

Organization of Bacteriophage Tail-Like Particles in Cells of *Chromobacterium violaceum*

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A strain of *Chromobacterium violaceum* has been isolated which produces bacteriophage tail-like particles in high numbers. The extracellular morphology and the intracellular arrangement of these particles are described.

Bacteriophage-like and bacteriophage tail-like particles have been observed by several investigators in bacteriocinogenic cultures of bacteria (1-3). These structures have generally been seen as single particles in either the contracted or uncontracted state. However, several workers have reported unique aggregations of these particles. Examples of these aggregations include the intracellular organization of rhabdosomes described by Lewin and Kiethe (4) in cells of *Saprosira grandis* as well as the extracellular rosette aggregation of particles found by Farkas-Himsley et al. (2) in a lysate of a mitomycin C-treated culture of a bacteriocinogenic strain of *Vibrio comma*.

We have isolated from an acid mine drainage in Central Pennsylvania a strain of *Chromobacterium violaceum* which produces bacteriophage tail-like particles that demonstrate a striking extra- and intracellular arrangement.

Cells cultured in tryptone broth (8.0 g per liter of distilled water), pH 7.0, at 28 C on a rotary shaker demonstrate normal growth characteristics when measured spectrophotometrically at 600 or 430 nm. However, phase microscope and electron microscope observations show that as the culture approaches and continues on into stationary phase the frequency of the appearance of spheroplasts and cell ghosts markedly increases. Figure 1 shows a negatively stained preparation of a stationary-phase culture which reveals, in addition to polar flagellation of cells, a number of particles resembling contracted phage tails. A few particles resembling uncontracted tails are also visible. The majority of the particles are

in the contracted state, with baseplate and fibers projecting from the contracted sheath. Very rarely, contracted particles can be seen with an empty bacteriophage-like head attached (Fig. 1).

Well-organized aggregates appearing as "strings of firecrackers" (Fig. 2, 3, 4) are commonly seen. These aggregates generally consist of contracted particles with the core piece toward the "string." Additionally, highly organized rosettes are seen both by negative staining (Fig. 5) and within cell ghosts by thin sectioning (Fig. 6). These rosettes are seen in cell ghosts produced upon lysis of exponential-phase cells removed from the growth medium and suspended in 0.8% NaCl (w/v) as well as in stationary phase cells. Figure 7 shows a thin section of a single cell ghost from a stationary phase culture containing numerous rosettes. As many as 19 rosettes have been seen within a single section of a cell ghost. Thin sections of intact cells also reveal rosettes (Fig. 8, 9). These rosettes always appear to displace considerable cytoplasm and to be in close association with the cell membrane. As many as five rosette structures have been seen in a section of a single cell.

We have observed that exponential-phase cells of *C. violaceum* undergo lysis with the release of tail-like particles following treatment with mitomycin C. Attempts to demonstrate a biological activity for the particles described herein have thus far been unsuccessful. Studies on the biochemical nature, the biological significance of the particles, and their unique aggregation are being continued.

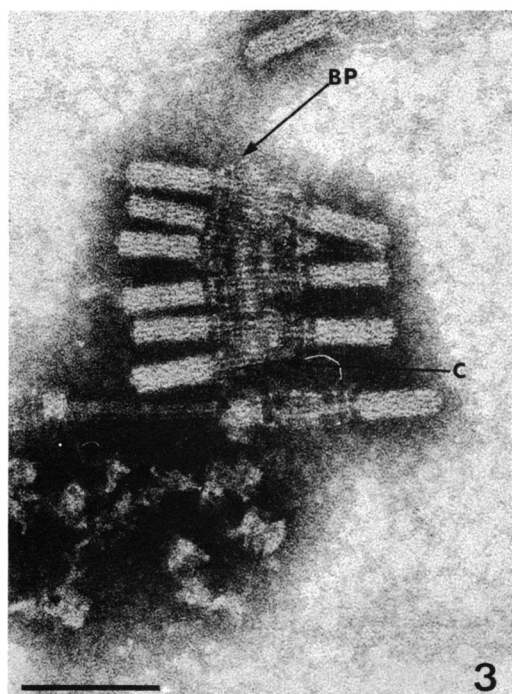
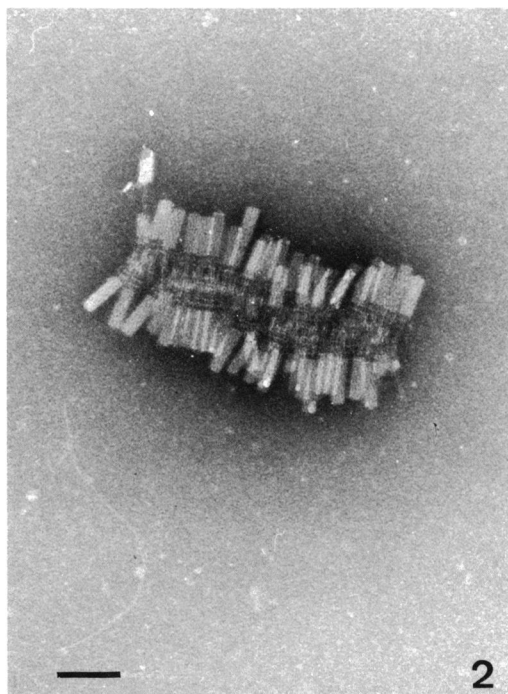
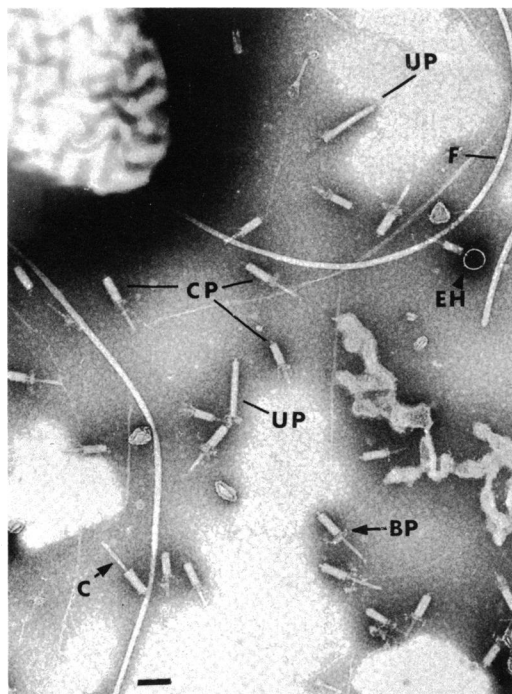


FIG. 1. Negatively stained stationary-phase culture of *Chromobacterium violaceum* (36 hr). A number of contracted tail-like particles are visible. Note base plate structure and exposed core piece. Also seen is an uncontracted particle as well as a particle with an attached empty head. Negative staining was with 2.0% phosphotungstate. Marker bar represents 100 nm. Abbreviations: F, flagella; CP, contracted particle; C, core piece; UP, uncontracted particle; BP, base plate; EH, empty head.

FIG. 2. Negative stain (with 2.0% phosphotungstate) of a "string of firecrackers" aggregate of contracted tail-like particles. Marker bar represents 100 nm.

FIG. 3. Negative stain (with 2.0% phosphotungstate) showing arrangement of particles in "string of firecrackers." Note baseplate (BP) and core pieces (C). Marker bar represents 100 nm.

FIG. 4. Shadow cast preparation of particle aggregate. One particle not in the aggregate appears to be uncontracted. Marker bar represents 100 nm.

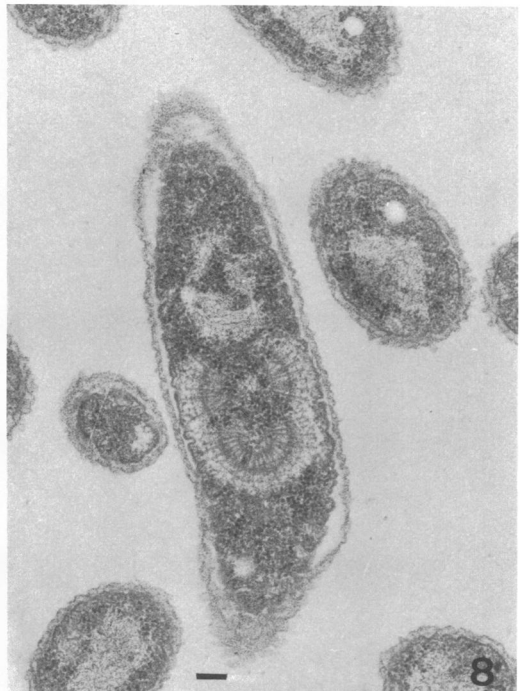
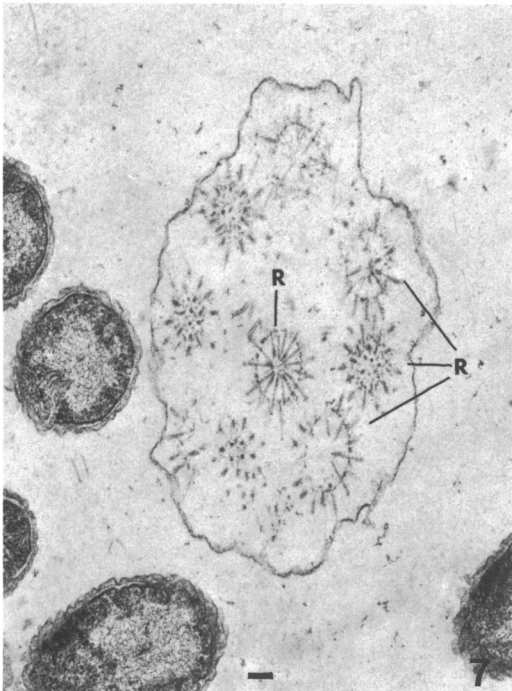
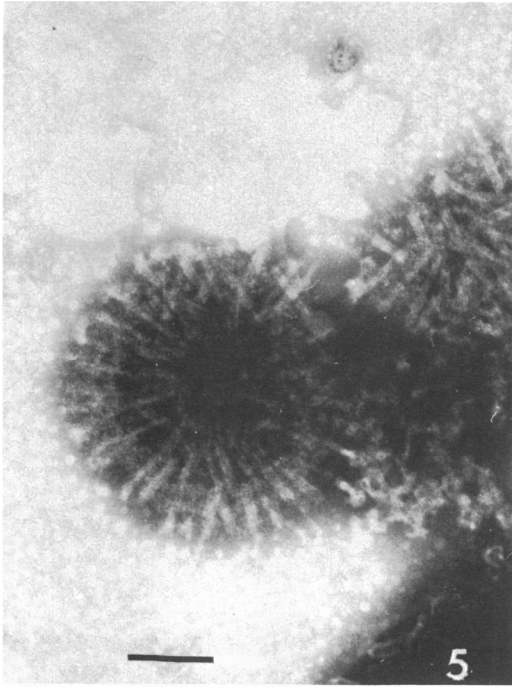


FIG. 5. Rosette-like aggregate of tail-like particles. Marker bar represents 100 nm.

FIG. 6. Thin section of ghosted cell with well-defined particle rosette. Cells prepared for electron microscopy by the Ryter-Kellenberger (5) method and embedded in low-viscosity epoxy resin (6). Marker bar represents 100 nm.

FIG. 7. Thin section of cell ghost containing at least eight rosettes (R) of tail-like particles. Marker bar represents 100 nm.

FIG. 8. Longitudinal section through a cell showing the central location of two rosettes. Marker bar represents 100 nm.

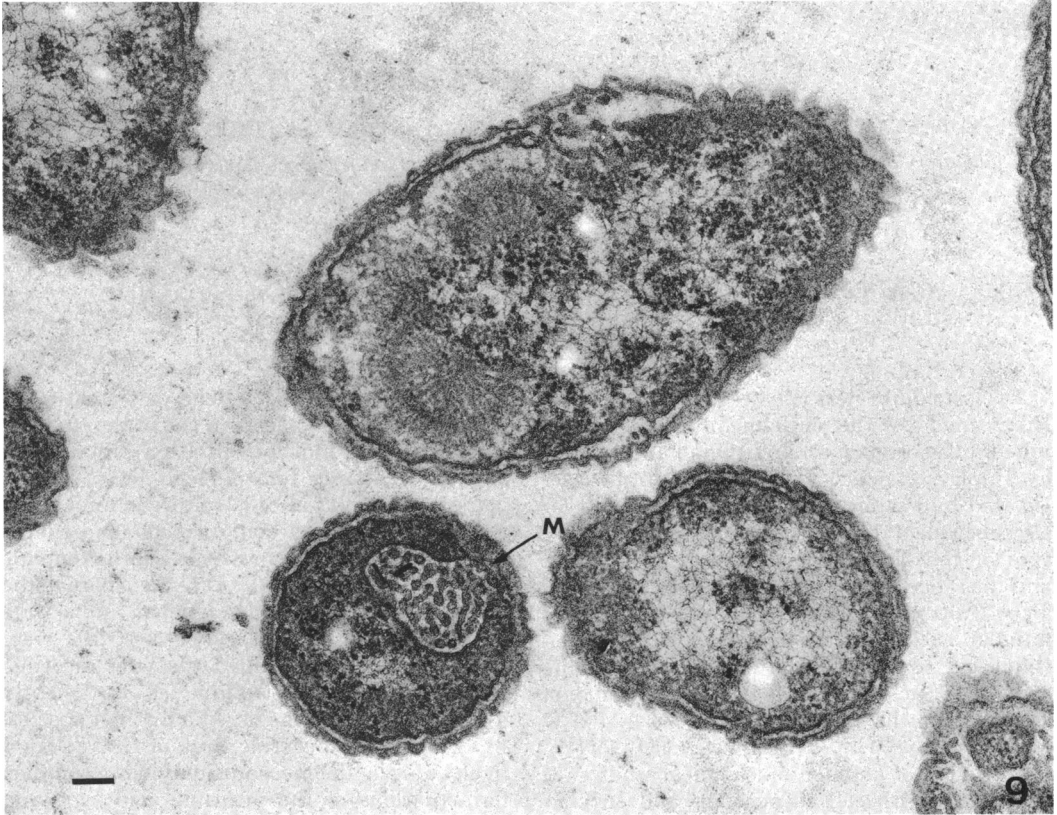


FIG. 9. Thin section through a cell showing two rosettes at the pole of the cell. Note mesosome (M) in adjacent cell. Marker bar represents 100 nm.

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