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Bacteriophages of *Clostridium botulinum* Types A, B, E, and F and Nontoxigenic 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

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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 nonproteolytic type F. They also observed phage tail-like rods in lysates of type E and nonproteolytic type B. Proteolytic 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, proteolytic and nonproteolytic types B, and proteolytic type F.

C. botulinum type A and nonproteolytic 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 (nontoxigenic 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 toxinogenicity) from V. J. Cabelli (Northeast Marine Health Science Laboratory, Narragansett, R.I.); strain 8E of type E and proteolytic strain 169B of type B from C. F. Schmidt (Continental Can Co., Chicago, Ill.); and culture 8G, a proteolytic 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 *p*H, 7.0).

Induction of lysis experiments were performed in screw-cap tubes (25 × 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 nontoxigenic culture 066BNT (a nontoxigenic variant derived from toxigenic strain 066B). The concentration of mitomycin C yielding maximal lysis was (i) 1 μg/ml for type A, nonproteolytic and proteolytic types B, nontoxigenic strain P4, and proteolytic type F; (ii) 0.1 and 0.5 μg/ml for type E strain; and (iii) 0.5 and 1 μg/ml for nonproteolytic type F.

Bacteriophage and phage tail-like structures from lysates of the different types of *C. botulinum*

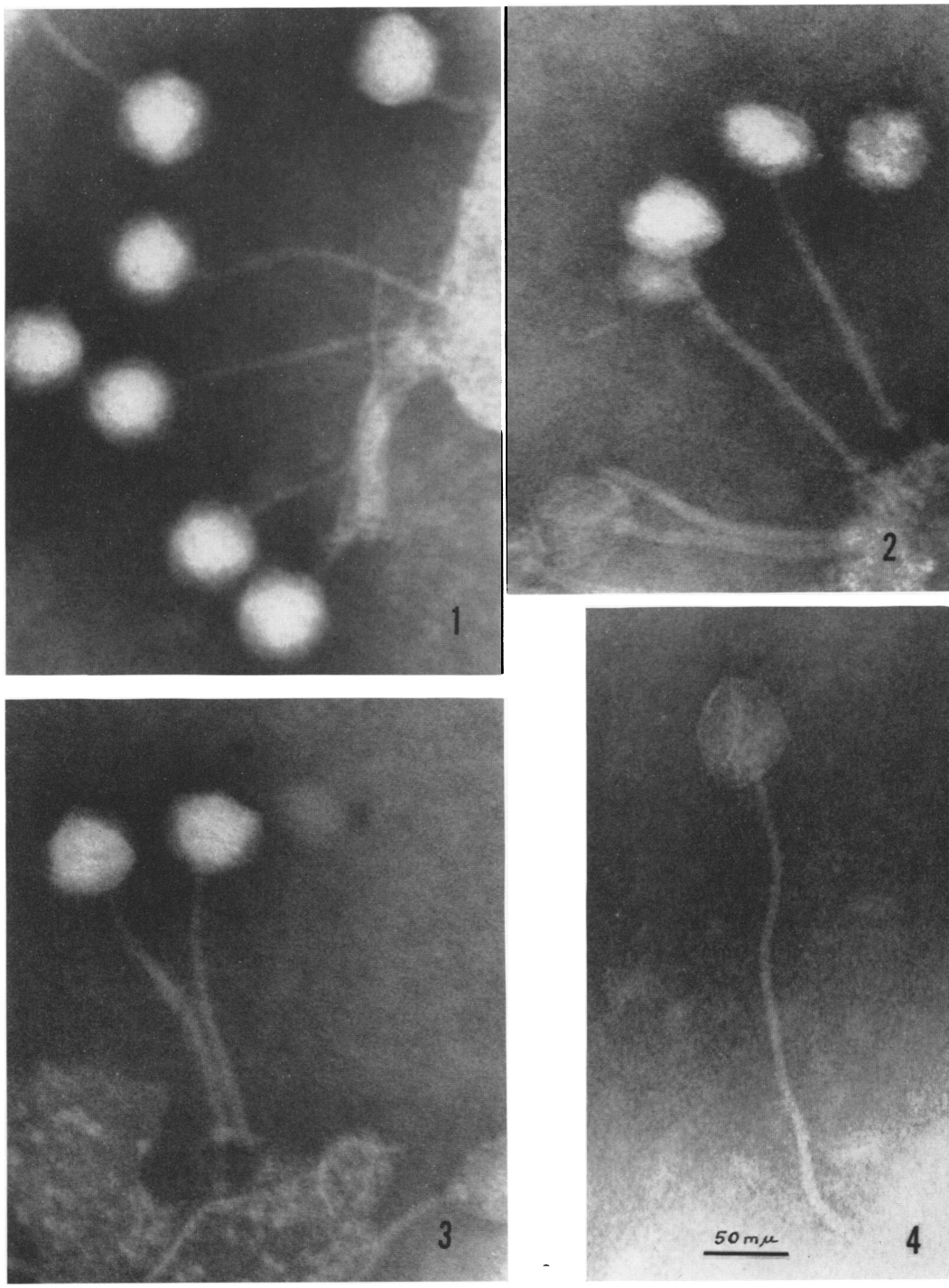

FIG. 1–4. *Bacteriophages from lysates of C. botulinum type A, proteolytic and nonproteolytic types B.* ×*258,000. (1) Type A, strain B1G4; (2) non-proteolytic type B, strain 2B; (3) nonproteolytic type B, strain 17B; (4) proteolytic type B, strain 169B.*

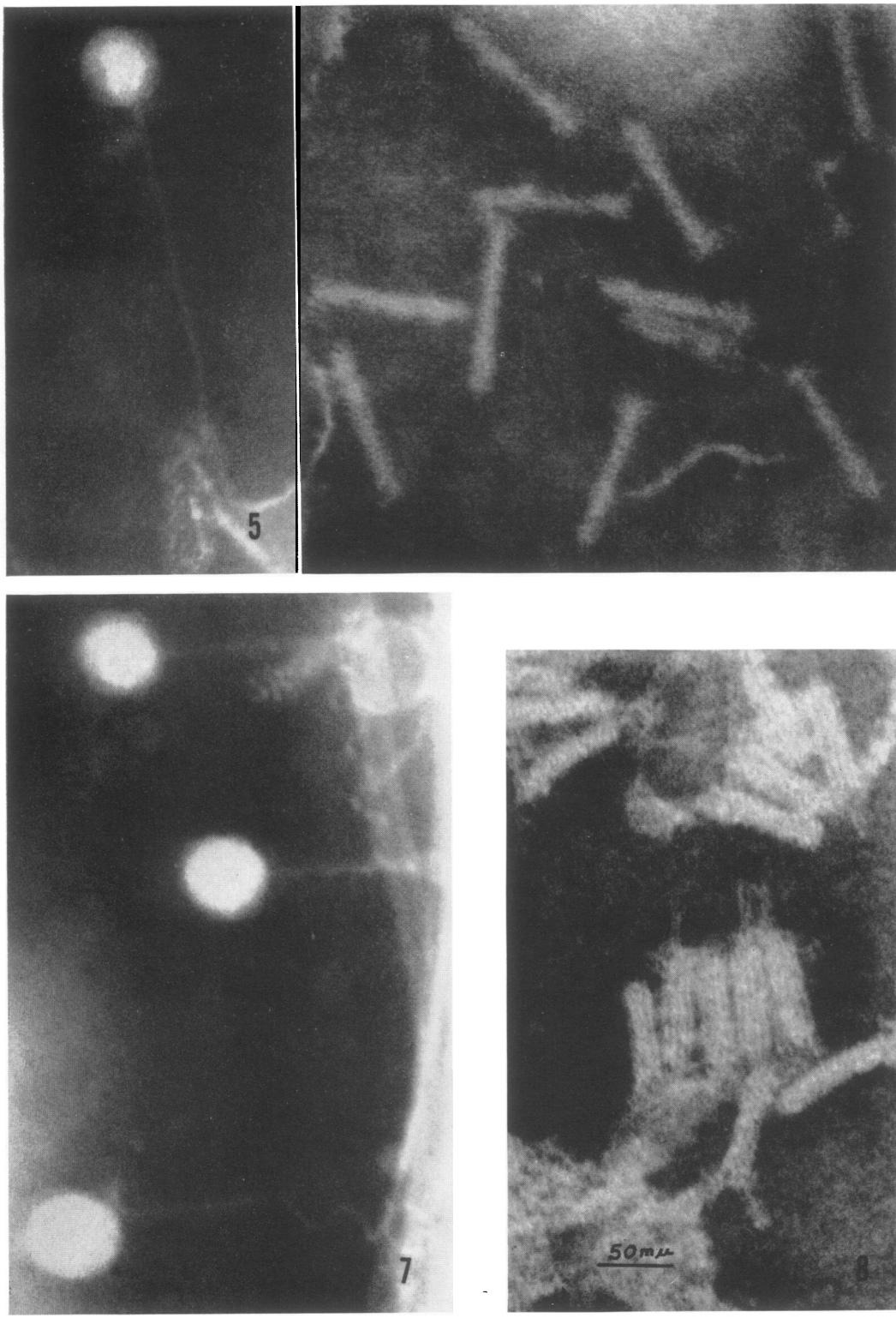

FIG. 5–8. *Bacteriophages and phage tail-like structures from lysates of C. botulinum type E and organisms resembling type E.* ×*258,000. (5) Type E, strain 066B; (6) type E, strain 8E; (7) type E, strain Beluga; (8) non-toxigenic strain P4.*

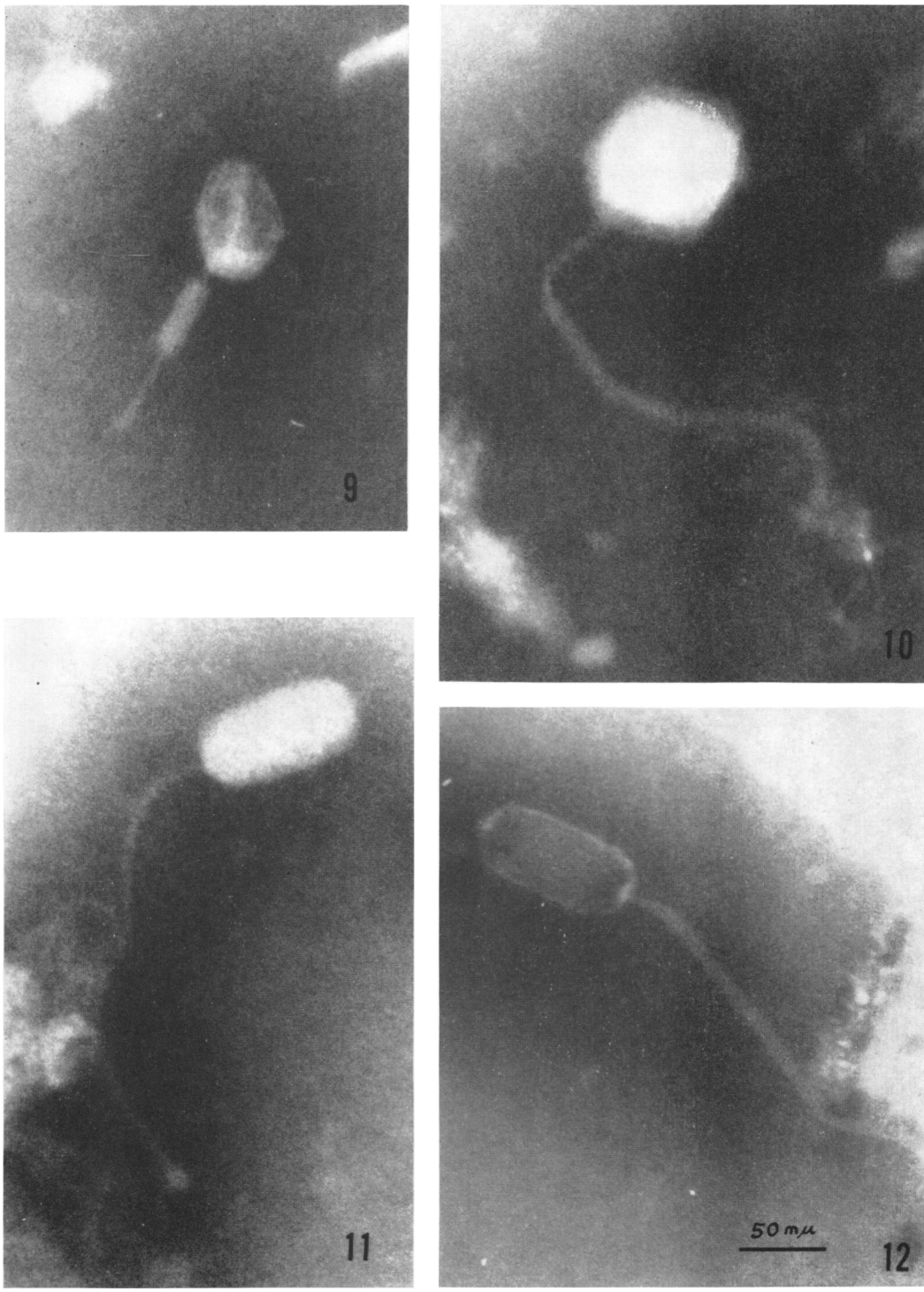

FIG. 9–12. *Bacteriophages from lysates of nonproteolytic and proteolytic strains of C. botulinum type F. (9 and 10) Nonproteolytic type F, strain 70F; (11 and 12) proteolytic type F, strain 8G.*

and nontoxigenic 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 stain 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 nontoxigenic 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

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