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NOTES

Disruption of Chemically Killed Bacterial Cells by a Synthetic Zeolite

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The applicability of a synthetic zeolite (type 4A, Union Carbide Corp., Linde Div., New York, N.Y.) as a disruptive agent in a procedure for the preparation of pure bacterial cell wall fractions from a variety of phenol-killed gram-negative, gram-positive, and acid-fast bacteria was demonstrated. The disruptive effect was found to be limited with formaldehyde-killed gram-positive cells and most gram-positive cocci killed either by phenol or formaldehyde.

The original procedures utilizing a synthetic zeolite (type 4A, Union Carbide Corp. Linde Div., New York, N.Y.) for the disruption of microbial cells (1, 2) would have a limited application in the case of live pathogens, because of the obvious aerosol which would result during the grinding step. The obvious answer, the disruption of heat-killed cells, is not always applicable since at least in one instance (heat-killed *Mycobacterium tuberculosis*) such cells have been shown not to be susceptible to zeolite disruption (1). The use of chemical disinfectants, however, has not been studied and would seem to offer a simple solution to the problem.

The present paper reports the results of our investigations concerning the effectiveness of zeolite (type 4A) as a disruptive agent in a procedure for obtaining pure cell wall material from phenol- and formaldehyde-killed bacterial species.

Of the 25 organisms used, 14 were obtained from the stock culture collection of the Division of Biological Sciences, Bacteriology Section, University of Southern California. These were *Bacillus subtilis*, *Corynebacterium pseudodiphtheriticum*, *Escherichia coli*, *Gaffkya tetragena*, *Micrococcus lysodeikticus*, *Mima polymorpha*, *Mycobacterium phlei*, *M. smegmatis*, *Neisseria catarrhalis*, *N. perflava*, *Proteus vulgaris*, *Sarcina lutea*, *Serratia marcescens*, and *Staphylococcus aureus*. Eight organisms were obtained from the Bacteriology Laboratory, St. Mary's Hospital, Long Beach, Calif. These were *Alcaligenes faecalis*, *E. coli*, 0-111 strain, *Proteus mirabilis*, *P. morganii*, *P. rettgeri*, *P. vulgaris*, *Salmonella* group D, and *Shigella* group D. Three cultures, *Neisseria catarrhalis* ATCC 8176, *N. flava* ATCC 14221, and *N. subflava* ATCC 19243, were obtained from the American Type Culture Collection, Rockville, Md.

The procedures used for the cultivation and harvesting of bacterial cultures were described in an earlier publication (1). After incubation, the cells of individual microbial cultures were suspended in either 1% phenol or 1% formaldehyde in 0.85% saline solutions and kept at approximately 4 C for 2 weeks. Only cells from chemically killed cultures found to be nonviable were used for zeolite disruption. Specimens for electron microscopy were prepared by the method described earlier (1) and were examined with an RCA (EMU-3F) electron microscope operated at 100 kv.

Relatively good cell wall preparations were obtained from 21 of the 25 phenol-killed cultures studied by the zeolite disruption procedure (1). An electron micrograph of a representative preparation is shown in Fig. 1. The only exceptions were *S. aureus*, *G. tetragena*, *M. lysodeikticus*, and *S. lutea*. The limited effectiveness of the technique was caused by the same factors reported earlier (1).

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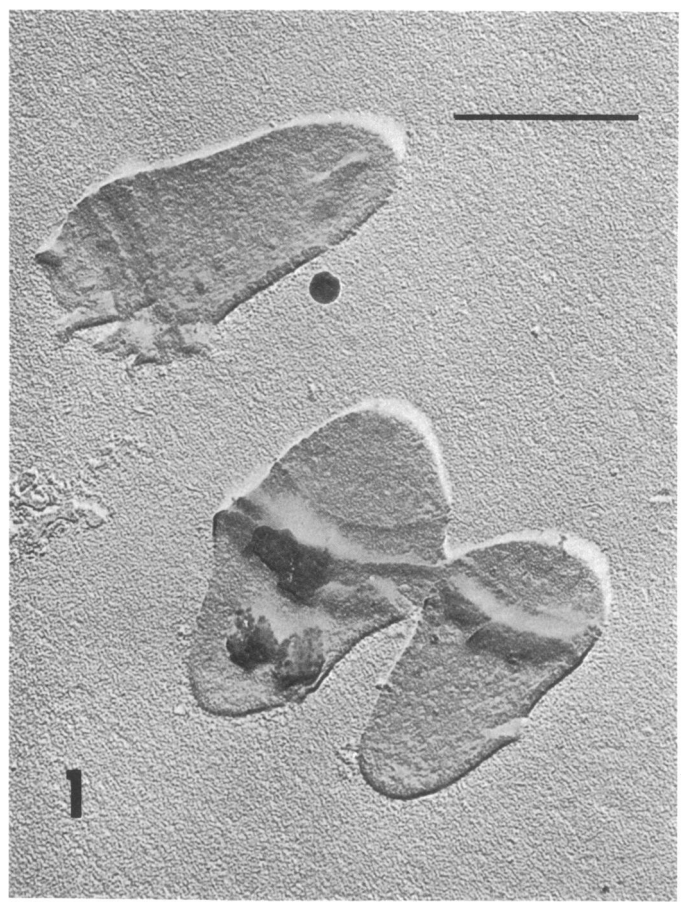

FIG. 1. *Results of the zeolite procedure with phenol-killed bacterial cells. Bar marker represents 1 nm. A platinum/palladium (Pt/Pd) shadowed preparation of cell walls obtained from phenol-killed Corynebacterium pseudodiphtheriticum.* ×24,700.

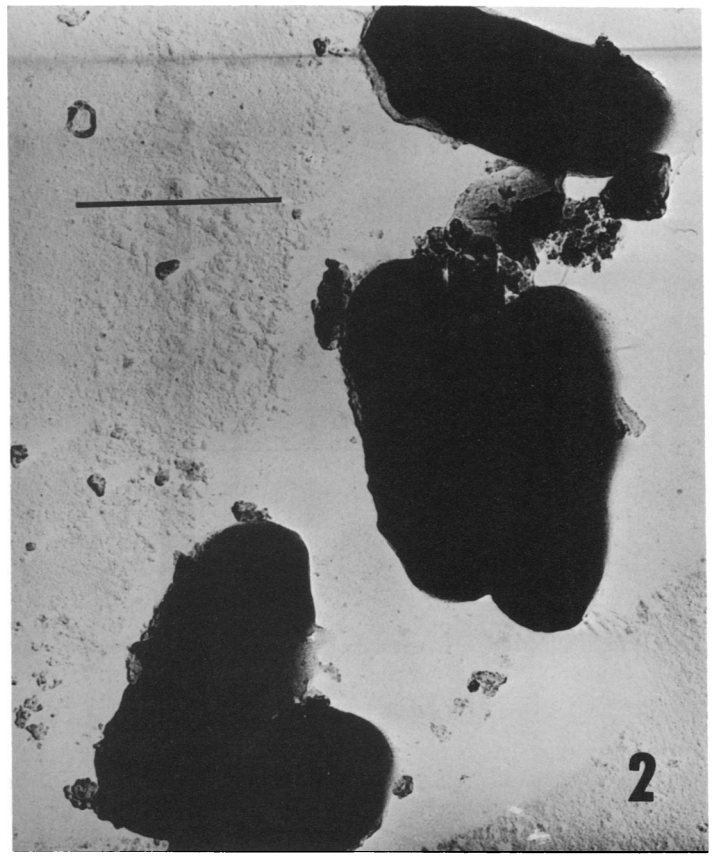

FIG. 2. *Results of the zeolite procedure with Formalin-killed bacterial cells. Bar marker represents 1 nm. Absence of zeolite disruption with Formalin-killed cells of C. pseudodiphtheriticum. Cells were shadowed with platinum/palladium.* ×27,400.

The use of phenol has one disadvantage if the chemistry of cell walls is of primary consideration. It serves as a chemical extractant for various compounds including nucleic acids and lipids. Comparisons of cell walls from live and phenol-killed preparations showed that the characteristic absorption shoulder formed between 260 and 280 nm for cell walls was greatly depressed when phenol was used to kill cells.

Attempts to disrupt formaldehyde-killed cells by the zeolite procedure met with a moderate degree of success. In general, only gram-negative cells were found to be susceptible. Gram-positive cells were ruptured but the cytoplasmic contents of such cells were not dislodged (Fig. 2).

The zeolite technique is both rapid and simple, and, when phenol is used as the killing agent, it presents a logical answer to the problems involved in obtaining large amounts of pure preparations of the cell wall material of pathogenic bacteria for chemical analysis.

LITERATURE CITED

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