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Mitomycin C-induced Phage-like Particles in a Mutant of *Mycobacterium tuberculosis* BCG

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Mitomycin C is known to be the most efficient antibiotic which induces bacteriocins in certain mutants of gram-positive and gram-negative bacteria (2). Although it has been reported to be bacteriocidal to mycobacteria (3), successful isolation of bacteriocins from this genus has not been accomplished.

During our studies on mutants isolated from various species of mycobacteria, a mutant of *Mycobacterium tuberculosis* BCG was found to produce bactericidal phage-like particles after mitomycin C treatment.

The BCG strain used in the present study was originally obtained from the Instituto Nacional de Tuberculosis, Caracas, and was maintained in a Lowenstein-Jensen medium. A spontaneous mutant occurred during cultivation at 37 C in Middlebrook 7H9 Broth (Difco) without enrichment. This mutant strain, characterized by short rod-shaped cells containing acid-fast granules, grew more rapidly in various liquid media, such as Penassay (Difco), Middlebrook, and Dubos (Difco) broths, than did the parent strain. It showed smooth surface colonies when grown on Dubos Oleic Agar (Difco) enriched with 0.25% bovine serum albumin fraction *V* (Sigma Chemical Co., St. Louis, Mo.) and 0.75% dextrose.

Ultrastructurally, the mutant cell is enclosed by a very thick cell wall, varying from 250 to 800 A (Fig. 1), which differs from that of the parent strain (Fig. 2).

Induction of phage-like particles was accomplished by adding mitomycin C (Sigma Chemical Co.) to mid- or late exponential cells grown in either Penassay or Middlebrook Broth, at a final concentration of 1.0 to 2.0 μg/ml. After 2 days of cultivation with mitomycin C at 37 C, cells were harvested by centrifugation at 10,000 $\times$ *g* for 30 min. Figure 3 shows an ultrathin section of osmium-fixed cells after mitomycin C treatment. Hexagonal dense bodies can be seen in the cytoplasm. Cells resuspended in 0.1 M ammonium acetate solution were partially disrupted in a Waring Blendor. After removing the undisrupted cells and large cellular debris by centrifugation at 10,000 $\times$ *g* for 15 min, pellets were sedimented at 30,000 $\times$ *g* for 2 hr. All procedures were performed at 4 C.

These pellets contained phage-like particles (Fig. 4). As observed in ultrathin sections, these particles have hexagonal heads, which are interpreted as being octahedral in three dimensions. The morphology of these particles resembles that of mycobacteriophages.

These particles were found to kill both *M. smegmatis* ATCC 607 and the parent BCG strain; e.g., colony-forming units of both sensitive species decreased significantly after mixing with the particle suspension. However, they did not cause any lysis of the major populations of indicators grown in liquid media, nor did they form any transparent plaques with the double agar method (1), implying that they are incapable of intracellular multiplication. These data strongly suggest that the phage-like particles induced in the BCG mutant by mitomycin C should be categorized as bacteriocins.

ACKNOWLEDGMENT

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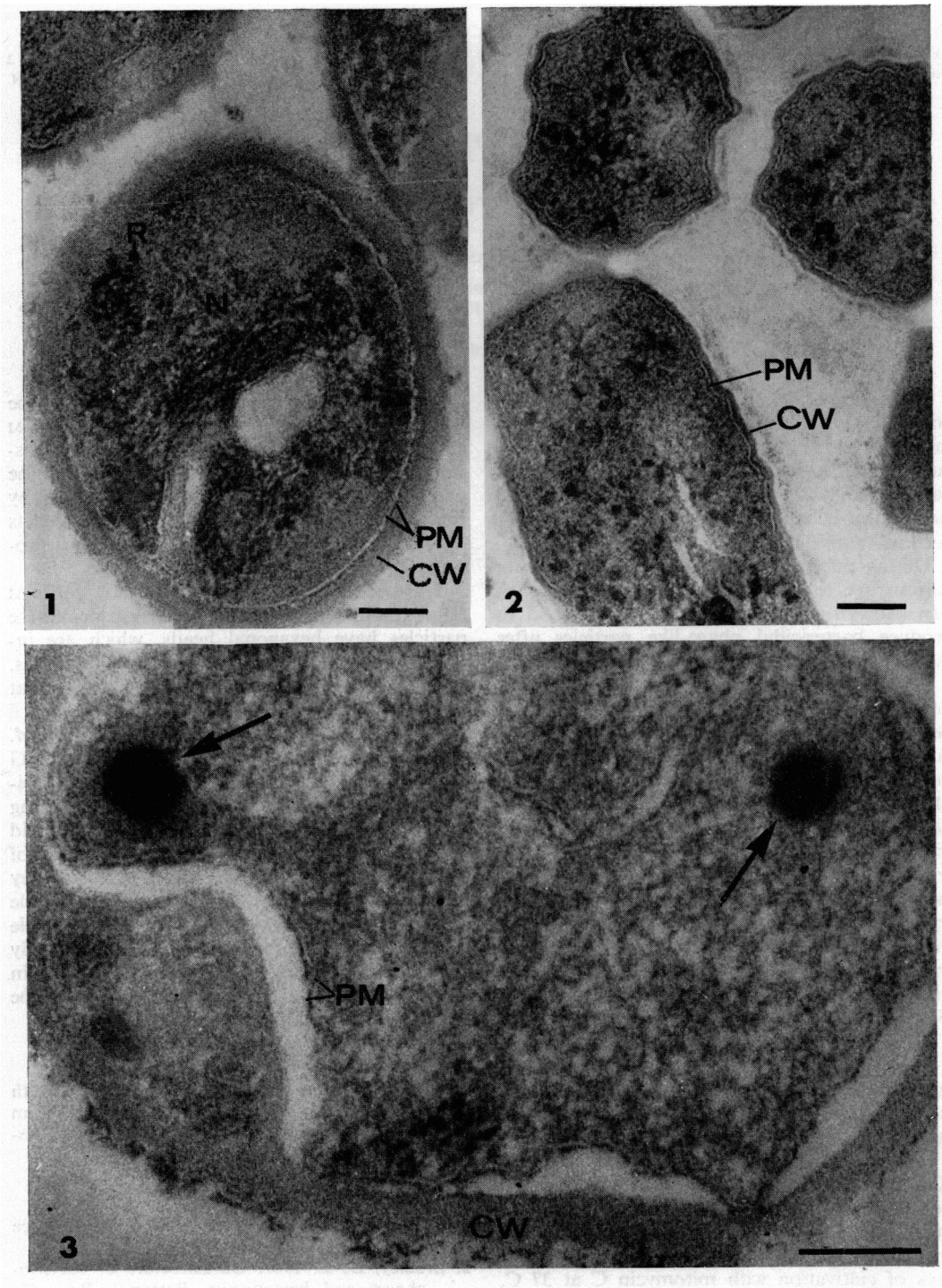

FIG. 1. *Ultrathin section of a mutant strain of Mycobacterium tuberculosis BCG at the exponential growth phase in Penassay Broth. Cells were fixed in 4% aqueous formaldehyde solution containing 1% NaCl and 0.2% $CaCl_2$, followed by 1% osmium tetroxide in the same salt solution. Staining with 2% uranyl nitrate was done before dehydration. Note thicker cell walls and denser cytoplasmic ground substances of this mutant than those of the parent strain shown in Fig. 2. N, nuclear substance; PM, plasma membrane; CW, cell wall; R, ribosome. Marker: 1,000 A.*

FIG. 2. *Ultrathin section of the parent strain of Mycobacterium tuberculosis BCG at the exponential growth phase. The fixation method was the same as in Fig. 1. PM, plasma membrane; CW, cell wall; R, ribosome. Marker: 1,000 A.*

FIG. 3. *Ultrathin section of a BCG mutant after mitomycin C treatment (1.0 μg/ml) for 2 days. Two dense bodies (arrows) of roughly hexagonal shape lie near the plasma membrane (PM), which detaches from the inner surface of the cell wall (CW). Marker: 1,000 A.*

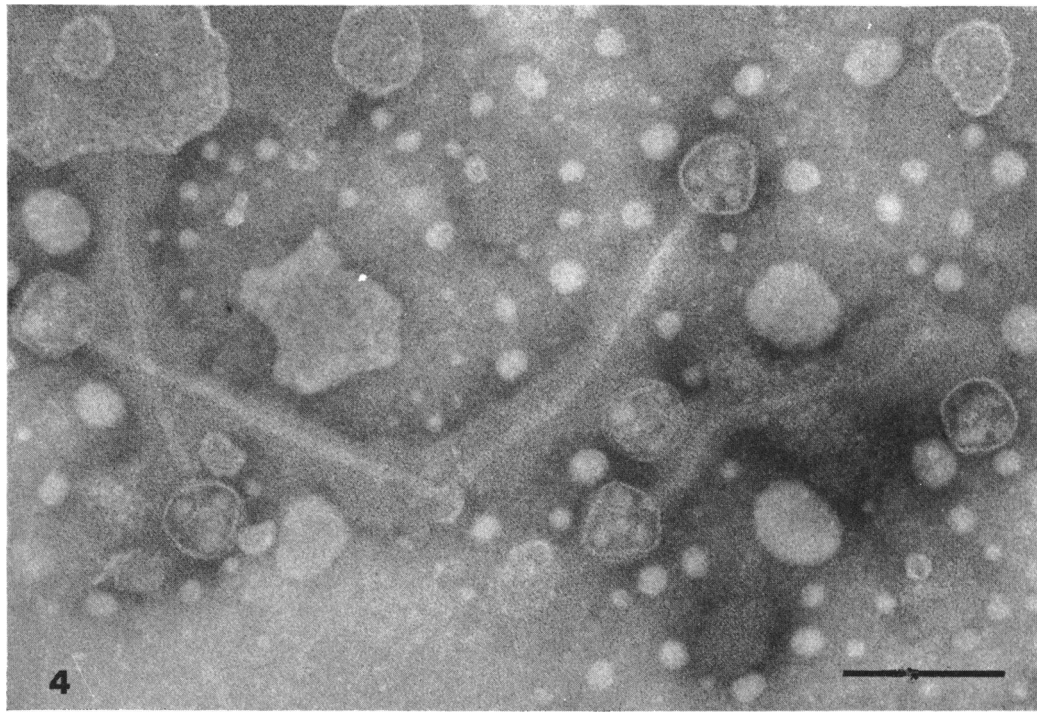

FIG. 4. *Negatively stained (2% sodium silicotungstate at pH 7.6) phage-like objects isolated from mitomycin C-treated BCG mutant. Some of them lack tails, possibly due to disruption during preparation. Marker: 1,000 A.*