Isolation of Bacteriophage for *Caryophanon latum*

R. K. NAUMAN AND E. F. WILKIE

Department of Microbiology, University of Maryland, School of Dentistry, Baltimore, Maryland 21201

Received for publication 21 January 1974

Bacteriophage which produce either clear or turbid center plaques have been isolated for native isolates of *Caryophanon latum*.

The bacterium *Caryophanon latum* is a unique procaryote because of its extremely large size, unusual structural complexity, and specialized ecological niche. This giant bacterium is gram positive and forms trichomes 3 μ m in diameter by 10 to 40 μ m in length. Peshkoff first isolated this organism from fresh cow dung in Russia in 1939 (2).

Prior to 1966, no bacteriophage was known for the members of the genus *Caryophanon*. At this time, Peshkoff reported the isolation from cow dung of a bacteriophage which produced clear plaques on *Caryophanon tenue* but was inactive against *C. latum* (4). Further experiments, however, culminated in the isolation from fresh cow dung of two virulent phage for several strains of both *C. tenue* and *C. latum* (3).

As a result of these prior reports, we decided to attempt the isolation of bacteriophage for native isolates of *C. latum*. The isolates used in our study were *C. latum* 2.9 and 1.6, as isolated by Provost and Doetsch in 1962 (5), and were kindly provided by R. N. Doetsch. *C. latum* 2.9 was grown in liquid culture with shaking, at room temperature, in a medium containing acid-hydrolyzed casein hydrolysate-vitamin and salt-free (Nutritional Biochemicals Corp., Cleveland, Ohio) (10.0 g/liter), yeast extract (Difco Laboratories, Detroit, Mich.) (5.0 g/liter), anhydrous sodium acetate (8.2 g/liter), thiamine-hydrochloride (0.2 mg/liter), and biotin (0.04 mg/liter), with the final pH adjusted to 7.8.

Phage enrichments were prepared essentially as described by Adams (1) with barnyard soil and fresh (1- to 2-day-old) cow dung samples used as potential sources of phage. Of the several enrichments prepared, only one, prepared with barnyard soil, proved positive. The plaques obtained from the soil enrichment were further purified and found to be variable in size (from less than 1 mm to 2 mm in diameter) with a turbid center. The variability in size of the plaques was a stable phenotypic characteristic

of the phage. The phage producing these plaques was designated ϕ CL-29.

Isolation of a clear plaque mutant of ϕ CL-29 was attempted with the aid of the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG). Three 250-ml flasks containing 20 ml of a log-phase culture of *C. latum* 2.9 were prepared, and NG was added to give a final concentration of 0, 45, and 135 μ g/ml. Immediately after the addition of NG, phage were added to give a final concentration of 5×10^7 PFU/ml. The flasks were incubated with shaking at 30 C for 4 h, and then each culture was centrifuged at $5,000 \times g$ for 15 min. The supernatants were assayed for plaque-forming ability. Only the supernatant from the culture with 135 μ g of NG per ml contained phage which produced a mixture of turbid center and clear plaques, with *C. latum* 2.9 used as the indicator. The clear plaques were further purified and also found to be variable in size. The clear plaque-forming phage was designated ϕ CLV-29.

Crude phage lysates of ϕ CL-29 or ϕ CLV-29 were concentrated and partially purified by differential and density gradient ultracentrifugation. In CsCl, ϕ CLV-29 had a buoyant density of 1.450 g/ml. The partially purified preparations were negatively stained with 1% potassium phosphotungstate, pH 6.9, and examined with a Siemens IA electron microscope. The phage morphology of ϕ CLV-29 was morphologically indistinguishable from ϕ CL-29 (Fig. 1).

The phage isolated for our strains of *C. latum* appear morphologically distinct from those described by Peshkoff et al. (3, 4). The two phages isolated in Russia had round-to-hexagonal heads measuring 65 nm in diameter, with one possessing a very long noncontractile tail 370 to 450 nm in length and 8 nm in width and the other possessing a short contractile tail 120 to 140 nm in length. In comparison, our phage isolates were found to possess an elongated, oval-shaped head approximately 70 by 130 nm with a long, thin noncontractile tail approxi-

FIG. 1. Electron micrograph of a partially purified preparation of ϕ CLV-29 negatively stained with 1% potassium phosphotungstate, pH 6.9. Bar, 0.1 μ m.

mately 330 nm long and 6.7 nm wide.

Our preliminary studies indicate that the turbid center plaque-forming phage ϕ CL-29 may be a temperate phage. However, attempts to induce lysis in immune populations of *C. latum* 2.9 either with mitomycin C or UV-irradiation have been unsuccessful.

This work was supported by Public Health Service training grant DE-00088-12 from the National Institute of Dental Research.

LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. Peshkoff, M. A. 1939. Cytology, karyology, and cycle of development of new microbes—*Caryophanon latum* and *Caryophanon tenue*. C. R. (Dokl.) Acad. Sci. U.R.S.S. **25**:244-247.
3. Peshkoff, M. A., S. B. Stefanov, I. A. Shadrina, and B. I. Marek. 1967. Virulent phage for *Caryophanon sublatum* X₁₃. Mikrobiologiya **36**:136-139.
4. Peshkoff, M. A., A. S. Tikhonenko, and B. I. Marek. 1966. A phage for the multicellular bacterium, *Caryophanon tenue* Peshkoff. Mikrobiologiya **35**:684-687.
5. Provost, P. J., and R. N. Doetsch. 1962. An appraisal of *Caryophanon latum*. J. Gen. Microbiol. **28**:547-557.