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NOTES

Pesticin-Dependent Generation of Osmotically Stable Spheroplast-Like Structures

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Homogeneous pesticin, a bacteriocin produced by *Yersinia pestis*, promoted rapid dose-dependent killing of *Escherichia coli* φ but permitted residual generation of cell mass. Both growing cells and those blocked in net synthesis of nucleic acids or protein were converted by pesticin to osmotically stable spheroplast-like forms. Morphology and viability of cells starved for fermentable carbohydrate were not affected by pesticin. Similar spheroplast-like structures were formed from sensitive cells of *Yersinia pseudotuberculosis, Yersinia enterocolitica,* and *Y. pestis.*

Cells of wild-type *Yersinia pestis* produce a bacteriocin, termed pesticin (1), that is active against serotype I strains of *Yersinia pseudotuberculosis* (4), a few isolates of *Yersinia enterocolitica* (8), and some colicin-indicator strains of *Escherichia coli* including φ but not K-12 (3, 5). Pesticin is a monomer of 63,000 daltons which, like certain colicins (6), is deficient in cysteine and can exist in alternative conformer states (7). Elgat and Ben-Gurion (5) reported that pesticin reduced net synthesis of DNA and promoted degradation of ribonucleic acid in cells of *E. coli* φ. These findings were interpreted as evidence suggesting a mode of action similar to that of colicin E2, which is known to cause extensive hydrolysis of DNA in vivo (10). In this communication we describe an additional ability of pesticin to affect conversion of sensitive bacteria to osmotically stable spheroplast-like structures. We also show that pesticin-induced lethality occurs in the absence of net macromolecular synthesis but is dependent upon the presence of a fermentable carbohydrate.

Homogenous preparations of pesticin, prepared as previously described from *Y. pestis* A1122 (7, 8), were dialyzed overnight at 5°C against 5.0 mM sodium morpholinopropane sulfonate (MOPS) buffer (pH 7.0) before they were stored at −20°C. The bacteriocin remained fully active under this condition for 5 months. Activity of pesticin was determined by the double-agar layer technique using medium containing ethylenediaminetetraacetate in excess Ca^{2+} (3); units are defined as the reciprocal of the highest dilution exhibiting a zone of inhibition against *Y. pseudotuberculosis* PB1/0 after incubation for 24 h at 37°C.

Before use in experiments, cells of *E. coli* φ (*met pur pan*) were transferred twice (8 to 10 generations) in defined MOPS-buffered medium (9) supplemented with L-methionine (0.5 mM), hypoxanthine (0.5 mM), and calcium pantothenate (5.0 μM). The medium contained added sodium thiosulfate (2.5 mM), L-threonine, L-methionine, L-isoleucine, L-valine, and L-phenylalanine (all 0.5 mM), and thiamine, calcium pantothenate, and biotin (all 5.0 μM) when used for growth of yersiniae. Bacteria were aerated (200 rpm) at 37°C on a model G76 gyratory shaker (New Brunswick Scientific Co., New Brunswick, N.J.) in 25 ml of medium per 250-ml Erlenmeyer flask. Optical density was measured at 620 nm with a model 2000 Gilford spectrophotometer (Gilford Instrument Co., Oberlin, Ohio). Dry weight was determined from organisms washed, suspended, and dried (100°C) in 0.15 M ammonium acetate buffer (pH 7.0). Viability was assayed with blood agar base (Difco).

Addition of pesticin to exponentially growing *E. coli* resulted in immediate dose-dependent loss of ability to form colonies (Fig. 1). Nevertheless, a residual increase in optical density of cultures receiving pesticin was always observed. For example, addition of sufficient pesticin to reduce viability by >99% permitted a 6-fold increase in cell mass; a corresponding 10-fold increase occurred in a parallel culture lacking the bacteriocin (Fig. 2). Attempts to subculture pesticin-treated cells in fresh medium were not successful. These findings indicate that although

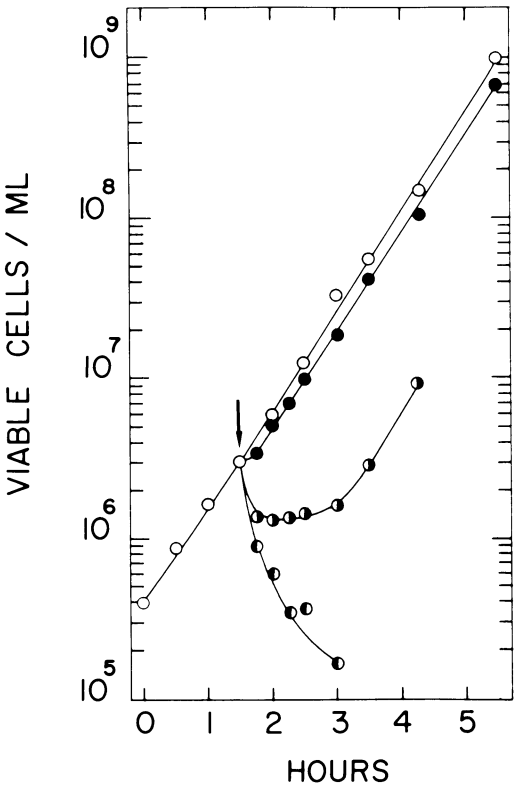

FIG. 1. *Viability of cells of E. coli φ after addition (arrow) of none (○), 4 U (●), 20 U (◑) and 40 U (◐) of pesticin per ml of MOPS-buffered defined medium.*

the lethal effect of pesticin is rapid, its mode of action is such that treated cells remain capable of about 2.5 doublings in mass.

Identical results were obtained with pesticin-sensitive *Y. pseudotuberculosis* PB1/0, *Y. enterocolitica* P76M, and *Y. pestis* 2C. Observation by phase contrast with a Zeiss microscope revealed that these organisms and *E. coli φ*, but not K-12, became converted after addition of pesticin to spheroplast-like forms (Fig. 3). These structures, which were not observed to undergo division, were stable in distilled water and thus differ from spheroplasts generated by extensive hydrolysis of murein or inhibition of its biosynthesis. A similar morphological change occurred after pesticin-treated organisms were diluted and plated on blood agar base. No significant differences in envelope structure of control and pesticin-treated cells were detected upon observation of thin sections with an electron microscope.

Exponentially growing cells of *E. coli φ* were washed twice in 5.0 mM MOPS buffer (pH 7.0) and then starved for 30 min by aeration in the same buffer. The organisms were then used to prepare parallel cultures of complete medium and media lacking D-glucose, hypoxanthine, or L-methionine. After incubation for 2 h, one culture of each set received pesticin, and loss of viability was determined. Colony-forming ability was rapidly lost with subsequent formation of large spheroplast-like forms in fully supplemented medium (Fig. 4A). Extensive killing also occurred in medium lacking hypoxanthine (Fig. 4C) or L-methionine (not shown) accompanied by conversion to small dense spheroplast-like forms. Accordingly, lethality was not dependent upon net synthesis of nucleic acids or protein. In contrast, cells incubated in the absence of D-glucose were resistant to pesticin (Fig. 4B). This result would be expected if an energized membrane was necessary for irreversible absorption of the bacteriocin.

These findings suggest that the mode of action of pesticin more resembles that of colicin M (2) than colicin E2. The ability of pesticin to catalyze partial hydrolysis of murein in vitro will be described in a subsequent report.

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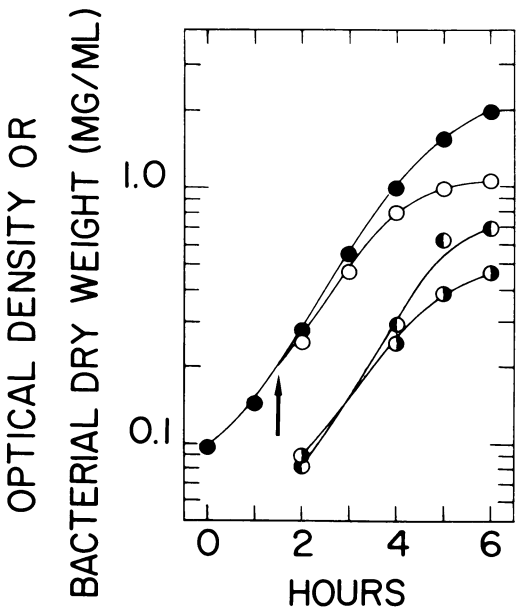

FIG. 2. *Comparison of optical density and dry weight of E. coli φ grown without and with (arrow) pesticin (50 U/ml) in MOPS-buffered defined medium. Symbols: (●) Optical density of control; (◐) dry weight of control; (○) optical density with pesticin; and (◑) dry weight with pesticin.*

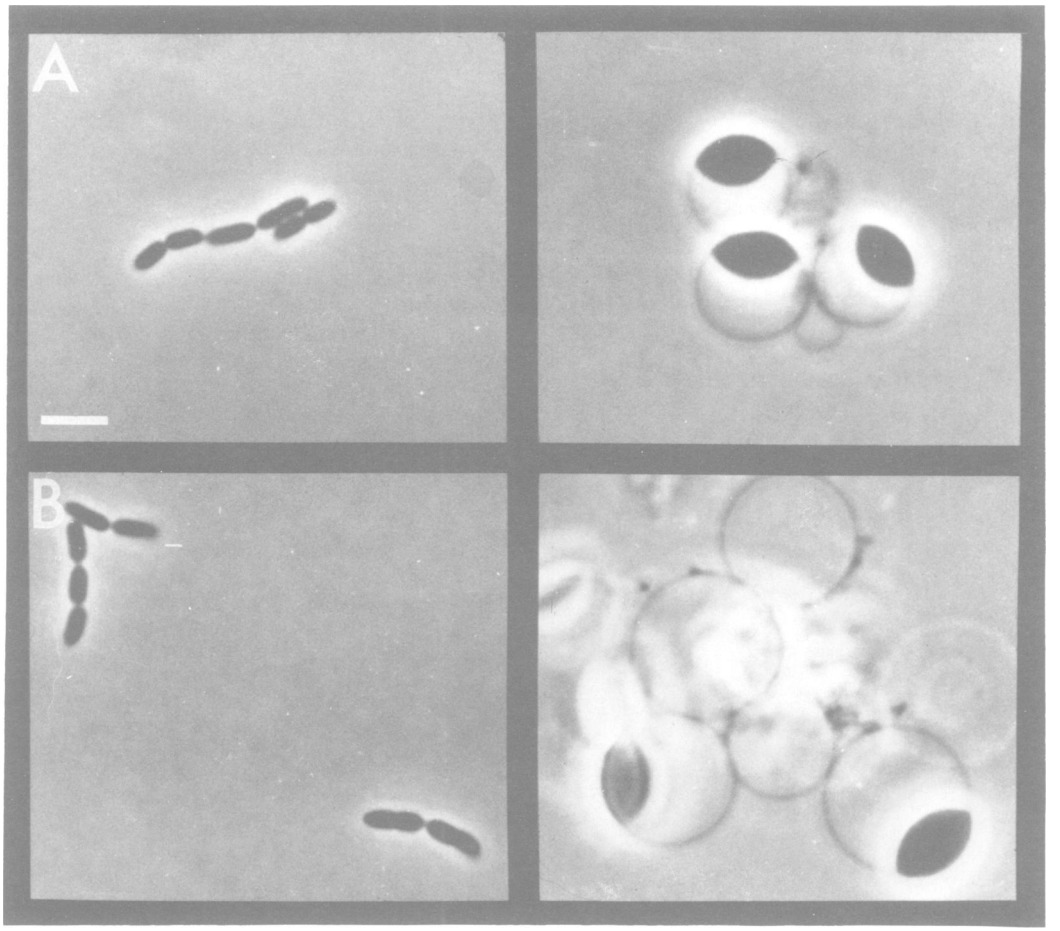

FIG. 3. *Morphology of cells of Y. enterocolitica P76M after cultivation for 4 h (A) and 6 h (B) without pesticin (left) or with 40 U of pesticin per ml of MOPS-buffered defined medium (right).*

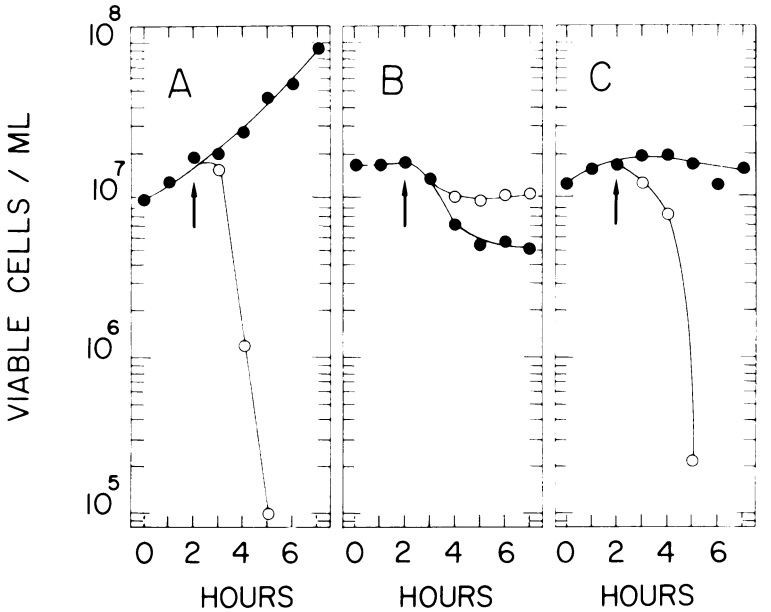

FIG. 4. *Viability of previously starved cells of E. coli φ during cultivation in fully supplemented MOPS-buffered defined medium (A), and the same medium lacking* D*-glucose (B), or hypoxanthine (C). Symbols:* (●) *No added pesticin;* (○) *pesticin (100 U/ml) added where indicated (arrow).*

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