Bacteriophages of Clostridium botulinum Types A, B, E, and F and Nontoxicogenic Strains Resembling Type E

M. W. EKLUND, F. T. POYSKY, AND E. S. BOATMAN

Bureau of Commercial Fisheries Technological Laboratory, Seattle, Washington 98102, and Department of Preventive Medicine, University of Washington, Seattle, Washington 98105

Received for publication 23 October 1968

Recent investigations have shown cultures of Clostridium botulinum to be lysogenic. Vinet and Fredette (2) presented electron micrographs of bacteriophage obtained from C. botulinum type C. Inoue and Iida (1) induced the lysis of C. botulinum cultures with mitomycin C and ultraviolet light and observed bacteriophage in lysates of types A, C, D, and nonprotoelytic type F. They also observed phage tail-like rods in lysates of type E and nonprotoelytic type B. Protoelytic types B and F were not included in these studies.

This paper presents (i) additional observations on the occurrence of lysogenicity in the different types of C. botulinum and (ii) the first observations of bacteriophage from type E, protoelytic and nonprotoelytic types B, and protoelytic type F.

C. botulinum type A and nonprotoelytic types B and F were isolated at this laboratory from marine sediments. Other strains were obtained as follows: Beluga strain of type E from C. E. Dolman (Univ. of British Columbia, Vancouver, B.C.); strain 066B of type E and 066BNT (nontoxicogenic variant of 066B) from D. A. Kautter (U.S. Food and Drug Administration, Washington, D.C.); strain P4 (resembling type E except for the absence of toxigenicity) from V. J. Cabelli (Northeast Marine Health Science Laboratory, Narragansett, R.I.); strain 8E of type E and protoelytic strain 169B of type B from C. F. Schmidt (Continental Can Co., Chicago, Ill.); and culture 8G, a protoelytic strain of type F from N. W. Walls (Georgia Institute of Technology, Atlanta, Ga.).

The cultures were maintained in cooked meat medium at 25 C. The nonparticulate medium used for induction of lysis experiments was TPGY (trypticase, 5%; peptone, 0.5%; glucose, 0.1% yeast extract, 0.5%; and sodium thioglycolate, 0.1%; final pH, 7.0).

Induction of lysis experiments were performed in screw-cap tubes (25 x 150 mm) containing 30 ml of TPGY medium. The tubes were inoculated with an 18-hr culture to give an optical density of 0.06 to 0.08 and incubated at 25 C until the optical density attained a value of 0.12. Then, different concentrations of mitomycin C (0.1 to 5.0 μg/ml) were added to the cultures. Optical density was measured in a Bausch & Lomb Spectronic-20 colorimeter at 525 nm.

After lysis was complete, the cultures were clarified by centrifugation at 5,000 × g (Sorvall SS-34 rotor) for 15 min at 5 C. The pellet was discarded and the phage was sedimented from the supernatant fluid by centrifuging at 40,000 × g in an International model B-60 ultracentrifuge (rotor no. A-211) for 2 hr at 5 C. The pellet obtained was suspended in neutral 0.1 m ammonium acetate solution and again centrifuged at low and high speeds. The final pellet was resuspended in 0.5 ml of 0.1 m ammonium acetate solution.

Electron microscope specimen grids were dipped into the culture lysates and partially drained on filter paper. The grids were then dipped into a 2% solution of neutral potassium phosphotungstate solution, dried on filter paper, and examined in an RCA 3G electron microscope. Specimens were photographed at an initial magnification of ×21,000, and the negatives were further enlarged photographically to ×258,000.

Lysis occurred in all of the strains treated with mitomycin C except the nontoxicogenic culture 066BNT (a nontoxicogenic variant derived from toxicogenic strain 066B). The concentration of mitomycin C yielding maximal lysis was (i) 1 μg/ml for type A, nontoxicogenic and protoelytic types B, nontoxicogenic strain P4, and protoelytic type F; (ii) 0.1 and 0.5 μg/ml for type E strain; and (iii) 0.5 and 1 μg/ml for nontoxicogenic type F.

Bacteriophage and phage tail-like structures from lysates of the different types of C. botulinum
FIG. 1–4. Bacteriophages from lysates of C. botulinum type A, proteolytic and nonproteolytic types B. ×258,000. (1) Type A, strain B1G4; (2) non-proteolytic type B, strain 2B; (3) nonproteolytic type B, strain 17B; (4) proteolytic type B, strain 169B.
Fig. 5-8. Bacteriophages and phage tail-like structures from lysates of C. botulinum type E and organisms resembling type E. X258,000. (5) Type E, strain 066B; (6) type E, strain 8E; (7) type E, strain Beluga; (8) non-toxigenic strain P4.
FIG. 9-12. Bacteriophages from lysates of nonproteolytic and proteolytic strains of C. botulinum type F. (9 and 10) Nonproteolytic type F, strain 70F; (11 and 12) proteolytic type F, strain 8G.
and nontoxicogenic organisms resembling type E are presented in Fig. 1 through 12.

These phages are classified into four groups. The first group consists of phages from types A, E, and nonproteolytic and proteolytic types B, which exhibited mainly electron-dense hexagonal heads, 50 to 60 nm in diameter, and long flexible tails, 140 nm (phage from type A) to 250 nm (phage from type E and proteolytic type B) in length and 6.0 to 8.0 nm in diameter (Fig. 1–5 and 7). The second group consists of nonproteolytic type F phages. Two different phages were observed in lysates of strain 70F. One of the phages exhibited a large hexagonal head, 90 nm in diameter, and a long flexible tail, 280 nm in length and 8.0 nm in diameter (Fig. 10). The other phage also exhibited a hexagonal head, 54 nm in diameter, and a tail, 100 nm long and 4 nm in diameter, surrounded by a contracted sheath 16 nm in diameter (Fig. 9). The third group consists of a proteolytic type F phage, which exhibited an elongated head 85 to 95 nm long and 46 to 50 nm wide and a tail 270 nm long and 8 nm in diameter (Fig. 11 and 12). The fourth group consists of one of the type E strains (strain 8E) and a nontoxicogenic organism resembling type E. Lysates from these cultures contained phage tail-like structures, 100 nm long and 15 to 19 nm in diameter (Fig. 6 and 8). Similar tail-like structures were also observed by Inoue and Iida (1) in lysates of type E and nonproteolytic types B and F.

Induction of lysis in the toxigenic type E strain 066B and the inability to induce lysis in the nontoxigenic strain 066BNT suggests a possible relationship between toxigenesis and bacteriophage. Numerous unsuccessful attempts, however, have been made to convert strain 066BNT to a toxigenic organism by exposing it to lysates containing phages of toxigenic type E cultures.

The relationship of the bacteriophage to the toxigenesis of the different types of C. botulinum is currently being investigated.

LITERATURE CITED