Co-Evolution of a Virus-Alga System

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Plectonema boryanum, a filamentous blue-green alga, was cloned and then allowed to reach a steady state in a quasi-continuous culture in the presence of the algal virus, LPP-1. The culture was maintained for a 3.5-month period during which time at least four distinct culture lysings were evident. After the fourth lysis the culture reached a steady-state level which was identical in its algal concentration to the preinfection level. Upon testing the characteristics of the evolved alga and virus variants, the following was determined: cell variants resistant to both the original virus and the derived virus had evolved, and there was no evidence of lysogeny present among these cells. The evolved virus strains still grew on the parental algal strain, though with altered plaque morphology. Furthermore, they were antigenically similar to the parental virus, and showed no significant difference in adsorption rate or growth characteristics on parental cells. However, a low-grade chronic viral infection persisted in the culture. Rapid re-establishment of a dense, stable culture is apparently the normal laboratory response of a procaryotic cell-virus system.

LPP-1 is the first discovered algal virus, isolated by R. Safferman in 1963 (19). It infects three closely related species of blue-green algae, which have been classified by F. Drouet into a single species, Schizothrix calcicola, the most prevalent blue-green alga on earth (22). Furthermore, LPP viral types are evidently a widely distributed virus group, having been isolated repeatedly in America (8, 22, 23), Israel (14), India (26), and Scotland (3), from algae-rich environments such as waste-stabilization ponds as well as less eutrophic lakes and rivers. Furthermore, they represent a stable population inasmuch as they can be re-isolated over a period of many months at the same location (14, 22). Their presence is correlated with the extent of cultural pollution (24).

Although the host species are ubiquitous in eutrophic waters, they are rarely found as the dominant alga (22). Furthermore, it is only with difficulty that these species can be found at all in environments containing established LPP-1 populations (14, 24). Thus, one might presume that LPP-type viruses are in dynamic equilibrium with their host in eutrophic waters, serving an important function as a natural algal control. Algal viruses therefore offer hope for both the understanding and the control of algal bloom populations (2, 15, 18).

The infectious cycle (25), virion morphology (4, 5, 11, 12), and photosynthetic needs of LPP-1 (13) have been worked out in detail. Furthermore, blue-green algal virus epidemics have been simulated (8, 20). However, no studies on the population dynamics of the virus and alga have been published.

The present paper describes an experiment to simulate the natural interaction of LPP-1 and Plectonema boryanum. We have looked at the long-term characteristics of a quasi-continuous, infected algal culture.

Similar experiments have been reported for a bacteriophage-bacterium system (7, 17). Wide fluctuations in Escherichia coli concentration and either T3 or T4 concentration were followed after 20 to 40 h (T4) or 500 to 700 h (T3) by a stable phase in which bacterial concentration returned to the pre-infection value, but low-grade, chronic viral growth continued. This stability did not end in 50 to 80 weeks. Similar results are found in our system.

MATERIALS AND METHODS

Algal and viral strains. The algae, P. boryanum, and its virus, LPP-1, were originally obtained from Dr. R. Safferman, Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio. The parental alga and virus were genetically purified by cloning on agar; and r+ type virus was chosen.

Culture methods. Algal cultures were grown in a medium designed by Allen (1). It contains per liter: 1.5 g of NaNO₃, 39 mg of KH₂PO₄, 75 mg of MgSO₄·7H₂O, 20 mg of Na₂CO₃, 36 mg of CaCl₂·2H₂O, 78 mg of Na₂SiO₃·9H₂O, 1 mg of EDTA,
6 mg of ferric citrate, 6 mg of citric acid, 2.86 mg of 
\( \text{H}_2\text{BO}_4 \), 1.81 mg of \( \text{MnCl}_2 \cdot 4\text{H}_2\text{O} \), 0.222 mg of 
\( \text{ZnSO}_4 \cdot 7\text{H}_2\text{O} \), 0.178 mg of \( \text{MoO}_3 \), 0.079 mg of 
\( \text{CuSO}_4 \cdot 5\text{H}_2\text{O} \), and 0.0494 mg of \( \text{Co(NO}_3)_2 \cdot 6\text{H}_2\text{O} \). Cultures were grown in 100-ml quantities in a 250-ml 
Erlenmeyer flask, magnetically stirred, aerated with 
purified, piped-in laboratory air (0.5% \( \text{CO}_2 \)) through a 
fritted glass disk, and lighted with one or two 20-watt 
cool-white fluorescent lamps giving about 150 foot-
candles incident on the culture. This culture was 
maintained as a quasi-continuous culture at about 8 
\( \times 10^7 \) cells/ml by replacing a portion of the culture 
with fresh growth medium daily. A second culture was 
allowed to grow more densely (10\(^4\) to 2 \( \times 10^4 \) cells/ml) 
and was used as the source of the indicator cells on 
plates for typical plaque assays. Culture temperature 
was about 25 C.

**Plaque assays.** Virus titer was assayed and plaque 
morphology was studied by plating 0.5 ml of virus, 2.0 
ml of indicator algae, and 2.5 ml of top agar on plates 
containing 20 ml of bottom agar. Both top and bottom 
agar contained Allen’s medium and 1.5% agar (Difco). 
Plates were incubated under illumination of 175 
footcandles for 3 to 5 days, at room temperature 
(approximately 22 C).

**Spectrophotometry.** Spectrophotometric readings 
were taken on portions of the infected algal culture 
using a Beckman DBG Spectrophotometer at 540 and 
632 nm.

**Algal colony assays.** Algal filament titer was 
assayed and resulting colonies were picked and 
regrown by spreading 0.1 ml of an appropriate dilution 
of culture on 20 ml of bottom agar in a petri plate, 
and then incubating for 10 to 14 days under illumination.

**Microscopy.** Filament concentration, cell concentration, 
and cell morphology were studied by light microscopy 
using a Neubauer-Hausser counting chamber. At a magnification of 450, the number and 
contour length of all the filaments in the field of view 
(20 to 100 filaments) were measured. At a magnification 
of 1,000, the number of cells in, and contour length of, 
several filaments were measured, yielding the 
average longitudinal length of a cell (2.5 \( \mu \text{m} \)). Finally, 
the cell concentration was calculated by:

\[
\text{cells per milliliter} = \frac{(\text{filaments/milliliter}) \times \text{(average contour length/filament)}}{(\text{average contour length/cell})}
\]

Cells per filament was calculated from the same data.

**Serological procedures.** LPP-1 antiserum was 
prepared by injection of rabbits with centrifugally 
purified and concentrated LPP-1 preparations. 
Neutralization tests were carried out at 22 C.

**Viral adsorption studies.** Adsorption rates were 
calculated from the decrease of nonadsorbable 
plaque-forming units (PFU) as a function of time in 
an algal culture shaken at room temperature (22 C) 
on an Eberbach reciprocal shaker to which virus had 
been added. Usually, a 30-ml algal culture in a 250-ml 
Erlenmeyer flask was used with a multiplicity of 
infection much less than 1.

**One-step growth experiments.** Virus was 
allowed to adsorb for 60 min in a 10-ml algal culture in a 
shaken flask (multiplicty of infection <0.001 phage 
per cell), and then treated with antiserum for 30 min. 
The culture was then diluted in growth medium to 
500, and to 5, infected cells per milliliter. Periodic 
assays of both dilutions were made. The source of cells 
for these one-step growth experiments proved to be 
important, as there was a loss of PFU in physiologi-

**RESULTS**

**Quasi-continuous algal culture.** Under the 
growth conditions described earlier, our 
*Plectonema* cultures have doubling times of 
about 10 h at cell concentrations in the 10\(^4\) to 
10\(^5\) cell/ml range. By diluting 1.5-fold each 24 h 
(corresponding to a continuous dilution rate of 
0.0167 h\(^{-1}\)), the culture was forced to stabilize 
at a doubling time of 41 h, the concentration 
stabilizing at 8 \( \times 10^7 \) cells/ml.

The 1.5-fold dilution was achieved by a daily 
removal of 33 ml of the 100-ml culture and 
addition of 34 ml of fresh growth medium. All 
assays were administered on the 33-ml portion. 
The 1-ml difference was to compensate for 
evaporation.

Figure 1 is the result of assays over a 113-day 
period. The top curve indicates the optical 
density (OD) at 540 nm and is an indication of 
cell concentration quantitatively so, when there 
is little cell lysis present. It is seen that 
the initial inoculum reached a steady state in about 
11 days, given by an OD of 1.6 which corre-
sponds to 8 \( \times 10^7 \) cells per ml. The dip at time = 
7 days was due to an increase of the culture 
from 50 to 100 ml by the addition of 50 ml of 
growth medium; the return to steady state is 
characteristic of a perturbed system.

**Results of LPP-1 infection.** A small volume of 
cloned LPP-1 r\(^+\) (parental) phage was added to the 
stabilized culture (time = 0 days in Fig. 1). The 
culture went through distinct lysings, of 
which four can be detected by a simultaneous 
examination of the three top curves in Fig. 1; 
these four lysing periods peaked at days 8, 22, 
33, and 41. The culture then reached a new, 
stable steady state by day 53, which it main-
tained to the end of the experiment. This corresponded in cell concentration to the prein-
fection steady state.

The third curve is the ratio of OD\(_{543}\) to OD\(_{450}\); 
this ratio represents the amount of reduced 
phycocyanin per cell when no lysis is present 
and decreases in value during periods of lysis. It 
reached an eventual stable value of 1.2 corre-
sponding to the preinfection ratio.

The second curve indicates the viral titer.
FIG. 1. Daily assays on LPP-1-infected algal culture. The top curve indicates the OD at 540 nm and is an indication of cell concentration (OD$_{471}$ = 1.6 represents $8 \times 10^7$ cells/ml). The second curve indicates the viral titer in PFU per milliliter. (The dashed line is the calculated effect of dilution on a non-replicating viral population.) The third curve, the ratio of OD$_{471}$ to OD$_{470}$, represents the amount of reduced phycocyanin per cell, and is an indicator of cell lysis. The fourth curve is the filament concentration as determined both microscopically and by colony assay on agar. The fifth curve is mean cells per filament as determined microscopically.

The initial wide fluctuations in titer smoothed out by the third lysis, and eventually stabilized at about $10^8$ PFU/ml. There was a gradual decrease, but this was not as fast as the decrease that would ensue if the phage were merely being diluted out without concomitant phage multiplication, as shown by the dashed line. Thus, phage multiplication was going on in the terminal culture.

Data on microscopically measured filament concentrations and colony-forming units and mean cells per filament are shown in the bottom two curves. During periods of lysis, filaments are very short due to breakage following random infection and lysis of cells; one to five cell filaments predominate. The bottom curve seems to indicate, however, a several-day delay in fragmentation after lysis. The steady-state culture has about $10^8$ filaments/ml which yields an average filament size of 80 cells/filament.

Periods of lysis were observable microscopically by the shortness of the filaments as well as by the presence of "swollen" cells in the culture; that is, cells rounded up and increased in volume by about one-half. On the other hand, no light-microscopically observable alteration in cell morphology was evident at other times during the course of the experiment.

**Characteristics of the derived cells.** On 3 of the days during the course of the experiment, 5 to 10 individual colonies from the plates for filament assay were picked and grown up in liquid medium for further examination of their properties, results of which are shown in Table 1. The 20 cell strains were isolated from the culture on days 4, 32, and 82. The third column shows the results of using these strains as indicator algae for parental LPP-1 viruses. Two of the early strains eventually yielded a low number of plaques (i.e., 2% of phage-formed plaques). By 32 days, only one of five yielded a similar sensitivity; and by 82 days, none of the 10 derived cell strains were sensitive to parental virus. Thus, cell lines resistant to the original virus evolved.

We were aware of the possibility of immunity by lysogeny in the evolved culture, and tested this as shown in column 4 by asking whether a drop of the derived algae would form a lysis spot or ring on a plate freshly inoculated with parental algae. All results were negative. As a partial control, a drop of resistant algae (strain 3 from day 82) to which parental virus had been added was similarly tested and formed a lysis ring.

Four of the cell lines (5th column) were tested negative for their ability to adsorb parental virus, and showed that the block was at the point of adsorption.

**Characteristics of the derived phage.** Al-
though the parental phage in this study was chosen as a large plaque former \((r^+)\), plaque variants quickly appeared. The rapid appearance of plaque-size variants in an infected culture has been studied by Safferman and Morris (21), who designated two cloned isolates as \(r^+\) and \(r\).

Ten single plaques from the final day of the experiment were picked for further study of the characteristics of these 10 evolved phage strains. A variety of plaque sizes was purposely chosen. The designations \(r^+\), \(r^m\), and \(r\) refer to plaque-size variants whose diameters under our standard plating conditions are: \(r^+ = 4.7\) mm, \(r^m = 2.6\) mm, and \(r = 1.5\) mm. The results are summarized in Table 2.

Each of the isolates bred true, in terms of homogeneity of plaque size, under replating except for number 7 which contained two true-breeding types. We next tested antigenic similarity to the parental LPP-1. The results, expressed in terms of an inactivation rate constant, indicate that all 10 are antigenically similar to the original virus. The next three columns indicate no large shift in the adsorption rate constant, the burst size, or the latent period when the phage were tested against parental cells. However, when tested against the 10 derived (evolved) algal strains picked on day 82, the 10 derived phage do not show normal infection of any of the 10 derived algal strains by spot testing for lysis on these strains.

There are, however, nine combinations that show some “low-grade” infectivity. These were characterized by a few, very small, very slow-appearing plaques instead of a spot. The characteristics of these low-grade infective virus-alga systems are being further studied.

**DISCUSSION**

Ecological survey of algal virus and its host (see reference 15 for a summary), interest in algal virus as an anti-algal agent (20), and

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**Table 1. Properties of derived algal strains**

| Algal strain cloned from culture on day no. | No. of isolate | Sensitivity to parental LPP-1 | Spot test on parental alga* | Ability to adsorb parental LPP-1 |
|-------------------------------------------|----------------|-----------------------------|----------------------------|----------------------------------|
| 4                                         |                |                             |                            |                                  |
| 32                                        | 1              | -                           | -                          |                                  |
| 82                                        | 1              | -                           | -                          |                                  |
| Control (parental alga)                    |                | +                           | -                          | +                                |

* Using derived strain as indicator alga for plaque assay. +, indicates lysis of alga.

**Table 2. Properties of derived viral strains**

| No. | Type* | Inheritance of plaque size (breeds true) | Inactivation rate constant \((\text{min}^{-1}\) by anti-parental LPP-1 serum) | Growth on parental alga | Growth of evolved plaque strains on derived algae (by spot test) |
|-----|-------|----------------------------------------|--------------------------------------------------------------------------------|-------------------------|------------------------------------------------------------------|
|     |       |                                        | k_adsorption \((10^{-9}\ \text{ml/min})\) | Burst size | Latent period \((\text{h})\) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1   | \(r^m\) | +                                      | 80                           |             |                        | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 2   | \(r^+\) | +                                      | 110                          |             |                        | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 3   | \(r^+\) | +                                      | 120                          | 0.04        | 200                    | 6 | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 4   | \(r^m\) | +                                      | 140                          |             |                        | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 5   | \(r^m\) | +                                      | 130                          |             |                        | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 6   | \(r^m\) | +                                      | 140                          | 0.02        | 300                    | 6 | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 7   | \(r^m,r\) | -                                      | 190                          |             |                        | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 8   | \(r\)   | +                                      | 110                          | 0.02        | 200                    | 7 | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 9   | \(r^m\) | +                                      | 80                           |             |                        | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 10  | \(r\)   | +                                      | 80                           |             |                        | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| Parental | \((r^+)\) | +                                      | 80                           |             |                        | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |

* \(r^m = 2.6\) mm; \(r^+ = 4.7\) mm; \(r = 1.5\) mm.
previous studies on bacterial virus infection in chemostat culture (7, 17) have combined to stimulate our interest in the long-term characteristics of an infected algal culture.

A stable, quasi-continuous algal culture can be maintained with little perturbation using the simple system described here. Although we have used periodic dilution, rather than a strictly continuous culture, we do not believe this affects our conclusions.

Introduction of virus into the culture leads to inversely correlated fluctuations in virus and cell densities for 50 days, including four observable lysing phases. The regrowth of the cells after the first lysis is surprisingly fast. This regrowth can be used, by extrapolation backward, to estimate the concentration of resistant (i.e., viable) cells during the first round of lysis. A rough estimate of 0.3% of the cells on day 4 survive lysis. This is much too high to be a frequency of phage-resistant mutants in the culture (16), and would seem to indicate that cell viability during the first round of lysis is due to some kind of physiological resistance. When the factor causing this physiological resistance is diluted out, more cells lyse, i.e., the second round of lysis, etc.

We have also seen evidence for a physiological resistance under other conditions: in the occasional observation of ring-centered plaques (Cowlishaw, unpublished data).

Although the initial resistance may be physiological, genetically resistant algal strains eventually dominate the culture. In the cases studied, this resistance is at the point of adsorption. Even strain 4 from day 32, on which parental phage formed plaques with an efficiency of 2%, did not exhibit detectable adsorption (2% adsorption would not have been detectable by loss of nonsedimentable PFU). The resistance does not seem to be due to lysogenic immunity, if the absence of spontaneously induced virus from the derived cells is any indication.

The derived phage are antigenically similar to the parental phage and still adsorb to and grow in the parental cells with similar burst size and latent period, though with somewhat altered plaque morphology.

Some phage multiplication must be going on in the terminal culture since the phage are not diluted out. One possibility is that some small fraction of the cells has normal sensitivity to the virus population. Based on the assumption that the source of these cells is a constant conversion of resistant cells to sensitive cells, one can write down steady-state equations (9) for the terminal-infected culture using values contained herein for dilution rate, phage adsorption rate, burst size, and terminal concentrations of phage and total cells. Solving these equations, one finds that a conversion of resistant cells to sensitive cells at the rate of 0.004 per generation would be necessary to maintain a population of 10^4 sensitive cells per ml, which would yield the steady-state phage and cell concentrations observed. Thus, in such a case only one cell out of 80 would be sensitive, and the probability of finding one normal sensitive cell among the 10 strains chosen from the terminal day would be about 12%, suggesting that we would not find one. On the other hand we did find evidence for a more frequent, but less infective, pairing of derived phage and algae. Of the 100 spot tests, 9 showed significant, albeit low-grade, infectivity. The nature of this low-grade infectivity is being explored further.

The study on T3 and T4 in E. coli chemostat cultures indicated the recovery of the bacterial concentration to preinfection levels, after periods of 500 to 700 h, and 20 to 40 h, respectively (7). Our algal culture recovered in 50 days. Given the 10-fold slower growth rate of blue-green algae, the results of our study are certainly analogous to the bacterial studies. M. Horne also found: irregular amplitude and frequency of the fluctuations of the pre-steady-state concentrations, many phage-sensitive cells during the pre-steady-state period, the final dominance of phage-resistant cells, persistence of viral infection in the terminal culture characterized by fluctuations of phage concentration of low frequency and amplitude, and the terminal phage being characterized by minute plaque formation, increased latent period and decreased burst size, and serological equivalence to the parental phage (7). Our results are similar in most respects. Thus, it appears to be not unusual for simulated co-evolution of a virus-procaryotic cell system to result in a high cell density, steady-state, terminal culture with a persistent low-grade viral infection.

B. Gromov and S. Kozyakov (6) and E. Padan et al. (16) have both isolated LPP-resistant P. boryanum strains and host range mutants of LPP-1 which could infect the original and mutant hosts. Gromov reports that the resistance is stable (genetic) "but in some cases the limited multiplication of the virus in the culture of r-clones was observed, evidently due to the appearance of sensitive cells." This is reminiscent of the low-grade infectivity present in our experiment.
What are the implications of this study for the understanding and control of natural algal blooms by viral epidemics? In the first place, Padan and Shilo (14) contend that the LPP-Plectonema system is also in dynamic equilibrium in natural habitats. The difficulty in recovering host cells from sites of LPP activity may be due to the chance of viral lysis of the cells during attempted isolation. This would seem to be a more appealing hypothesis than that other organisms may serve as LPP "reservoirs" (8). The multiplication of resistant host cells to dominate the habitat may be discouraged by the presence of other available algal species for bloom production (10). The apparent infrequency of naturally occurring phage-resistant strains of P. boryanum and viral host range mutants is difficult to understand, however. (See for example, Shane et al. [24] who were able to grow LPP-resistant Plectonema in LPP-containing river water samples, as if no viral host range mutants were present.) Apparently, the potential for genetic warfare between virus and cell is poorly realized in a mixed-species natural habitat where predator-prey interactions are presumably reduced.

The search for an ideal algicide has been extensive (2). The potential for complete viral control is not encouraging on the basis of our results. In our uni-algal system, the eventual return of high algal concentrations has been shown. Nevertheless, in a hetero-algal environment, the lysis of a hypothetical nuisance bloom by appropriate viruses might conceivably be followed by the development of other less troublesome species of blue-greens.

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LITERATURE CITED

1. Allen, M. M. 1968. Simple conditions for growth of unicellular blue-green algae on plates. J. Phycol. 4:1-4.
2. Brown, R. M., Jr. 1972. Algal viruses. Adv. Virus Res. 17:243-277.
3. Daft, M. J., J. Begg, and W. D. P. Stewart. 1970. A virus of blue-green algae from freshwater habitats in Scotland. New Phytol. 69:1029-1038.
4. Goldstein, D. A., and I. J. Bendet. 1967. Physical properties of the DNA from the blue-green algal virus LPP-1. Virology 32:614-618.
5. Goldstein, D. A., I. J. Bendet, M. A. Lauffer, and K. M. Smith. 1967. Some biological and physicochemical properties of blue-green algal virus LPP-1. Virology 32:601-613.
6. Gromov, B., and S. Kozyakov. 1970. A study of the peculiarities of the interrelationship between a blue-green algal population Plectonema boryanum, and cyanophage LPP-1. Bull. Leningrad Univ. 3:128-135.
7. Horne, M. T. 1970. Coevolution of Escherichia coli and bacteriophages in chemostat culture. Science 168:992-993.
8. Jackson, D., and V. Sladecek. 1970. Algal viruses-Eutrophication control potential. Yale Sci. 44:16-21.
9. Kubitschek, H. E. 1971. Introduction to research with continuous cultures. Prentice-Hall, Inc., Englewood, N.J.
10. Lin, C. K. 1972. Phytoplankton succession in a eutrophic lake with special reference to blue-green algal blooms. Hydrobiologia 39:321-334.
11. Luftig, R., and R. Haselkorn. 1967. Morphology of a virus of blue-green algae and properties of its deoxyribonucleic acid. J. Virol. 1:544-561.
12. Luftig, R., and R. Haselkorn. 1968. Studies on the structure of blue-green algal virus LPP-1. Virology 34:654-674.
13. Padan, E., B. Raboy, and M. Shilo. 1971. Endogenous dark respiration of the blue-green alga, Plectonema boryanum. J. Bacteriol. 106:45-50.
14. Padan, E., and M. Shilo. 1969. Distribution of cyanophages in natural habitats. Int. Soc. Appl. Theor. Limnol. Verh. Int. Ver. Limnol. 17:747-751.
15. Padan, E., and M. Shilo. 1973. Cyanophages-viruses attacking blue-green algae. Bacteriol. Rev. 37:343-370.
16. Padan, E., M. Shilo, and N. Kislev. 1967. Isolation of "cyanophages" from freshwater ponds and their interaction with Plectonema boryanum. Virology 32:234-248.
17. Paynter, M. M. B., and H. R. Bungay III. 1971. Characterization of virulent bacteriophage infections of Escherichia coli in continuous culture. Science 172:405.
18. Safferman, R. S. 1973. Phycoviruses. In N. G. Carr and B. A. Whitten (ed.). The biology of blue-green algae. Blackwell Science Publisher, Oxford.
19. Safferman, R. S., and M. E. Morris. 1963. Algal virus: isolation. Science 140:679-680.
20. Safferman, R. S., and M. E. Morris. 1964. Control of algae with viruses. J. Am. Water Works Assoc. 56:1217-1224.
21. Safferman, R. S., and M. E. Morris. 1964. Growth characteristics of the blue-green algal virus LPP-1. J. Bacteriol. 88:771-775.
22. Safferman, R. S., and M. E. Morris. 1967. Observations on the occurrence, distribution and seasonal incidence of blue-green algal viruses. Appl. Microbiol. 15:1219-1222.
23. Shane, M. S. 1971. Distribution of blue-green algal viruses in various types of natural waters. Water Res. 5:711-716.
24. Shane, M. S., R. E. Cannon, E. DeMichele. 1972. Pollutant effects on phycovirus and host algae ecology. J. Water Pollu. Control Fed. 44:2294-2302.
25. Sherman, L. A., and R. Haselkorn. 1970. LPP-1 infection of the blue-green alga Plectonema boryanum. I. Electron microscopy. J. Virol. 6:820-833.
26. Singh, R. N., and P. K. Singh. 1967. Isolation of cyanophages from India. Nature (London) 216:1020-1021.