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Detection of Bacteriophages from Two Strains of *Clostridium tetani*

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Recent investigations in this laboratory (L. M. Prescott and R. A. Altenbern, J. Bacteriol. **93:** 1220, 1967) have shown that all of seven strains of *Clostridium tetani* exhibited lysis after induction by mitomycin C. Inhibitors of protein, deoxyribonucleic acid, and ribonucleic acid synthesis prevented lysis after exposure to mitomycin C. Neither any of the *C. tetani* strains nor the two strains of *C. tetanomorphum* showed bacteriophage plaques when plated with appropriate dilutions of filtrates from lysed cultures. Attempts to show "killer" or bacteriocin activity in such filtrates against the strains mentioned above were also unsuccessful. In this study, electron microscopy of pellets derived from high-speed centrifugation of mitomycin C-induced lysate of two strains of *C. tetani* revealed distinct bacteriophages.

Flasks of Fluid Thioglycollate Medium (FTM; Difco), 160 ml per 250-ml flask, were inoculated with 20 ml of a 24-hr culture, in FTM, of *C. tetani* strain 10779 or 453 (ATCC). After incubation at 37 C for 150 min, mitomycin C was added (3 μg/ml for 10779, 5 μg/ml for 453). The flasks were then incubated for 7 to 8 hr, when lysis was complete. The culture was clarified by centrifugation at 6,000 rev/min for 60 min in a Servall GSA head. The pellet was discarded and the supernatant fluid was centrifuged for 150 min at 30,000 rev/min in the no. 30 rotor of a Spinco Model L centrifuge. The pellet obtained from 200 ml of clarified lysate was resuspended in 2 ml of 10% glycerol. The resulting suspension was examined in the electron microscope, by use of the pseudoreplica technique with 2% ion agar and parlodion film. The preparation was stained with 2% phosphotungstic acid adjusted to *p*H 5.0 with KOH and examined in the Siemens Elmiskop 1A at 80 kv and 20, 40, 60, and 80 thousand times. Prints were enlarged three times.

Bacteriophages from lysates of *C. tetani* strains 10779 and 453 are presented in Fig. 1. The phage from strain 453 exhibited mainly phage tails and empty ghosted heads of hexagonal outline 60 to 65 mμ in diameter, although about 20% of the heads were electron-dense (not shown) and presumably contained nucleic acid. The tails consisted of a tail tube 100 to 110 mμ in length and 10 to 12 mμ in diameter surrounded by a sheath, about 50 mμ in length and 20 mμ in diameter, which commenced at the proximal end of the tail tube. The sheath appeared to be constructed of a regular array of clearly visible subunits arranged in typical helical symmetry. Occasional sheaths were noted which appeared to be contracted to a length of approximately 30 mμ. Many instances of association of two to four tails at the distal ends of the tail tubes were noted. Clearly visible in some photographs were tail tubes with an apparent head plate extended into empty heads. The phage from strain 10779 possessed a structurally simple tail 100 mμ in length and approximately 5 mμ in width. The heads were 60 to 65 mμ in diameter and did not show clearly a hexagonal outline as did the phage from 453. Most of the heads of phage from strain 10779 were electron-dense, although ghosted heads were noted. The distal ends of the tails appeared to unravel in the majority of the phage particles examined. Preparations from both *C. tetani* strains showed considerable debris, presumably from lysis of the bacterial cells.

Both of these strains of *C. tetani* were efficient producers of tetanus toxin. Earlier investigations (L. M. Prescott and R. A. Altenbern, J. Bacteriol. **93:**1220, 1967) showed that toxinogeny was not related to mitomycin C-induced lysis, presumably because of these phages. Electron microscope examination of sediment obtained from uninduced cultures of these strains failed to reveal any phage particles. Probably similar examination of lysates from other *C. tetani* strains would reveal other phages harbored in this organism. There was no evidence that either of the strains investigated was multiply lysogenic. To the authors' knowledge, these are the first electronmicrographs of *C. tetani* bacteriophages.

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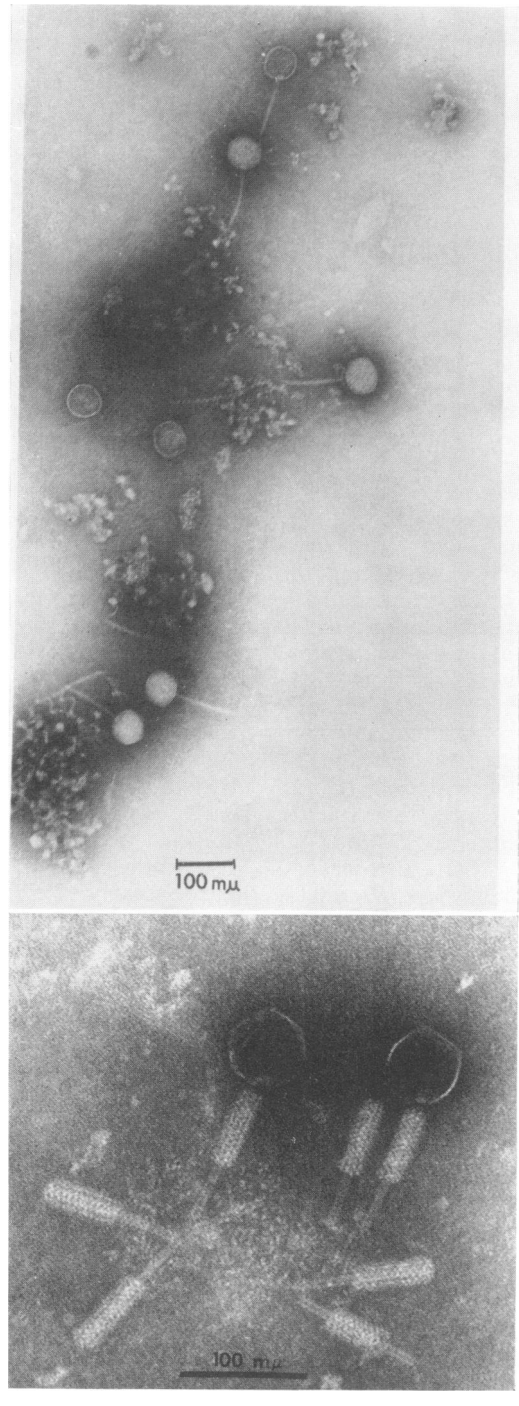

FIG. 1. *Bacteriophages from lysates of Clostridium tetani, strains 10779 and 453. (a) Phage from strain 10779, (b) phage from strain 453.*