and Dowding (1972), and suggests that since spore and mycelium walls are almost identical in composition, adsorption must be a highly specific process.

Phage f13 replicated normally on both acidophilic and neutrophilic hosts within the limits of its stability. Furthermore, the titres achieved at optimum host pH were similar for both acidophilic and neutrophilic hosts. In contrast, phage f6 did not replicate on either neutrophilic host if the media were maintained below pH 6.0, although it can survive at pH 5 to 6 (Fig. 1). The step susceptible to low pH occurs during the infection period or the early stages of replication. If the pH is sufficiently high (>6.0) during the infection period, replication will occur at a lower pH (Fig. 2). Since attachment itself was not pH-dependent, low pH during the adsorption period may prevent infection or 'effective adsorption'. The low infection efficiency (Table 5) compared with adsorption suggests that a large proportion of phage f6 were ineffectively adsorbed.

The length of the latent period in the phage/host systems studied related to the metabolism of the host. For example, the latent period of phage f13 was similar on both neutrophilic and acidophilic hosts at their optimum pH. The increase in the length of the latent period at sub-optimum pH is due to the reduced metabolic activity of the host. The rise period of phage f13 on neutrophilic and acidophilic hosts was independent of pH and no consistent effects were observed. The burst sizes of phage f6 and f13 were variable and independent of pH. In general, burst sizes were lower than those previously reported for actinophage. Calberg-Bacq (1963) found that phage ϕ 17 had a burst size of 120, whereas Wilde (1966) reported it to be 2000. However, many factors can influence burst size, including the filamentous nature of the host, clumping and host age (Jones & Bradley, 1965).

Thus, intracellular development is the stage of the phage cycle least influenced by pH. The ability of phage to replicate equally well in several hosts with different pH optima would increase their chances of survival in the soil.

A research grant from N.E.R.C. is gratefully acknowledged.

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