CHARACTERISTICS OF A NEW VIBRIO-BACTERIOPHAGE SYSTEM*

By LUCILE SCHULTZ SMITH and ALBERT PAUL KRUEGER

(From the Department of Bacteriology, University of California, Berkeley)

PLATES 1 TO 3

(Received for publication, May 7, 1954)

The recent and very extensive literature relating to bacteriophage has included a number of surveys of diverse phage–host cell systems. We feel that such studies are necessary tests of the applicability of current theories and accordingly we have investigated two new phages, one active on *B. mycoides* (1–3) and the other on a salt water vibrio. Certain special aspects of the work on the latter phage have been reported elsewhere (4, 5); the present paper deals with the general characteristics of the system.

The vibrio was isolated from San Francisco Bay mud and grew optimally in media containing 3 per cent sodium chloride; prompt lysis occurred when the organism was suspended in distilled water.

The phage, isolated from the same sample of mud, has a head 95 to 100 mμ in diameter, a tail 100 mμ long and 15 mμ in diameter.

Lysogenic organisms developed readily and grew in large clumps which settled out of the medium. The burst size of young cells as noted in one-step growth curve experiments averaged 60 to 70 phage particles. Determination of the burst size of individual organisms gave an average of 126 phage particles per cell.

Materials and Methods

The Phage-Vibrio System.—The vibrio was first isolated from San Francisco Bay mud approximately 3 years ago and was classified in the genus *Vibrio* on the basis of morphology, staining reactions, cultural characteristics, and motility. While some degree of growth occurred in nutrient broth or on nutrient agar with NaCl concentrations ranging from M/12 to 2.5 M the salt concentration for optimal growth was M/2. Stock cultures of the organism were carried on nutrient agar containing M/2 NaCl.

The organism is Gram-negative and somewhat pleomorphic, varying in shape from rod to coccoid with a diameter of 0.5 mμ and a length of 1.0 to 4.5 mμ. In nutrient broth containing M/2 NaCl short chains of 2 to 4 organisms are common with oc-

* This investigation was supported by grants from the Office of Naval Research, the State of California Cancer Research Fund, and the Board of Research, University of California.

casional long chains of 10 organisms arranged as a wavy filament. The vibrio has a single polar flagellum and is actively motile. It grows aerobically and is a facultative anaerobe. It does not liquefy gelatin, reduces nitrates to nitrites, forms acid but no gas in glucose, fails to coagulate milk, produces indol and catalase. We were unable to classify the vibrio among the genera listed in Bergey's Manual of Determinative Bacteriology.

Growth of the organism on nutrient agar containing M/2 NaCl occurred as translucent, iridescent, faintly blue colonies measuring 1 to 2 mm. The colonies were circular with a smooth surface, an amorphous structure of butyrous consistency, and were easily emulsifiable into a homogeneous suspension in water.

Growth curves in liquid media were determined for temperatures ranging from 22–37°C. The present experiments were conducted at a temperature of 30°C. for the sake of convenience since the logarithmic period of growth begins at 2 hours and continues for some 6+ hours.

A phage lytic for this vibrio was isolated from the same sample of San Francisco Bay mud and was purified by successive serial transfers of single plaques. The phage characteristically produces plaques varying in diameter from ⅓ to 1.5 mm. (Fig. 1). Prolonged incubation results in the development of a halo at the periphery of the plaque due to the growth of resistant organisms.

Media.—Experiments on phage production were conducted in a yeast extract—mineral medium of the following composition: yeast extract (Difco bacto dehydrated) 1 gm., $MgSO_4$ 0.05 gm., K_2HPO_4 0.1 gm., NH_4NO_3 0.1 gm., NaCl 3.0 gm., tap water 10.0 ml., distilled water q.s. 100 ml.

For plaque counts the layering technique of Gratia (6) was used with nutrient agar containing M/2 NaCl.

Determination of Average Burst Size (One-Step Growth Curves, Delbrück (7)).—100 ml. of yeast extract–mineral 3 per cent NaCl medium containing a total of 1×10^{10} phage particles and 1×10^{10} bacterial cells was placed in a 250 ml. Erlenmeyer flask and the mixture was incubated at 30°C. with aeration. Turbidity readings in the Klett-Summerson colorimeter were made at intervals to follow [bacteria]. At the end of 15 minutes the suspension was diluted 100-fold to stop adsorption of phage and incubation of the diluted sample was continued. Actually, even without dilution, very little adsorption occurred after the period of 15 minutes. The phage titer was determined by plaque counts on samples removed at 0 time and at intervals thereafter. Both total and free phage determinations were made on the earlier samples. For this purpose free phage was separated from the cells by passing the mixture through super-cel pads as described by Krueger, Scribner, and Brown (8). Control experiments proved that the pads permitted passage of all free phage and < 0.1 per cent of the cells.

Method of Determining Burst Size for Individual Organisms.—A suspension containing 1×10^8 phage particles/ml. and 1×10^8 vibrios/ml. in yeast extract–mineral 3 per cent NaCl medium was incubated at 3°C. for 15 minutes to permit adsorption of phage onto the host cells. Dilutions of the suspension were made at this time to provide aliquots containing 1 cell/ml. of medium. 0.5 ml. quantities of this dilution were placed in each of a series of small test tubes and incubated at 30°C. for an additional hour to allow time for the infected cells to burst. At the end of this period there were added to each tube 0.5 ml. of a vibrio suspension just heavy enough to

produce a confluent growth in the final dilution and 1 ml. of 0.8 per cent nutrient agar containing M/2 NaCl. The contents of the tubes were thoroughly mixed and a 1 ml. aliquot was spread on a layer of nutrient agar M/2 NaCl in a Petri dish. After incubation at 30°C. overnight the plaques were counted in the customary fashion.

EXPERIMENTAL

Phage Production.—The production of phage was observed by means of one-step growth curve experiments using cells of different ages and by determining the burst size of individual organisms.

(*a*) *One-Step Growth Curves.*—The procedure described above under Materials and Methods was utilized in conducting experiments with bacterial cells of the following ages: late lag phase, early logarithmic phase, late logarithmic phase, and resting phase. Text-figure 1 depicts the experimental results obtained. Average burst sizes were calculated for the four cases studied from the equation:

$$\frac{\text{Titer of phage at end of burst period—unadsorbed phage}}{\text{Titer of phage at end of latent period—unadsorbed phage}} = \text{average burst size}$$

The values obtained were as follows:—

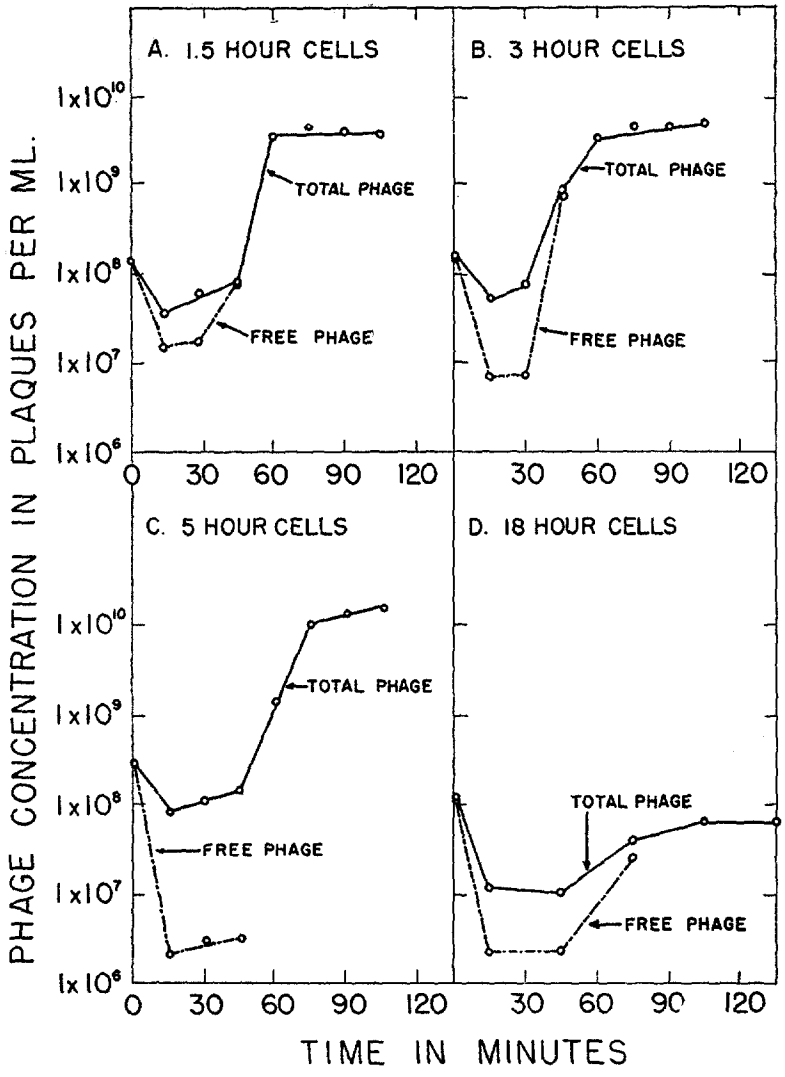
	Average burst size
Late lag phase (1.5 hour cells)	57.3
Early logarithmic phase (3.0 hour cells)	63.4
Late logarithmic phase (5.0 hour cells)	70.6
Growth stationary phase (18.04 hour cells)	7.4

(*b*) *Burst Size of Individual Organisms.*—An adaptation of the conventional method used for determining the burst size of individual organisms is described under Materials and Methods. Using this procedure 343 tubes theoretically containing the bursts from single cells were plated for plaque counts. 232 of the plates showed no plaques or only one plaque which probably represented a free phage particle unadsorbed during the adsorption period. Discarding one count of 786 as representing an experimental error, the average burst size of the other 110 counts was 126. Distribution of the burst size is shown in Table I.

Development of Lysogenic Organisms.—Organisms resistant to lysis regularly appeared in both liquid and solid media. A single cell isolation of the resistant strain was made and the clone thus derived was subjected to study.

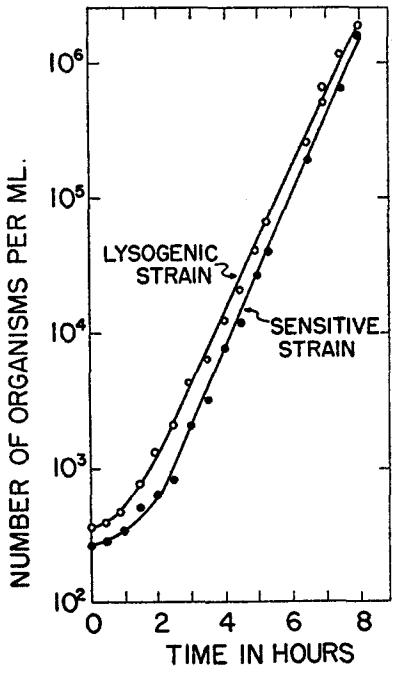
When resistant organisms were mixed with a suspension of the indicator strain and prepared for plaque count by the Gratia layering technique (6), the number of plaques formed corresponded to the number of resistant organisms present, indicating that these cells were lysogenic. Pinpoint colonies varying in number from 1 to 5 customarily appeared in each of the plaque areas.

There are certain striking differences between lysogenic and indicator strains so far as growth characteristics are concerned. The indicator strain



TEXT-FIG. 1. One-step growth curves with cells of various ages.

produces an even turbidity in fluid medium; the lysogenic strain forms a granular sediment which settles to the bottom and leaves a clear supernatant. In stained preparations the indicator strain occurs as single cells or very short chains while the lysogenic strain develops in the form of large clumps. On a



TEXT-FIG. 2. Growth curves of sensitive and lysogenic strains of vibrio. Klett-Summerson colorimetric measurements.

TABLE I

Distribution of the Burst Size of Cells of the Vibrio-Bacteriophage System Obtained from Single Cell Infections

Groups of Nos. of phage particles per cell	No. of cells in this group	Average yield of phage particles per cell for the group
0 to 50	26	24.5
51 to 100	26	76.3
101 to 150	26	128.6
151 to 200	8	180
201 to 250	12	227.7
251 to 300	6	282.7
301 to 350	6	352.3
786	1	Considered an error

solid medium the indicator strain produces a smooth, confluent growth while the lysogenic organisms forms a granular growth (Fig. 2). Similar differences between the indicator strain of *B. mycoides* and the lysogenic strain arising from it were reported by Baer and Krueger (1). Individual colonies of the

lysogenic and sensitive strains of the vibrio show no significant differences; this is true also of the growth curves obtained in the fluid medium (Text-fig. 2).

Structure of the Bacteriophage.—Suspensions of purified phage particles were prepared by the freeze-drying technique for electron microscopy. The examinations were conducted by Dr. Herbert Gold and showed that the heads of the particles were not truly spherical; there is some evidence of angularity as though the structure were polyhedral. The flattening evident in the accompanying picture (Fig. 3) is considered to be an artefact. The indefinite boundaries of the head suggest the presence of some sort of sheathing material. This could be something absorbed from the broth but washing failed to remove it.

The heads are 95 to 100 mμ in diameter and the tails are 100 mμ long by 15 mμ in diameter. There appears to be a constriction of the tail at the juncture with the head and the connection between head and tail is quite fragile. Manipulation of the particles during ultracentrifugation resulted in most of the particles dividing or breaking at this point.

SUMMARY

A vibrio-bacteriophage system isolated from San Francisco Bay mud is described. The vibrio lyses readily in distilled water and appears to be a new species. In contact with the phage it becomes lysogenic and forms large clumps. The phage presents no unique features.

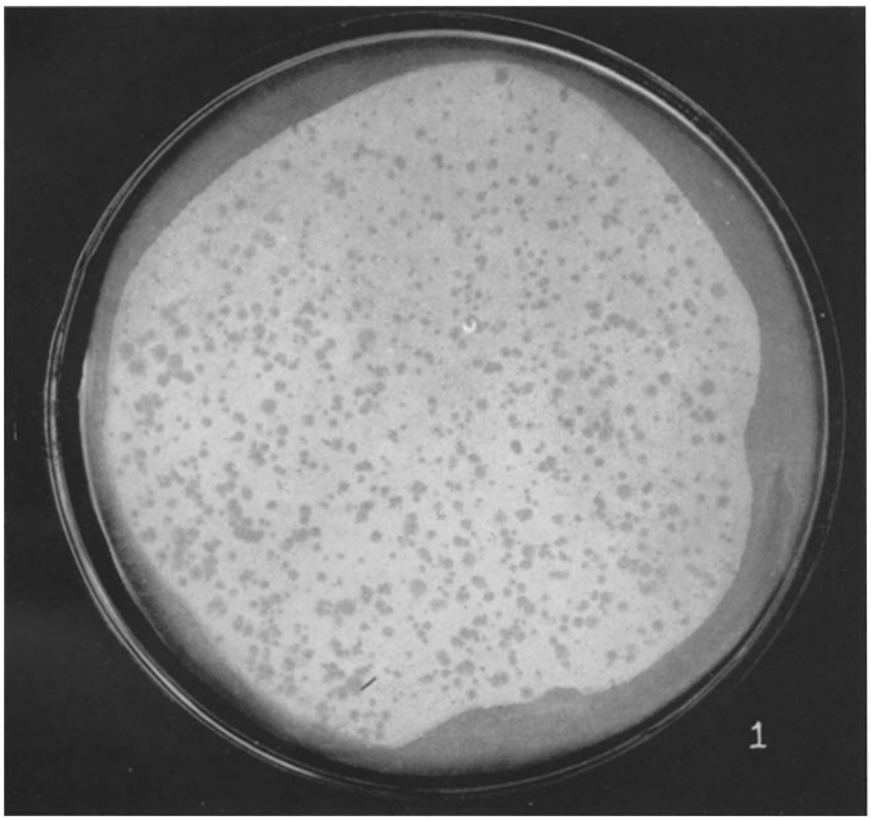
BIBLIOGRAPHY

1. Baer, B. S., and Krueger, A. P., The *B. mycoides* N host-virus system. I. Differences in appearance and "rate of growth" of the lysogenic and parent indicator strains, *J. Gen. Physiol.*, 1952, **35**, 857.
2. Baer, B. S., and Krueger, A. P., The *B. mycoides* N host-virus system. II. Interrelation of phage growth, bacterial multiplication, and lysis in infections of the indicator strain of *B. mycoides* N with phage N in nutrient broth, *J. Gen. Physiol.*, 1952, **36**, 111.
3. Baer, B. S., and Krueger, A. P., The *B. mycoides* N host-virus system. III. Release of phage from the lysogenic strain, *J. Gen. Physiol.*, 1952, **36**, 127.
4. Smith, L. S., and Krueger, A. P., Thermal inactivation of a new vibrio phage, *Proc. Soc. Exp. Biol. and Med.*, 1952, **81**, 371.
5. Smith, L. S., and Krueger, A. P., Thermal shock in a new vibrio phage, *Proc. Soc. Exp. Biol. and Med.*, 1952, **81**, 254.
6. Gratia, A., *Ann. Inst. Pasteur*, 1936, **57**, 652.
7. Delbrück, M. *Arch. Biochem.*, 1942, **1**, 111.
8. Krueger, A. P., Scribner, E. J., and Brown, B. B., *J. Gen. Physiol.*, 1946, **30**, 25.

EXPLANATION OF PLATES

PLATE 1

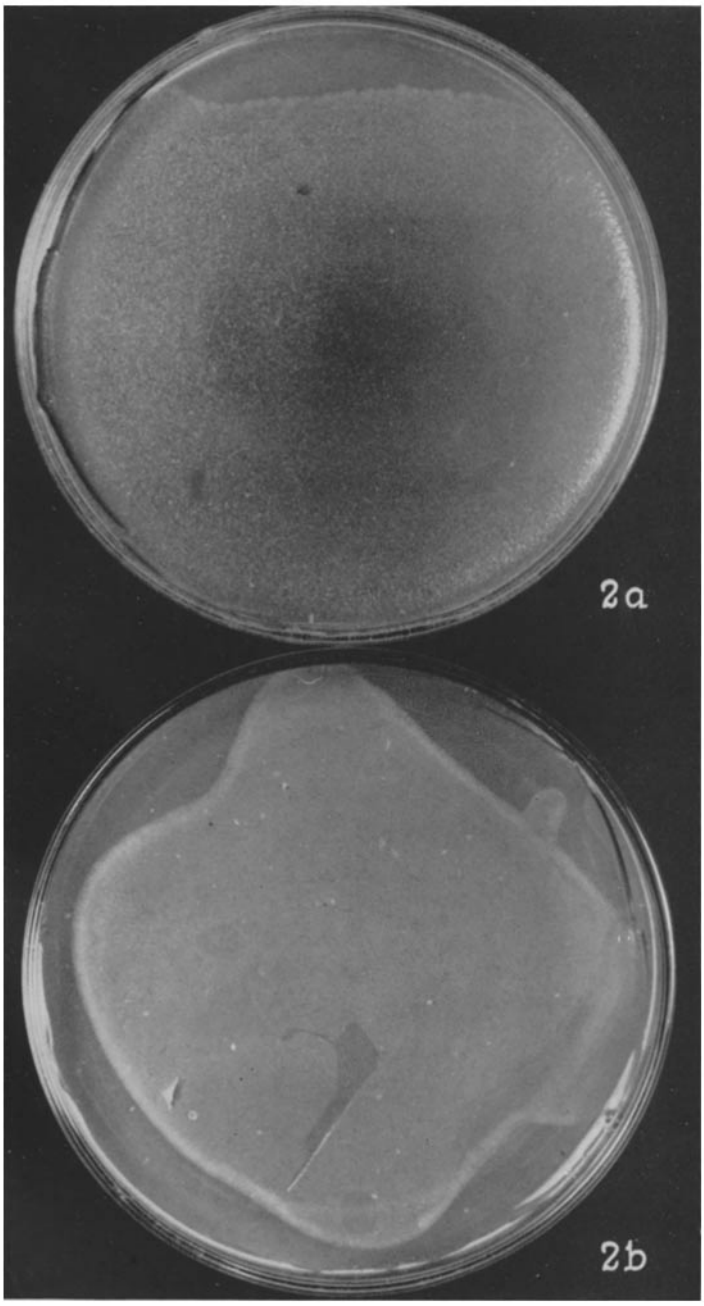
FIG. 1. Plaque formation by new vibrio-phage. × 1.



(Smith and Krueger: Characteristics of new vibrio-bacteriophage system)

PLATE 2

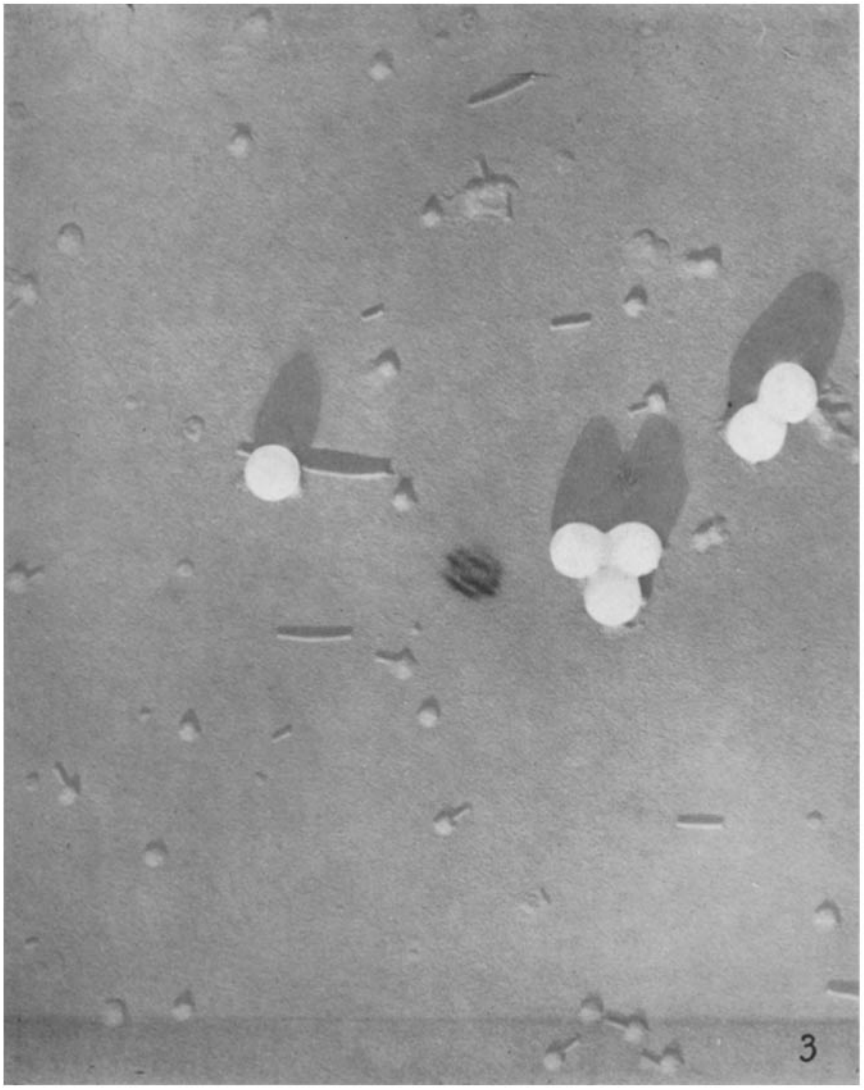
FIG. 2. Surface growths of (*a*) lysogenic vibrio and (*b*) indicator strain. × 1.



(Smith and Krueger: Characteristics of new vibrio-bacteriophage system)

PLATE 3

FIG. 3. Electron micrograph of new vibrio-phage. Purified suspension prepared by freeze-drying technique. Approximately × 30,000.



(Smith and Krueger: Characteristics of new vibrio-bacteriophage system)