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A new cyanophage has been isolated and biologically characterized. This virus, designated LP-1, infects Plectonema and Lyngbya species, and was isolated from a polluted river in North Carolina. It is antigenically similar to LPP-2 cyanophage. LP-1 has wider temperature and pH ranges than LPP-cyanophages. Adsorption kinetics and single-step growth experiments indicate that LP-1 has a slower adsorption time and a longer lytic cycle than previously characterized cyanophages.

BIOLOGICAL CHARACTERIZATION OF CYANOPHAGE LP-1 INFECTING PLECTONEMA AND LYNGBYA SPECIES

by

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III

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INTRODUCTION

A virus specific for blue-green algae was first isolated in 1963 (Safferman and Morris, 1963). Since that time, blue-green algae, of the phylum Cyanophyta, have drawn considerable attention from microbiologists, ecologists, and virologists. This interest has been centered upon both the hosts, cyanophytes, and their parasites, cyanophages, and has resulted in the discovery of several other viruses of blue-green algae (Singh and Singh, 1967; Safferman and Morris, 1967; Granhall and Hofsten, 1969; Safferman et al., 1969a; Adolph and Haselkorn, 1971; Safferman et al., 1972; Adolph and Haselkorn, 1973). This thesis presents evidence for the discovery of another blue-green algal virus which infects species of filamentous blue-green algae of the genera Plectonema and Lyngbya. This new viral isolate has been designated LP-1 in accordance with the system of nomenclature previously established for the cyanophages (Safferman and Morris, 1963; Safferman et al., 1969b). This system of nomenclature is based upon host specificity. Designations for each algal virus are derived from the first letter of the generic name of the algal host or hosts known to be susceptible to the viruses. For example, the LPP-viruses are cyanophages found to lyse blue-green algal species of the genera Lyngbya, Plectonema, and Phormidium. Viral strains within each group are further subdivided on the basis of serological differences and are designated numerically in the chronological order of their discovery, such as LPP-1 and LPP-2.

Blue-green algae are procaryotic, photoautotrophic organisms possessing a chlorophyll-a based oxygen evolving photosynthetic apparatus. They are biochemically and physiologically similar to eubacteria. Blue-green algae can be described further using procaryotic cell structure as a primary criterion. They have a cell wall surrounding a cell membrane. The entire cell is enclosed within an extracellular mucilaginous sheath which varies in thickness and viscosity among various genera and species. The cellular organelles and inclusions: such as vacuoles, ribosomes, polyphosphate granules, starch granules, and lipid bodies; are randomly dispersed within the cytoplasm. The genetic material, which in cyanophytes consists of deoxyribonucleic acid, is not enclosed within a nuclear envelope. The photosynthetic apparatus is a lamellar structure running parallel to the cell wall, and completely encircles the nucleoplasm. This lamellar photosynthetic structure is perhaps the most distinctive structure separating blue-green algae from eubacteria (Smith et al., 1966). A single photosynthetic lamella is a sac-like (Thylakoid) structure composed of two joined membranes containing the photosynthetic reactants, chlorophyll-a and carotenoids. Photosynthetic reactions of blue-green algae are similar to those of the eucaryotic plants in that molecular oxygen is liberated and water functions as the hydrogen donor in the radiant energy driven process.

The viruses that infect the blue-green algae were discovered almost fifty years after the discovery of bacteriophages (Twort, 1915). The years since 1963 have evidenced the growth of algal virology from mere speculation to its present status as a firm branch of subcellular experimental research. The innumerable contributions of the bacterium-

bacteriophage interactions serve as a prelude to advancements in the elucidation of the mysteries of cellular function and molecular biology. The use of algal viruses as aides in solving problems associated with algal blooms and eutrophication, photosynthetic processes of higher plants, and potential mechanisms of animal pathology does not seem unreasonable in light of advances in virology and molecular biology.

The infection process of algal viruses appears analogous to that of the T-even bacteriophages of Escherichia coli. The infection process of the model cyanophage, LPP-1, begins with the attachment of the virus to a susceptible algal host cell. Adsorption time of the various blue-green algal viruses varies as does the adsorption time of LPP strains (Goldstein et al., 1967). These variations are possibly due to the differences in the number of algal cells per trichome of competent host, as is the case in the LPP-1 infection of Plectonema boryanum (Goldstein et al., 1967), to perhaps more subtle aspects such as chemical affinity of the tail units and host cell wall, virus-cell concentrations, cation concentrations within the culture medium, and physiological conditions of the host. Viral deoxyribonucleic acid is injected through the cell wall of the host via the tail core of the virus. The eclipse period of LPP-1 has been reported to be approximately seven hours. It is followed by a release period that lasts approximately sixteen hours. The average burst size is approximately three hundred to five hundred virus particles per infected cell (Sherman and Haselkorn, 1970). The most definitive physiologic and microscopic change within the host cell during infection is the formation of the virogenic stroma with concomitant invagination of the photosynthetic lamella (Smith and Brown, 1967). Replication of

viral nucleic acid and other viral components occurs in the stroma, which is the area between the displaced lamellae and the cell membrane. Host cell lysis begins approximately seven hours post-infection, with very few unlysed cells remaining at the end of the fifteenth hour post-infection (Sherman and Haselkorn, 1970).

The plaques produced by algal cell lysis after LPP-1 infection are clear and have irregular borders due to the filamentous character of the host algae. They range in size from 0.1 mm to 0.8 mm in diameter (Safferman and Morris, 1963). The variability in plaque size is attributed to the presence of two variant types of LPP-1; the large plaque type is designated LPP-1(r+) and the small plaque type is noted as LPP-1(r). The two plaque types show no discernible differences in host range, pH stability, or thermal stability (Safferman and Morris, 1964; Luftig and Haselkorn, 1968). A primary difference noted in the two viral types was that LPP-1(r+) replicated at a somewhat faster rate than did type LPP-1(r). Repeated attempts to isolate and maintain the variants in axenic culture have failed, thus leading the investigators to speculate that the variants revert to a mixture of both forms after a series of transfers (Safferman and Morris, 1964a).

Magnesium ions are essential for biological viability of LPP-1 cyanophages. Removal of magnesium ions results in the disruption of the viral capsid and the loss of biological activity (Schneider et al., 1964).

LP-1 virus was initially isolated from the South Buffalo Creek basin in Guilford County, North Carolina. This drainage consists of watershed run-off as well as industrial and domestic waste water discharge. LP-1 was found in a routine viral analysis (Safferman and Morris, 1963)

of a sample taken from the creek in May, 1973. The new viral isolate has been shown to possess a reduced host range, wider pH range, greater thermal stability, continuity in plaque size, extended adsorption time, and other general characteristics that distinguish it from other cyanophages described by other investigators (Singh and Singh, 1967; Safferman and Morris, 1967; Granhall and Hofsten, 1969; Safferman et al., 1969a; Adolph and Haselkorn, 1971; Safferman et al., 1972; Adolph and Haselkorn, 1973).

MATERIALS AND METHODS

Blue-green Algal Culture Conditions

All blue-green algal cultures were incubated under aseptic conditions in revised Modified CHU No. 10 broth (Safferman and Morris, 1964) with modifications (Cannon et al., 1971). Iron was supplied in the form of ferric citrate (0.35 grams/liter)-citric acid (0.35 grams/liter). Nitrate was supplied as $Ca(NO_3)_2 \cdot 4H_2O$ (23.2 grams/liter) and $NaNO_3$ (16.6 grams/liter). Trace elements were supplied as $CuCl_2 \cdot 2H_2O$ (0.001 grams/liter), $ZnCl_2$ (0.04 grams/liter), H_3BO_3 (0.24 grams/liter), $CoCl_2 \cdot 6H_2O$ (0.002 grams/liter), and $MnCl_2 \cdot 4H_2O$ (0.014 grams/liter). Algal cultures were incubated under continuous "cool white" fluorescent lights (intensity of 100 - 120 foot candles measured from lights to cultures) at 24°C to 26°C for ten days to two weeks prior to infection with the viruses.

Blue-green Algal Cultures

All algal cultures, sensitive to LPP-1 cyanophage, were obtained from Dr. Robert S. Safferman, National Environmental Research Center, Environmental Protection Agency, Cincinnati, Ohio. The algal cultures subjected to viral infection consisted of thirty-two blue-green algal species from twelve genera. 100 ml algal cultures were maintained under the described conditions in 250 ml Erlenmeyer flasks and were transferred to fresh broth medium at three to four week intervals.

Bacterial Cultures and Conditions

All bacterial cultures were obtained from the Department of Biology, University of North Carolina at Greensboro, Greensboro, North Carolina. One species of each of ten genera of eubacteria were tested for susceptibility to LP-1 infection. 25 ml bacterial cultures were maintained in nutrient broth medium (8 grams/liter) (Difco Laboratories). Bacterial cultures were incubated for twenty-four hours at 37°C prior to infection.

Cyanophage Cultures and Viral Purification

LP-1 cyanophage was originally discovered in May, 1973 in a drainage basin of the South Buffalo Creek watershed basin in Guilford County, North Carolina. LP-1 cyanophage was purified after treatment of a 10 ml aliquot of the drainage water with 1 ml of chloroform which destroyed contaminating algae, bacteria, and protozoa; the non-chloroformed layer was removed utilizing a sterile serological pipet. The initial chloroformed sample was transferred aseptically to broth cultures of blue-green algae and incubated for two to three days. After two to three days incubation, infected algal broth cultures were examined visually for evidence of cellular lysis. Lysates of the algal cultures were filtered through 0.45μ membrane filters (Millipore Corporation) to remove cellular debris. Viral lysates were stored at 0°C to 5°C. Filtrates of the various lysed broth cultures were plated with various blue-green algae as hosts. A single plaque was isolated after lysis of Plectonema boryanum IU 581 and a viral stock culture was prepared as described for further characterization.

Cultures of LPP-1, LPP-2, and AS-1 cyanophages were obtained from the National Environmental Research Center, Environmental Protection Agency, Cincinnati, Ohio and from the Department of Biological Sciences, University of Delaware, Newark, Delaware.

Cyanophage cultures were maintained by aseptic inoculation of 1 ml of cyanophage (10^6 to 10^8 PFU/ml) into a two week old 100 ml culture of sensitive host alga and incubated for two to three days under the described conditions. Lysates were filtered through 0.45μ membrane filters (Millipore Corporation) and stored at 0° to 5°C.

Plectonema boryanum IU 581 consistently yielded the highest virus titers (PFU/ml), and was chosen as the host alga throughout these studies.

Biological Plaque Assay

The Adams plating technique for phage investigation (Adams, 1950) and modified by Safferman (Safferman, 1964) was employed as a standard plaque assay technique. Plaque counts were determined on plates in which 5 ml of an inoculated agar had been evenly distributed over a 15 ml layer of 1.0% revised modified CHU No. 10 agar. The surface layer was prepared in test tubes and consisted of 2.0 ml of a ten day to two week old culture of Plectonema boryanum IU 581, 0.5 ml of an appropriately diluted viral suspension, and 2.5 ml of a 1% modified CHU No. 10 agar (melted and cooled to 45°C). Viral dilution medium consisted of 0.42 grams of $MgCl_2 \cdot 6H_2O$, 5.85 grams of NaCl, and 1000 ml distilled water. Petri plates were incubated beneath "cool white" fluorescent lights (100 - 120 foot candles) for two to three days at 24°C to 26°C before plaque counts were made. Only those assay plates yielding thirty to three hundred plaques

were counted. All results are expressed as plaque-forming units per milliliter (PFU/ml).

Thermal Stability

Cyanophage LP-1, at a titer of 10^7 PFU/ml, was placed in sterile test tubes in a heated water bath. The aliquots of cyanophage were allowed to remain undisturbed for one hour intervals at temperatures ranging from 25°C through 63°C. After one hour incubation at the various temperatures, diluted suspensions were plated. Plates were incubated and plaque counts were made to determine the thermal stability of the virus at the selected temperatures.

pH Stability

2 ml aliquots of cyanophage LP-1, at a titer of 10^7 PFU/ml, were brought to a temperature of 25°C and transferred to test tubes containing sterile modified CHU No. 10 broth. The viral aliquots were then adjusted to varying pHs ranging from pH 3 to pH 12 by the addition of either 0.1 N HCl or 0.1 N NaOH. These aliquots were incubated for one hour before plaque assay on sensitive host algae.

Ionic Requirements

LP-1 cyanophage (10^7 PFU/ml), in LPP-dilution solution, was dialyzed against 5000 ml of distilled water for ninety-six hours. A 100 ml aliquot of LP-1 was introduced into moistened dialysis tubing (Fisher Scientific Company), suspended in 5000 ml of distilled water, and agitated by a magnetic stirrer. At twelve hour intervals, aliquots

of the isolate were removed, plated, and incubated as previously described. Four replicates were prepared simultaneously. A control assay of non-dialyzed cyanophage was conducted to measure non-dialyzed cyanophage activity.

Serological Studies

Rabbits were injected intraperitonally four times at two week intervals with either 3 ml LPP-1 (2.7×10^7 PFU/ml), 3 ml LPP-2 (2.1×10^7 PFU/ml), 3 ml AS-1 (5.3×10^6 PFU/ml), or 3 ml LP-1 (3.1×10^8 PFU/ml) emulsified in 2 ml Freund's Complete Adjuvant (Difco Laboratories). Rabbits were bled from the ear vein two weeks following the last injection. The blood obtained was centrifuged at 8000 rpm for thirty minutes in a Sorvall Model RC2-B centrifuge to obtain blood serum. The serum was aseptically transferred to sterile injection bottles and heated to $56^{\circ}C$ for one hour to inactivate Complement. 1.5 ml of LPP-1, LPP-2, AS-1 and LP-1 antisera were added to various dilutions of 1.5 ml of LPP-1, LPP-2, AS-1, and LP-1 cyanophages and the mixtures were allowed to react for one hour at $25^{\circ}C$ prior to biological plaque assay. Loss of plaque forming ability was indicative of neutralization of the appropriate cyanophage by its complementary antiserum. A cross-neutralization reaction was conducted using the four cyanophages and their heterologous antisera as described above. Twelve replicates of these studies were conducted.

Adsorption Kinetics

250 ml cultures of the susceptible host alga were incubated for three weeks to obtain a cell concentration of 2×10^7 cells/ml. Four

50 ml aliquots of the original 250 ml culture of Plectonema boryanum IU 581 were separated and each was infected with 5 ml aliquots of LP-1 cyanophage (2.1×10^7 PFU/ml). This mixture of host alga and cyanophage resulted in a multiplicity of infection (MOI) of 0.1. 0.1 ml aliquots were removed at five minute intervals for three hours from the alga-cyanophage mixture and transferred to a 9.9 ml dilution blank of modified CHU No. 10 broth to effectively stop further adsorption of the virus. These aliquots were centrifuged at 2100 rpm for ten minutes in an International Model IHC table-top centrifuge. Supernates were assayed for cyanophage. The number of unadsorbed viral particles (PFU/ml) compared to the known number of viral particles (PFU/ml) of the original inoculum represented the percent adsorption of LP-1 to its host over the time intervals tested.

Single-Step Growth Determination

100 ml aliquots of host alga, incubated for three weeks to obtain a cell density of 2×10^7 cells per ml, were infected with 10 ml of LP-1 (2.1×10^7 PFU/ml). This mixture of host alga and cyanophage resulted in a multiplicity of infection (MOI) of 0.1. 0.1 ml aliquots of the alga-cyanophage mixture were removed at one hour intervals for twenty-four hours. These aliquots were placed in a 9.9 ml dilution blank containing modified CHU No. 10 broth to effectively stop further adsorption of virus to host. Dilution tubes were centrifuged at 2100 rpm for ten minutes in an International Model IHC table-top centrifuge and supernates were assayed for cyanophage content. This procedure permits investigation of the replication cycle of LP-1 resulting the production of progeny virions.

The average yield of phage per cell, or burst size, is calculated using the ratio of total liberated phage divided by the number of initially infected cells. If all of the initial virus inoculum is not adsorbed, then the burst size is corrected by the incorporation of this factor into the ratio. The final titer less the number of unadsorbed phage is divided by the plaque count during the latent period (infected cells + unadsorbed inoculum) less the number of unadsorbed phage remaining the original inoculum.

Nucleic Acid Determination

A total of 36 ml of LP-1 was concentrated in cellulose nitrate centrifuge tubes (Beckman Corporation) in a No. 40 fixed angle rotor at 81500 X g for three hours in a Beckman Model L-2 preparative ultracentrifuge. 3 ml of concentrated LP-1 were placed in cuvettes and analyzed spectrophotometrically in a Beckman Model DB spectrophotometer. Ultraviolet wavelengths ranging from 220mμ to 290mμ were scanned. Absorbancy at wavelengths 230mμ, 260 mμ, and 280mμ served to determine the type of nucleic acid of LP-1 (Thomas and Abelson, 1966).

RESULTS

Source of Virus and Host Range

Cyanophage LP-1 was originally isolated in May, 1973 by Dr. Robert Cannon from water samples taken from the South Buffalo Creek watershed drainage basin in Guilford County, North Carolina. After treatment of each sample with chloroform, aliquots were aseptically transferred to broth cultures of various species of blue-green algae and incubated as described. Clearing of broth culture indicated possible viral activity. Lysed broth cultures were filtered through 0.45μ membrane filters (Millipore Corporation), and aliquots of the filtrates were plated with various blue-green algae as hosts. A single plaque was isolated after lysis of Plectonema boryanum IU 581 and a viral stock culture was prepared as described for further characterization.

Observable lysis in broth cultures was limited to only two species of Plectonema and one species of Lyngbya. Viral plaque formation was observed on a total of eleven species of Plectonema and three species of Lyngbya. LP-1 produced cloudy plaques on three species of algae. No lysis either in broth culture or plated specimens was observed for the remaining microorganisms tested (Table 1).

Reproducibility of Plating Technique and Plaque Size

LP-1 virus titering techniques gave reproducible plaque counts. Clear plaques with uneven, frayed borders and a diameter of 0.3 mm to 0.8 mm (average 0.5 mm) were observed after two to three days incubation

TABLE 1

Host Range of Susceptible and Non-susceptible Microorganisms Tested

Microorganisms	Susceptibility
Plectonema boryanum IU 581	+
Plectonema boryanum IU 594	+
Plectonema boryanum IU 595	+ (cloudy)
Plectonema boryanum IU 596	+
Plectonema boryanum IU 597	+
Plectonema boryanum IU 790	+
Plectonema calothricoides IU 598	+
Plectonema notatum IU 482	+ (cloudy)
Lyngbya sp. 10487 IU 621	+
Lyngbya sp. IU 487	+
Lyngbya sp. IU 488	+ (cloudy)
Anabaena catenula IU 375	-
Anabaena variabilis IU 377	-
Anabaena flos-aqua IU 1444	-
Anabaenopsis sp. SEC 1375	-
Anacystis nidulans IU 675	-
Anacystis nidulans IU 1550	-
Chlorogloea fritschii SEC 139	-
Fremyella diplosiphon IU 481	-
Microcoleus vaginatus SEC 191	-
Microcoleus vaginatus SEC 192	-
Microcoleus vaginatus SEC 197	-
Nostoc muscorum IU 486	-
Nostoc sp. IU 588	-
Oscillatoria chalybia IU 386	-
Oscillatoria formosa IU 390	-
Oscillatoria tenius IU 428	-
Phormidium faveolarum IU 427	-
Phormidium retzii Wis 1094	-
Phormidium minnesotense CU 1462/3	-
Synechococcus cedrorum M-4·1·1	-
Staphylococcus aureus	-
Streptococcus agalactiae	-
Pseudomonas aeruginosa	-
Proteus vulgaris	-
Escherichia coli	-
Bacillus subtilis	-
Neisseria catarrhalis	-
Salmonella typhosa	-
Shigella flexneri	-
Mycobacterium smegmatis	-

under described conditions. The uneven, frayed borders of the plaques may be attributed to the filamentous nature of the host alga (Safferman and Morris, 1963).

Stability

After one hour incubation at various temperatures, ranging from 25°C to 63°C, dilutions of the heated virus suspensions were made and plated. Plaque formation indicative of viral activity was observed to determine the thermal stability of LP-1. At temperatures up to and including 59°C, the virus titer remained stable. Exposure to temperatures of 60°C to 62°C for one hour caused the virus titer to decrease markedly, from a concentration of 10^7 PFU/ml at 59°C to a concentration of 10^2 PFU/ml at 62°C. Exposure of LP-1 to 63°C for one hour rendered the cyanophage inactive and no plaque formation was observed (Figure 1).

Aliquots of LP-1 at varying pHs ranging from pH 3 to pH 12 were incubated for one hour, dilutions were made, plated, and incubated as described. Plaque assays of the various samples were carried out to determine the pH stability of LP-1. LP-1 is active in a pH range of pH 4 to pH 11 with an optimum pH 6. No plaque formation was observed at pH 3 and pH 12 indicating LP-1 inactivation at these two pHs (Figure 2).

LP-1 has an ionic requirement similar to that of LPP-1 cyanophage (Safferman and Morris, 1964b). Clarified LP-1 lysates dialyzed against 5000 ml volumes of distilled water lost all biological activity during the first twelve hours of dialysis.

FIGURE 1

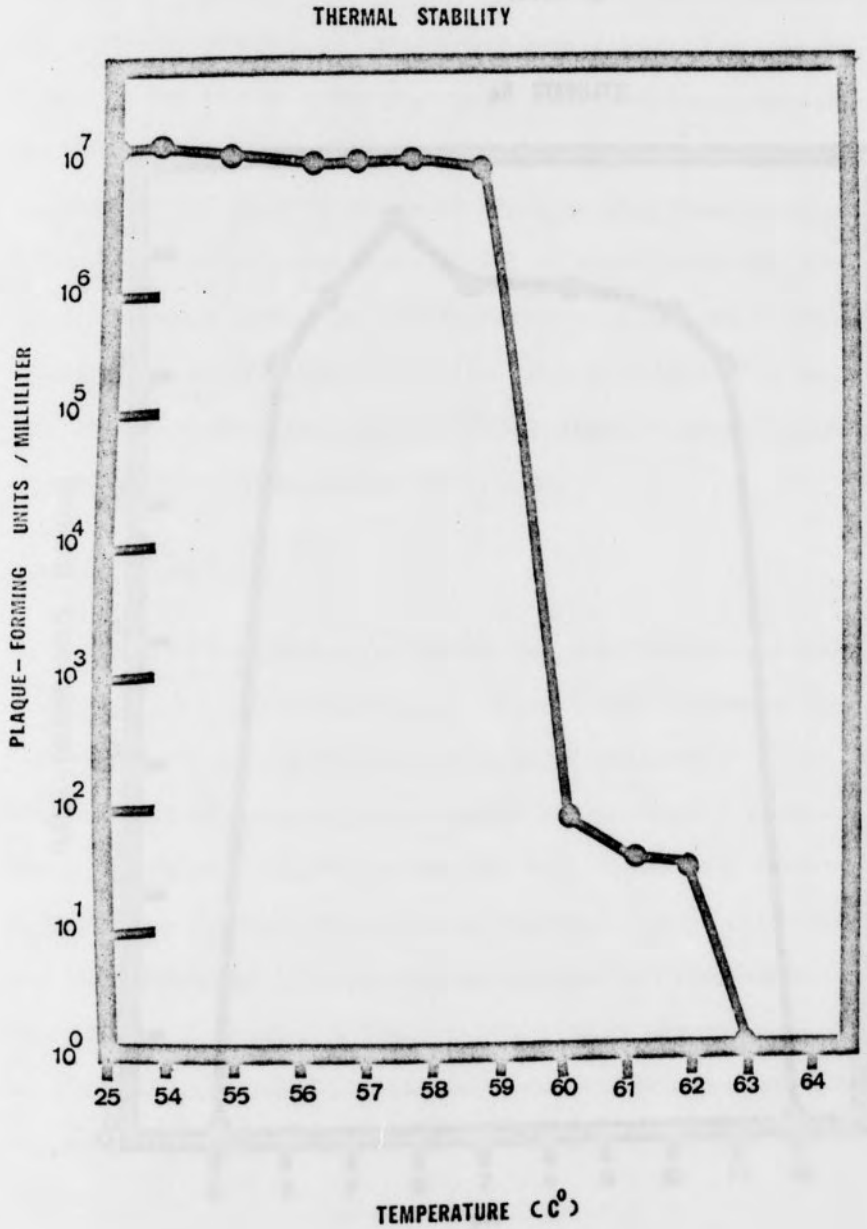
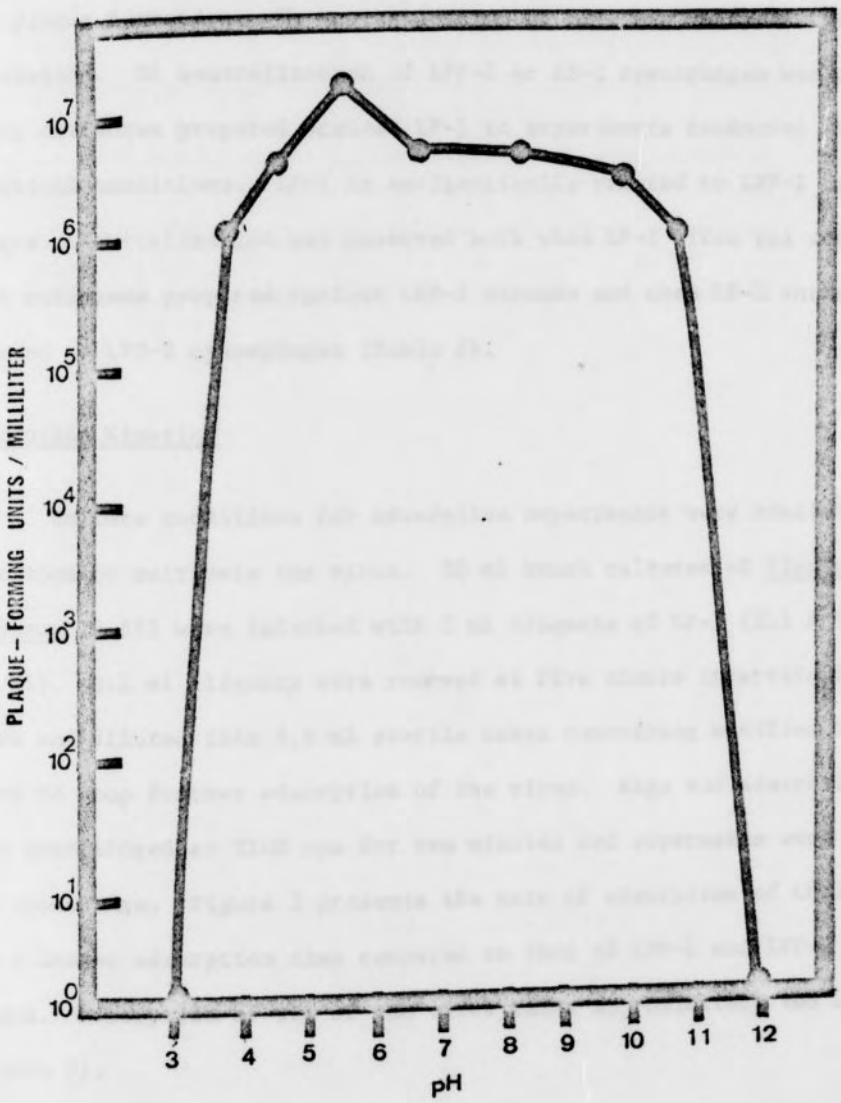

THERMAL STABILITY
PLAQUE-FORMING UNITS / MILLILITER
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
25
54
55
56
57
58
59
60
61
62
63
64
TEMPERATURE (C°)

FIGURE 2

pH STABILITY



Serology

LP-1 cyanophage was mixed with antiserum prepared against LPP-1 and AS-1 cyanophages. The antigen/antibody mixtures were incubated one hour prior to plating on susceptible host algae. Petri plates were incubated for two to three days under described conditions and observed for plaque formation. No neutralization of LP-1 was observed following incubation. No neutralization of LPP-1 or AS-1 cyanophages was observed using antiserum prepared against LP-1 in experiments conducted under identical conditions. LP-1 is antigentically related to LPP-2 cyanophages. Neutralization was observed both when LP-1 virus was reacted with antiserum prepared against LPP-2 viruses and when LP-1 antiserum was exposed to LPP-2 cyanophages (Table 2).

Adsorption Kinetics

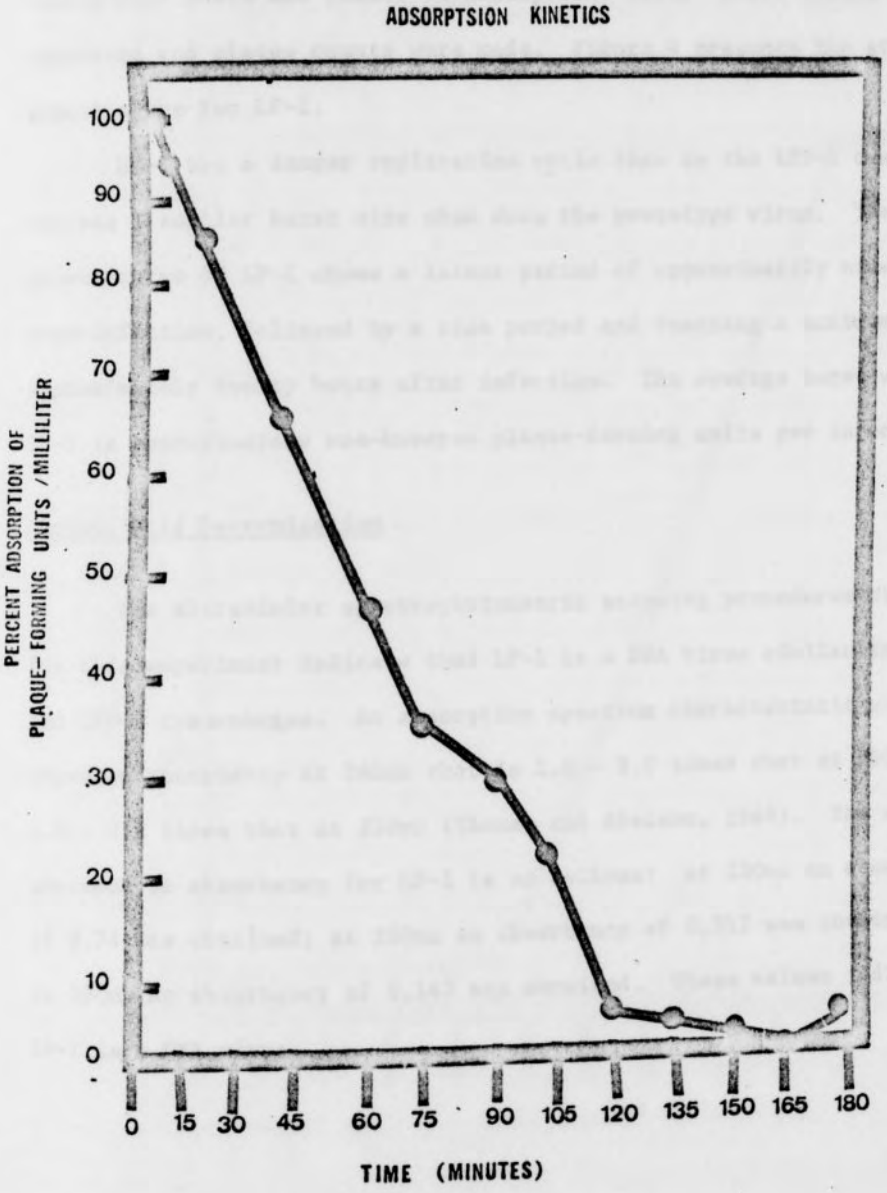
Culture conditions for adsorption experiments were similar to those described to cultivate the virus. 50 ml broth cultures of Plectonema boryanum IU 581 were infected with 5 ml aliquots of LP-1 (2.1×10^7 PFU/ml). 0.1 ml aliquots were removed at five minute intervals for three hours and diluted into 9.9 ml sterile tubes containing modified CHU No. 10 broth to stop further adsorption of the virus. Alga and adsorbed virus were centrifuged at 2100 rpm for ten minutes and supernates were assayed for cyanophage. Figure 3 presents the rate of adsorption of LP-1. LP-1 has a longer adsorption time compared to that of LPP-1 and LPP-2 cyanophages. Adsorption of 90% of the virus takes approximately two hours (Figure 3).

TABLE 2

Serology and Cross-Neutralization Reactions

Antisera	Antigens: LP-1	LPP-1	LPP-2	AS-1
LP-1	+	-	+	-
LPP-1	-	+	-	-
LPP-2	+	-	+	-
AS-1	-	-	-	+

FIGURE 3


ADSORPTSION KINETICS
PERCENT ADSORPTION OF PLAQUE-FORMING UNITS /MILLILITER
100
90
80
70
60
50
40
30
20
10
0
0
15
30
45
60
75
90
105
120
135
150
165
180
TIME (MINUTES)

Single-Step Growth Determination

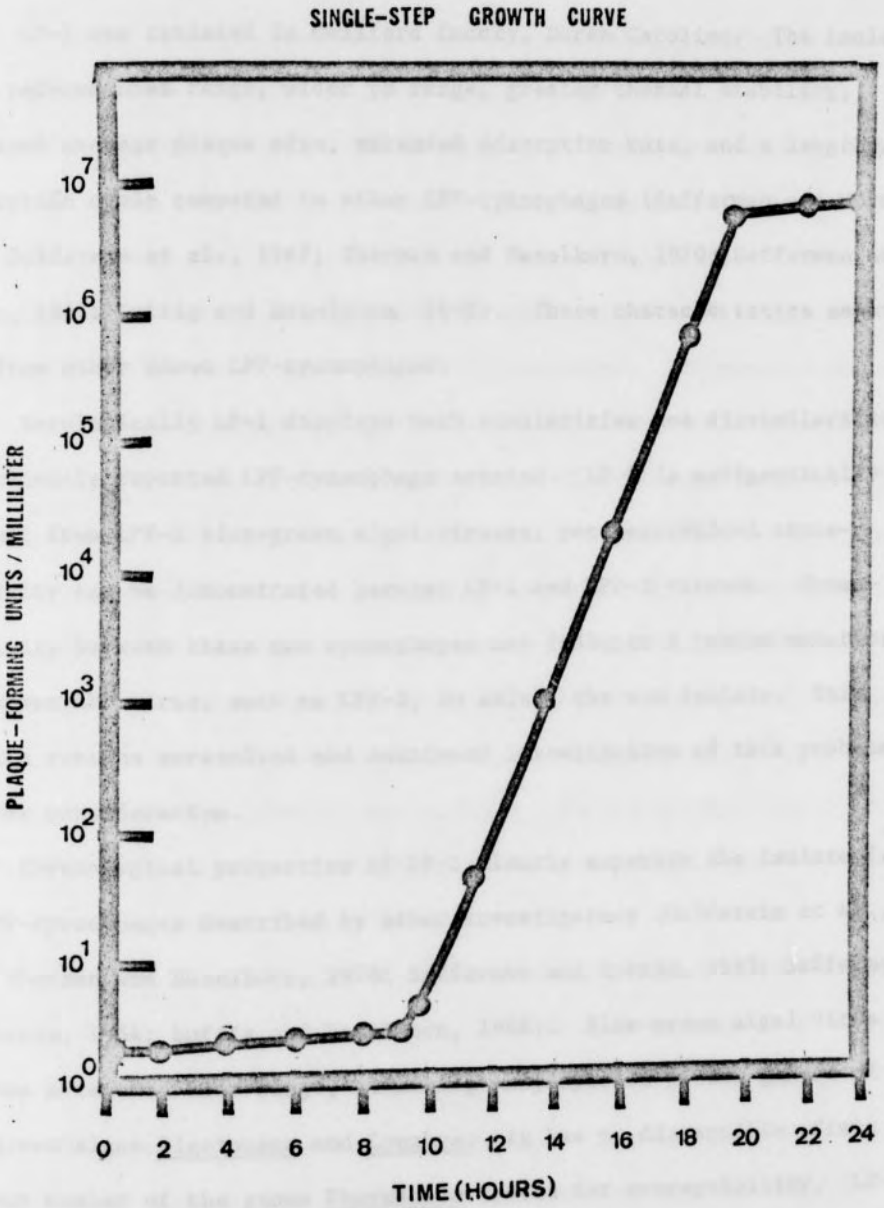
100 ml broth cultures of host alga were infected with 10 ml of cyanophage LP-1 (2.1×10^7 PFU/ml). Aliquots were removed hourly for twenty-four hours and plated on susceptible host. Petri plates were incubated and plaque counts were made. Figure 4 presents the single-step growth curve for LP-1.

LP-1 has a longer replication cycle than do the LPP-1 cyanophages, but has a smaller burst size than does the prototype virus. The one-step growth curve of LP-1 shows a latent period of approximately nine hours post-infection, followed by a rise period and reaching a maximum titer approximately twenty hours after infection. The average burst size of LP-1 is approximately one-hundred plaque-forming units per infected cell.

Nucleic Acid Determination

The ultraviolet spectrophotometric scanning procedures utilized for this experiment indicate that LP-1 is a DNA virus similar to LPP-1 and LPP-2 cyanophages. An absorption spectrum characteristic of DNA shows an absorbancy at 260mμ that is 1.6 - 2.0 times that at 280mμ and 1.8 - 2.2 times that at 230mμ (Thomas and Abelson, 1966). The spectrophotometric absorbancy for LP-1 is as follows: at 230mμ an absorbancy of 0.74 was obtained; at 260mμ an absorbancy of 0.212 was obtained; and at 280mμ an absorbancy of 0.147 was obtained. These values indicate that LP-1 is a DNA virus.

FIGURE 4


SINGLE-STEP GROWTH CURVE
PLAQUE-FORMING UNITS / MILLILITER
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0 2 4 6 8 10 12 14 16 18 20 22 24
TIME (HOURS)

DISCUSSION

LP-1 was isolated in Guilford County, North Carolina. The isolate has a reduced host range, wider pH range, greater thermal stability, decreased average plaque size, extended adsorption rate, and a lengthened replication cycle compared to other LPP-cyanophages (Safferman and Morris, 1963; Goldstein et al., 1967; Sherman and Haselkorn, 1970; Safferman and Morris, 1964; Luftig and Haselkorn, 1968). These characteristics separate LP-1 from other known LPP-cyanophages.

Serologically LP-1 displays both similarities and dissimilarities to previously reported LPP-cyanophage strains. LP-1 is antigentically distinct from LPP-1 blue-green algal viruses, yet serological cross-reactivity can be demonstrated between LP-1 and LPP-2 viruses. Cross-reactivity between these two cyanophages may indicate a random mutation of a known LPP virus, such as LPP-2, to select the new isolate. This question remains unresolved and continued investigation of this problem deserves consideration.

Physiological properties of LP-1 clearly separate the isolate from the LPP-cyanophages described by other investigators (Goldstein et al., 1967; Sherman and Haselkorn, 1970; Safferman and Morris, 1963; Safferman and Morris, 1964; Luftig and Haselkorn, 1968). Blue-green algal virus LP-1 has a reduced host range, attacking only species of two genera of blue-green algae Plectonema and Lyngbya. It has no discernible effect upon any member of the genus Phormidium tested for susceptibility. LP-1's inability to infect and lyse members of the genus Phormidium may result

from biological-physiochemical pressures of the environment. These pressures may result in the physiological incompetence of members of the genus (Delbrück, 1940; Puck et al., 1951). Along these same lines of conjecture, a more valid explanation for the lack of Phormidium infectivity may be found in the structural qualities of LP-1 and other LPP-cyanophages. These structural qualities are the basis for interactions between the viral tail fibers and host cell receptor sites during the adsorption process (Brenner et al., 1962; Anderson and Felix, 1953). The isolate has an increased pH stability and a significantly greater thermal stability than do the known LPP-cyanophages. LPP virus strains generally withstand changes in acidity and alkalinity within a range of pH 7 to pH 11 (Safferman and Morris, 1964a). LP-1 is capable of biological activity within a pH range of pH 4 to pH 11. This possibly indicates a capacity to remain viable in a more acidic environment. LP-1 remains biologically active at temperatures in excess of the 55°C thermal inactivation temperature of other LPP-cyanophages (Safferman and Morris, 1964a). LP-1 is active at temperatures up to and including 62°C, although there is a decrease in activity beyond 59°C. The ionic requirement of LP-1 is comparable to that of LPP-1 and LPP-2 cyanophages (Safferman and Morris, 1964a; Schneider et al., 1964).

Adsorption kinetics of LP-1 resemble those of other LPP-cyanophages (Goldstein et al., 1967). The adsorption of LP-1 is approximately 90% complete at the end of two hours in contrast to other LPP-cyanophages which are 90% adsorbed one hour post-infection. The departure from an adsorption rate typical of a first-order reaction has been attributed to varying cell numbers in the trichome of the filamentous blue-green algal

hosts (Goldstein et al., 1967). An increase in the adsorption rate of LP-1 may perhaps be attributed to exotic virus-host cell physiochemical affinities that make the process more difficult and may include viral cofactors within the medium, cations, and possibly the mucilaginous sheath (Arber, 1965; Luria, 1953).

The replication cycle of LPP algal viruses displays an eclipse period of approximately seven hours followed by a release period that reaches a maximum approximately sixteen hours post-infection (Sherman and Haselkorn, 1970). LP-1 cyanophage, on the other hand, is different in the time periods encompassed by these two general replication markers. The eclipse period of the LP-1 infection process, which corresponds to the time needed for adsorption of parental virions and the initial steps of replication of progeny particles (Sherman and Haselkorn, 1970), lasts for approximately nine hours. The rise period shows logarithmic release of progeny virions liberated by the lysis of infected cells. This period of virus release reaches a maximum approximately twenty hours post-infection. The curve plateaus when nearly all host cells are lysed. The average burst size for LPP-1 cyanophage is approximately three hundred to five hundred virus particles per infected cell. This is in contrast to the average burst size for LP-1 which is approximately one hundred virus particles per infected cell.

LP-1 infection of blue-green algal species of the genera Plectonema and Lyngbya resulted in the visual lysis of broth cultures of two strains of Plectonema boryanum (IU 581 and IU 594) and one strain of Lyngbya sp. (IU 487). Clear plaques, indicative of virulent phage activity, appeared on eight of the eleven plated blue-green algae of the genera Plectonema

and Lyngbya. The plated specimens of the remaining three susceptible host algae Plectonema boryanum IU 595, Plectonema notatum IU 482, and Lyngbya sp. IU 488, developed cloudy, turbid plaques. Turbid plaque formation occurred repeatedly when these algae were infected with LP-1 cyanophage. Cloudy plaque formation is one criterion of temperate phages. The recurrent incidence of turbid plaques has let to the speculation that LP-1 is a temperate virus similar to that reported by Cannon (1972). Investigation of the temperate nature of LP-1 is being conducted at the present time. Evidence accumulated indicates that LP-1 is temperate and can lysogenize with these three hosts.

Research on the ecology of cyanophages has occurred in many parts of the world. In Israel, Padan and Shilo isolated LPP-cyanophages from fish ponds (Padan and Shilo, 1969). In India LPP-cyanophages have been isolated from fresh water ponds, sewage discharge, and rice fields (Singh and Singh, 1967; Singh, 1973). In the United States, Safferman (1967) and Shane (1971) have demonstrated LPP-virus strains in a large number of varied aquatic environments, such as rivers, lakes, streams, waste stabilization ponds, and industrial waste waters. LP-1 was found in an environment similar to those described by other investigators as sources for cyanophages.

Future research involving LP-1 must be directed towards the examination of the isolate's genetic material, to include base sequencing and possible differences between the nucleic acids of LP-1 and other LPP-cyanophages. In addition, provisions should be made to determine the effects of LP-1 infection upon the hosts' metabolic processes, such as photosynthesis, cellular protein production and regulation, and cellular

repair processes. Further study into the differences in the physiochemical stability of LP-1 compared to other LPP-viruses may play an important role in the elucidation of questions associated with the ecology of blue-green algal viruses, especially those of the LPP-group. Continued investigation of the host range of LP-1 may reveal reasons for the serological relationship of LP-1 and LPP-2 cyanophages.

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