Crystalline Inclusions in *Bacillus thuringiensis*

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Crystalline inclusion bodies resembling those seen in *Clostridium cochlearium* were detected in cultures of *Bacillus thuringiensis* infected with bacteriophage.

The appearance of a note by Pope, Yolton, and Rode (3) prompts us to place on record observations made during an electron-microscopic study of a serotype 9 (Tolworth) strain of the protein crystal-forming insect pathogen *Bacillus thuringiensis*.

This bacterium produces a protein crystal in the form of a parasporal body measuring approximately 1 by 0.5 μm. This protein crystal is one of the toxins produced by the pathogen and its structure, as seen in electron microscope sections, has been described by Norris (2). Several bacteriophages are known to be active against *B. thuringiensis* (1). During a study of the growth cycle of one of these bacteriophages in cells of the Tolworth organism, a different kind of crystalline inclusion was shown to be released from late-logarithmic-stage cells on lysis following bacteriophage infection.

Cultures were infected with bacteriophage and the cells were harvested at various time intervals after infection. Fixation was by glutaraldehyde-osmium, and the fixed material was embedded in araldite. Sections were poststained with uranyl acetate and lead hydroxide. The crystalline inclusions were distributed in small groups as though clusters of them had been released from lysed cells. They stained intensely with the protein stains and bear an obvious resemblance to the bodies described in *Clostridium cochlearium* by Pope et al. (3). Figures 1–6 show examples typical of many released crystalline bodies examined. Some of the figures show that bacteriophage was plentiful in the preparations and many of the cells were heavily infected. The appearance of the individual crystal lattice depends on the angle of sectioning. Figures 1 and 2 show lattices closely resembling those described by Pope et al. (3). Figure 6 suggests that the "rod" structure described by Pope et al. could arise from the overlapping of lattice planes in a crystal whose structure is basically one of hexagonally packed subunits. This hexagonal packing of subunits, which may be hollow cylinders, is clearly seen in Fig. 3–6.

In only one case did we detect these crystalline inclusions actually inside a bacterial cell. Figure 7 shows an obvious inclusion within a vegetative cell of *B. thuringiensis*. This cell was present in a preparation showing considerable amounts of released crystal material.

When the crystalline inclusions were first noted, we considered the possibility that they were early stages in the development of the protein crystal toxin of this insect pathogen. There appears, however, to be little similarity between the hexagonal array of subunits with a center-to-center spacing of approximately 9.5 nm seen in the inclusions and the structure of the toxic crystals, the basic subunit of which appears to be a rod-shaped or dumbbell-shaped structure measuring approximately 4.7 by 12.0 nm (2). Sections of parasporal bodies are illustrated in Fig. 8 and 9 for comparison.

Crystalline bodies were found only in cultures infected with bacteriophage; this supports the suggestion by Pope et al. (3) that the clostridial inclusions might conceivably result from defective phage production.

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LITERATURE CITED


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Fig. 1–6. Crystalline bodies lying among vegetative cells of Bacillus thuringiensis.
FIG. 7. Crystalline body within a cell of B. thuringiensis.

FIG. 8. Parasporal body of B. thuringiensis within a sporangium.

FIG. 9. Parasporal body after release from sporangium.