Lysed areas suggestive of phage activity were observed on a plate in the course of isolation of a strain of Chondrococcus columnaris from trout at the Fisheries Center of the University of Washington. C. columnaris is an aquatic fruiting myxobacterium responsible for epizootics in salmonid and other fishes (Ordal and Rucker, 1944). Subsequently a bacteriophage was isolated which infected and lysed this particular strain of C. columnaris.

Up to this time bacteriophages have not been reported for members of either the Myxobacteriales or the Spirichaeales, the orders of flexible bacteria characterized by the absence of rigid cell walls. In view of the basic difference in structure of the eubacteria and the myxobacteria, a study was undertaken to compare the properties of the myxophage with those of the well studied eubacterial phages. For this purpose a system for a quantitative assay of myxophages had to be developed.

MATERIALS AND METHODS

Stock cultures of C. columnaris were grown at 20 to 25°C in agar deeps consisting of tryptone, 0.05% per cent; yeast extract, 0.05% per cent; sodium acetate, 0.02% per cent; beef extract, 0.02% per cent; and agar at pH 7.2, 0.4% per cent. Liquid cultures were grown in tryptone broth containing tryptone, 0.4% per cent; yeast infusion, 3% per cent; and “Tween 80” at pH 7.2, 0.1% per cent. All broth cultures were shaken in a reciprocal shaker at approximately 27°C.

The method employed for plating phase suspensions was similar to that described by Adams (1950), except that the compositions of the media were adapted for the myxophage-host system. The overlay agar consisted of 0.4% per cent tryptone, 2.5 ml; NaCl, 0.06 M; and agar, 0.7% per cent. The base agar contained 0.4% per cent tryptone, 30 ml; and agar, 1.5% per cent. The base agar was poured into plates and dried overnight at 30°C. One-tenth ml of a broth culture containing approximately 4 × 10⁸ cells/ml and 1 ml of the phage dilution were added to the overlay agar. Plaques were counted after incubation for approximately 40 hr at 28°C.

A preparation for electron microscopy was made from a plaque by the pseudoreplica technique of Hillier and Baker (1946).

One-step growth experiments were performed according to the method of Delbrück and Luria (1942).

RESULTS

Phage morphology. Since preliminary attempts to purify the phage by centrifugation were unsuccessful, an electron micrograph was made of a plaque replicate. Figure 1 shows that the myxophage is about 80 μm in diameter and polyhedral in shape. Short tails appear to protrude from several of the particles, but the presence or absence of a tail cannot be definitely established until the particles are purified and prepared for electron microscopy by treatment less drastic than air drying.

Plaque morphology. Twenty plaques from areas selected at random from each of two plates were measured and found to vary in diameter from 0.1 to 1.5 mm with an average of 0.8 mm. Myxophage plaques produced in overlay agar are round with clear centers and relatively sharp margins. When the plaques are viewed from a low angle, the surfaces of the plaques are seen to be elevated slightly above the surrounding bacterial growth.

Development of plating technique. In preliminary experiments it was found that the plating procedure described under Materials and Methods would give reproducible plaque counts.
Reproducibility of plaque counts from parallel dilutions

<table>
<thead>
<tr>
<th>Dilution</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arithmetic mean of duplicate plaque counts</td>
<td>176</td>
<td>187</td>
<td>185</td>
<td>176</td>
<td>199</td>
<td>185</td>
</tr>
<tr>
<td>Arithmetic mean of five sets of duplicate plaque counts</td>
<td>185</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total of deviations squared</td>
<td>362</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>±9.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cent error for duplicate plaque counts</td>
<td>5.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Assay of errors in plating from a single dilution of phage

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>190</td>
<td>52</td>
</tr>
<tr>
<td>Total of deviations squared</td>
<td>1859</td>
<td>476</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>±14.4</td>
<td>±7.4</td>
</tr>
<tr>
<td>Standard error of duplicates</td>
<td>±10.2</td>
<td>±5.2</td>
</tr>
<tr>
<td>Per cent error of duplicates</td>
<td>5.2</td>
<td>10.0</td>
</tr>
</tbody>
</table>

In order to determine the precision of the plating procedure, two types of experiments were performed. The first experiment was designed to assay the errors in the manipulations required to make a series of plates from a single phage dilution with the same pipette. Ten plates were prepared from a suitable dilution of the phage stock, and the standard deviation, the standard error of duplicate plate counts, and the per cent error of duplicate plaque counts was calculated. Table 1 gives the results of two experiments of this type differing only in the arithmetic means.

The second experiment for the evaluation of the plating procedure was designed to determine the reproducibility of duplicate plaque counts made from similar dilutions of a single phage stock. In this experiment five sets of duplicate plates were made from five parallel dilutions of a phage suspension. The calculations for this experiment are recorded in table 2. In this case the averages of the duplicate plaque counts were treated as individual samples. It is apparent that experimental errors in both cases are within a reasonable range.

Another condition which must be satisfied in a suitable dilution and plating system is that of proportional decrease in phage titer upon dilution of a phage suspension. For this experiment four plates were made from each of a series of increasing dilutions made from a single phage suspension with a titer of approximately 160 particles/ml. Figure 2 is a plot of the average counts of the sets of plates and demonstrates that the requisite of proportional decrease of titer upon dilution is fulfilled in this system.

**Figure 1.** Electron micrograph of a nickel-shadowed preparation of the myxophage from a plaque.

**Figure 2.** Proportional decrease of phage titer upon dilution.

---

In order to determine the precision of the plating procedure, two types of experiments were performed. The first experiment was designed to assay the errors in the manipulations required to make a series of plates from a single phage dilution with the same pipette. Ten plates were prepared from a suitable dilution of the phage stock, and the standard deviation, the standard error of duplicate plate counts, and the per cent error of duplicate plaque counts was calculated. Table 1 gives the results of two experiments of this type differing only in the arithmetic means.

The second experiment for the evaluation of the plating procedure was designed to determine the reproducibility of duplicate plaque counts made from similar dilutions of a single phage stock. In this experiment five sets of duplicate plates were made from five parallel dilutions of a phage suspension. The calculations for this experiment are recorded in table 2. In this case the averages of the duplicate plaque counts were treated as individual samples. It is apparent that experimental errors in both cases are within a reasonable range.

Another condition which must be satisfied in a suitable dilution and plating system is that of proportional decrease in phage titer upon dilution of a phage suspension. For this experiment four plates were made from each of a series of increasing dilutions made from a single phage suspension with a titer of approximately 160 particles/ml. Figure 2 is a plot of the average counts of the sets of plates and demonstrates that the requisite of proportional decrease of titer upon dilution is fulfilled in this system.
and the typhoid phages, the addition of calcium increases the myxophage titer.

One-step growth experiment. Figure 3 is a plot of duplicate plaque counts of a typical one-step growth experiment made using the tryptone growth medium containing 0.001 m added calcium. From several such experiments the length of the latent period has been determined to be approximately 90 min and the rise period to be about 30 min. An apparent burst size of 97 is obtained from figure 3. Preliminary experiments, however, have shown that phage suspensions which have been incubated in the presence of appropriate cell concentrations give plaque counts several times higher than those obtained when the phage is adsorbed after plating. Since for the most part intracellular or adsorbed phage particles are plated during the latent period and free particles are plated during the plateau period, phage assays during these periods are not absolutely comparable. It is possible that the number of particles actually present during the plateau period was higher than the count indicated.

**DISCUSSION**

The discovery of a myxophage was not an unexpected event. Luria (1953) predicted that phages infecting myxobacteria, as well as phages of other groups of bacteria not presently known to be phage-sensitive, would be found when these groups were thoroughly investigated. Phage-like plaques have been observed on plates streaked with *Cytophaga psychrophila*, another myxobacterial fish pathogen, but the attempted isolation was not successful. On the basis of the present findings, it seems reasonable that other myxobacteria will be found to be susceptible to phage infection.

A study of the basic properties of the myxophage reveals that most of its properties lie within the range of variation of these same
properties of the eubacterial phages. In size and in shape the myxophage is similar to many phages of eubacteria. A latent period of 85 to 95 minutes is longer than that of other phages, but perhaps this long period is a reflection of the relatively slow growth of the host cells in tryptone broth. Burst size calculations for the myxophage approximating those for eubacterial phages further indicate the similarity of these phages.

An extensive study of the adsorptive behavior may reveal some differences between myxophages and other phages, since myxobacteria do not possess rigid cell walls like those found in the eubacteria. The addition of calcium to the growth medium has a pronounced effect on the final titer of phage obtained. However, conclusions on the myxophage adsorption mechanism cannot be drawn from the data included above.

Calcium may be involved in one or more stages of phage infection. It has not been established that calcium is specifically required for myxophage adsorption. In other phage systems several other ions serve equally well (Puck et al., 1951). Luria and Steiner (1954) have demonstrated that adequate concentrations of calcium or magnesium are necessary for full penetration of coliphage T5 nucleic acid after adsorption of the phage to host bacteria. Calcium may also serve to prevent inactivation of phage particles released from the host cells. Further investigation is necessary to define the exact role of calcium in myxophage multiplication.

ACKNOWLEDGMENT

The authors wish to express their sincere appreciation to Dr. Neal B. Groman for his helpful suggestions and advice during this study and to Mrs. Hilda Agar for making the electron micrograph.

SUMMARY

A bacteriophage infecting the myxobacterium *Chondrococcus columnaris* has been isolated and studied. The myxophage is similar in shape to the T3 and T7 phages, but the head diameter is approximately twice that of these coliphages. Plaques obtained in overlay agar are round, clear-centered, slightly raised, and average 0.8 mm in diameter.

A plaque-count procedure has been developed which gives reasonable accuracy. The proportionality of plaque counts with dilution has been demonstrated.

The addition of calcium to the broth medium in concentrations up to 0.001 M increases the yield of phage.

One-step growth studies show that the myxophage has a latent period of 85 to 95 minutes and a rise period of approximately 30 minutes. The burst size calculated from one of these experiments is 97, but an analysis of the experimental conditions indicates that the actual burst size is larger.

REFERENCES


