

RAPID METHOD FOR DEMONSTRATING CELL SURFACES  
OF BACTERIA USING PHASE CONTRAST MICROSCOPY

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ABSTRACT -- A new method is suggested for demonstrating the cell surface of bacteria. A cationic, surface-active agent, cetylpyridinium chloride, and phase contrast optics are required.

The most commonly employed technique for staining bacterial cell walls for light microscopy is Dyar's method (1947) which uses a cationic surface-active agent. Dyar's procedure involves positively charging the cell surface with a cationic agent, cetylpyridinium chloride (CPC), and then staining the cells with an acid dye, Congo red. The cytoplasm can be differentiated by counterstaining with a contrasting basic dye such as methylene blue. Best results for this technique are achieved when the cells are fixed for three minutes in osmium tetroxide prior to staining. Subsequent staining, washing, and mounting take at least an additional five minutes. It would be convenient to have a technique that would be quicker and safer than Dyar's procedure, but still demonstrate the cell wall.

MATERIALS AND METHODS

The bacteria for this study were grown on Tryptic Soy Agar (Difco) at 37 C, and with the exception of Bacillus subtilis in Figure 2c, 18-hour cultures were employed. An American Optical Phasestar microscope with a 97x dark high (positive) phase contrast objective was used for all photomicrographs in Figure 2; the same microscope with all phase rings removed from the condenser and a 97x achromatic bright-field oil immersion objective (conventional light microscopy) was used for Figure 1. Photomicrographs were prepared with Kodak High Contrast Copy film.

The procedure for preparing slides for observation is as follows:

- (1.) Suspend cells from a solid medium in one drop of  
0.01 M cetylpyridinium chloride (0.34 % w/v).
- (2.) Place a coverslip over the suspension.
- (3.) Observe using positive (dark) phase contrast optics.

## RESULTS AND DISCUSSION

Representative results of bacteria treated by this method are shown in Figure 2; a bright-field photomicrograph of a culture prepared by Dyar's technique is shown in Figure 1 for comparison.

The method suggested here takes seconds to prepare, utilizes only the surface-active agent of Dyar's stain, and does not require fixing the cells, hence eliminating the toxic osmium tetroxide. An explanation for this technique may be that the positively-charged quaternary compound is attracted to the bacterial surface, and either the pyridine nucleus or the long aliphatic carbon chain (or both) of the cetylpyridinium chloride causes a phase shift. In other words, the quaternary compound combining with the bacterial surface causes an exaggerated phase difference, and this can be observed as a darkened outline around the cell.

This is a rapid technique for demonstrating cell surfaces, and the availability of phase contrast microscopes makes this technique feasible. The method works almost as well with Gram-negative organisms as it does with Gram-positives, and the results of demonstrating the cell surface are more consistent than those using Dyar's method. The ease of preparation and the ability of the technique to demonstrate cell septa in addition to exposed cell surfaces make this an appropriate method.

## LITERATURE CITED

DYAR, M. T. 1947. A cell wall stain employing a cationic surface-active agent as a mordant. *J. Bacteriol.*, 53:498.

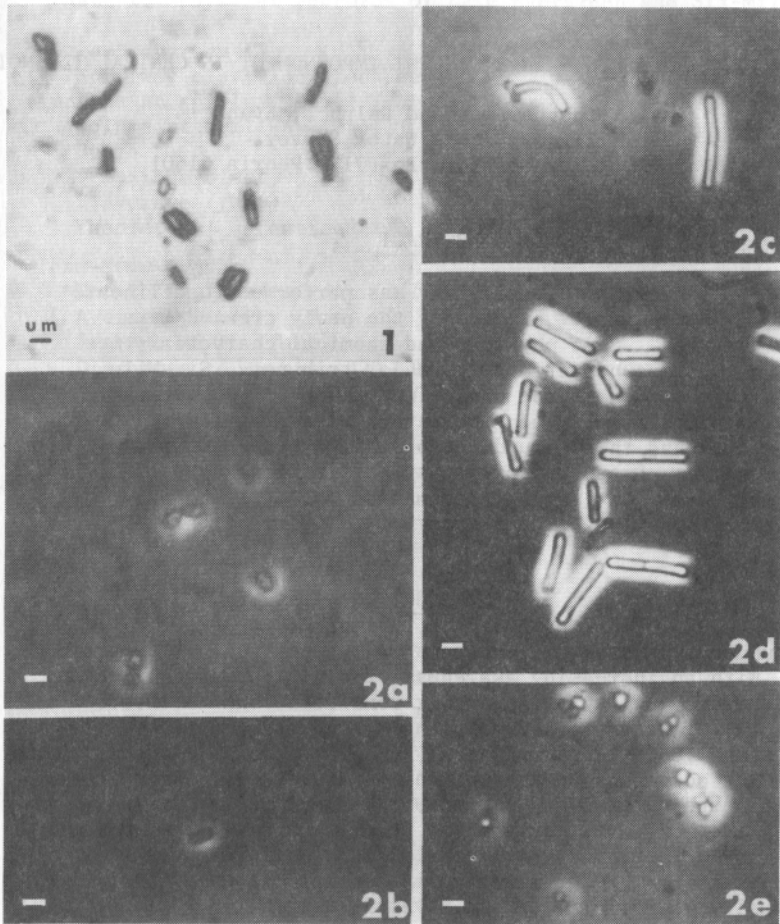


Figure 1. Dyar's cell wall stain of Bacillus subtilis as viewed by bright-field light microscopy.

Figure 2. Positive phase contrast photomicrographs of bacteria treated by the method suggested here.

- a Pseudomonas sp.
- b Escherichia coli
- c Bacillus subtilis second generation cells (4 hours); one pair of cells attached to endospore coat.
- d Bacillus megaterium
- e Staphylococcus aureus

Bars represent 1  $\mu$ m in all photomicrographs.