BRIEF NOTE

SURFACE CHARGE DETERMINATION OF *PROTEUS MIRABILIS* EXPOSED TO CARBENICILLIN[1]

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OHIO J. SCI. 77(5): 210, 1977

Proteus mirabilis undergoes a series of predictable morphological changes when exposed to different concentrations of carbenicillin (Perkins and Miller 1973). At low drug concentrations septum formation is inhibited but cell growth is not affected; thus, filamentation occurs. At higher drug concentrations, both septum formation and cell growth are inhibited and spheroplast formation occurs. In our experiments, the surface charge distribution of *P. mirabilis* exposed to various concentrations of disodium carbenicillin was studied using a positively charged colloidal-iron stain and transmission electron microscopy.

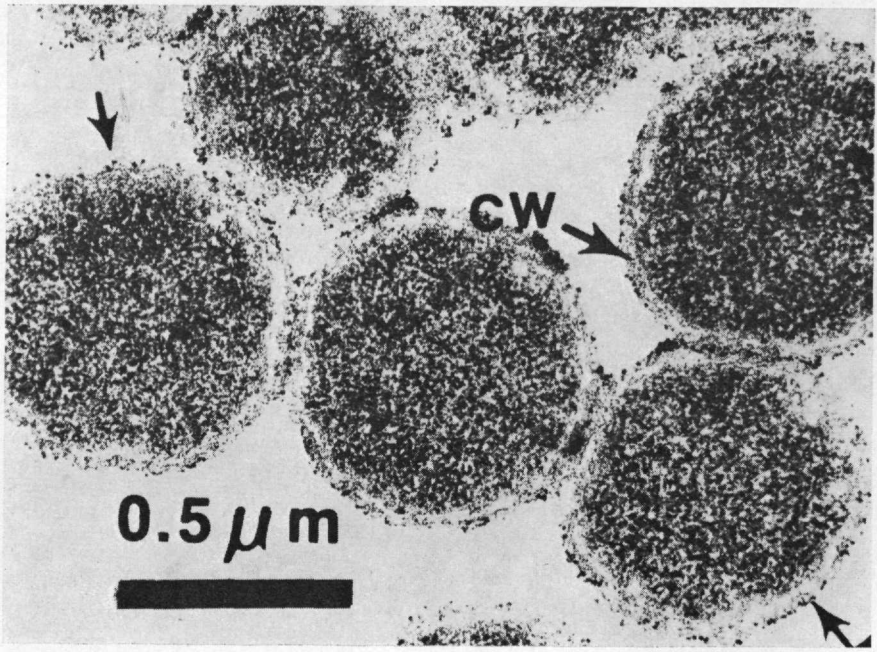

FIGURE 1. Transmission electron micrograph of control *P. mirabilis* exposed to varying concentrations of the minimal inhibitor concentration of carbenicillin stained with a positvely charged collodial-iron mixture showing attachment of iron particles (arrows) to surface of cell walls (CW).

P. mirabilis (minimal inhibitory concentration carbenicillin, 0.49 μg/ml) was cultured in trypticase-soy broth (TSB)

[1]Manuscript received March 4, 1977 and in revised form as a note April 28, 1977 (#77-27).

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for 4 hr and then 1 ml aliquots were transferred to 9 ml TSB containing various concentrations of carbenicillin. Final carbenicillin concentrations equivalent to 0.1, 1.0, 10, and 100 times the minimal inhibitory concentration (MIC) were studied. The antibiotic-containing cultures were incubated at 37°C for 4 hr. Control cultures of *P. mirabilis* exposed to drug-free diluent were treated in an indentical manner. Following exposure to the various drug concentrations, the cells were fixed in 1% glutaraldehyde in 0.05M phosphate buffer pH 7.3 with 4% sucrose as previously described by Prior and Perkins (1974). The cells were then washed and exposed to an acetic acid-iron colloid mixture at pH 1.8 for 15 min at 25°C. The colloidal iron-staining reagents were prepared and utilized according to the methods of Gasic *et al* (1968). Then the cells were washed first in acetic acid, then in distilled water, and were post-fixed subsequently in 1% osmium tetroxide. Following fixation, the cells were prepared for electron microscopy by the methods previously reported by Seed *et al* (1974) and ultrathin sections were examined with a Zeiss EM 9S electron microscope.

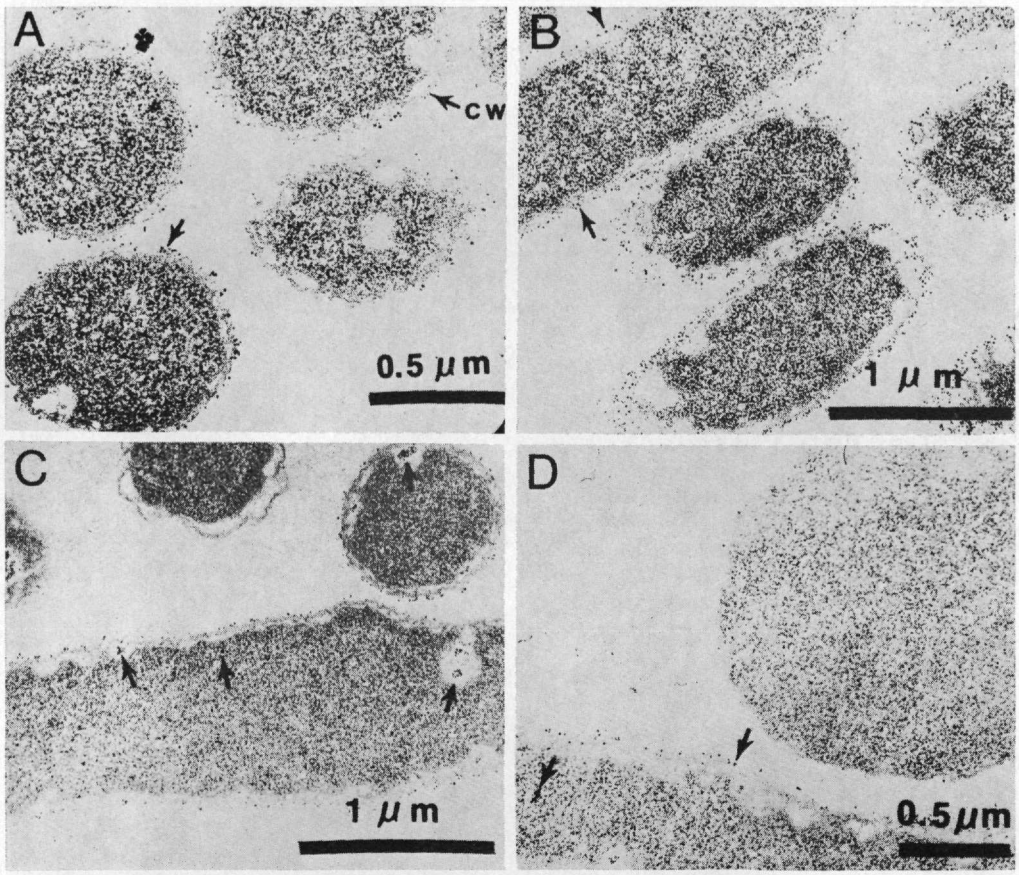

FIGURE 2. Transmission electron micrographs of *P. mirabilis* exposed to various concentrations of disodium carbenicillin and subsequently stained with a positively charged collodial-iron mixture. (A) Exposure to 0.1 times the MIC showing cytoplasmic vacuoles and attachment of iron particles (arrow) to surface of cell wall (CW). (B) Exposure to 1 times the MIC showing cytoplasmic vacuoles and attachment of iron particles to filamentous form (arrows). (C) Exposure to 10 times the MIC showing iron particle aggregates within cytoplasm and cytoplasmic vacuoles (arrows) as well as attachment to cell wall surface. (D) Exposure to 100 times the MIC showing filamentous form with iron particles attached to cell wall surface and within cytoplasm (arrows). A large spheroplast is also shown which lacks a cell wall and also attachment of iron particles to cytoplasmic membrane. (MIC=minimal inhibitory concentration).

The control *P. mirabilis* (fig. 1) had relatively uniform distribution of iron particles (arrows) attached to their cell wall surface (CW). These iron particles measured about 40Å in diameter. At 0.1 times the MIC of carbenicillin the cells had surface charge distribution similar to that of the control cells (fig. 2A). Small cytoplasmic vacuoles seen in these cells were attributed to the effects of the drug because previously published reports have described similar vacuoles (Fitz-James and Hancock 1965). At 1 times the MIC of carbenicillin the typical filamentous forms were observed (fig. 2B) and the surface charge distribution was similar to that observed in cells treated with the lower drug concentration. Cytoplasmic vacuoles along the inner portion of the cell wall were also present. At exposure to 10 times the MIC of carbenicillin, filamentous forms were also observed and the attachment of iron particles to a typical filamentous form is shown in figure 2C. Aggregates of iron particles were observed within the cytoplasm and within several vacuoles of cells treated with this higher drug concentration (fig. 2C). Penetration of the iron particles into the cytoplasm may be caused by change in permeability of the cell wall due to the action of the drug. A filamentous form and spheroplast produced during exposure to 100 times the MIC of carbenicillin are shown in figure 2D. Iron particles became attached to the cell surface and also aggregated within the cytoplasm of the filamentous form. The failure of iron particles to attach to the large spheroplast formed at 100 times the MIC of carbenicillin (fig. 2D) indicates lack of a negative charge on the cytoplasmic membrane.

The present study demonstrated little alteration in cell-wall charge due to carbenicillin, greater penetration of iron particles at high drug concentrations, and the absence of iron particle attachment with loss of cell wall. Studies to evaluate the positive charge distribution using negatively charged iron colloids and to determine the chemical nature of the charges by selective enzymatic treatment of the cells are in progress. We believe this cytochemical approach may contribute to a better understanding of the mode of action of cell-wall-active antibiotics.

LITERATURE CITED

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