DOI: 10.1017/S143192760344004X
Microsc Microanal 9(Suppl 2), 2003
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High Resolution CryoSEM: Examination of Microbial Samples in an In-lens and a Below-the-lens FESEM

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Low temperature or cryoSEM has been used by investigators to investigate the surfaces of various microorganisms using conventional cryoSEM, but resolution has been limited due to microscope design and to coating methodology [1]. Development of cryopreparative methods for cryo field emission SEM (FESEM) now makes it possible to perform high resolution cryo-examination of cell surfaces, and many instruments have advertised resolution approaching 1 nm as being attainable. Therefore, we have compared the morphology attainable in cryoFESEM by examining the same sample using either an in-lens FESEM (Hitachi S-900) or a below-the-lens FESEM (Hitachi S4700).

The gram-negative bacterium, *Proteus mirabilis*, was grown overnight in tryptic soy broth, washed in saline and labeled with a polyclonal antisera directed against surface antigens followed by indirect labeling with 10 nm immunogold Bacteria were then fixed in a cocktail of paraformaldehyde-glutaraldehyde in cacodylate buffer and rinsed in distilled water prior to high pressure freezing in liquid N_2. Cryoimmobilized cells were sublimated in a cryopreparation chamber and double-coated with 5 nm of platinum followed by 5 nm of carbon [2]. Bacterial cells in planchets were then transferred to a Gatan cryoholder for examination in an in-lens cryoFESEM, or to a modified Emitech cold stage for examination in below-the-lens cryoFESEM. The modified holder permitted two planchets to be loaded at a time, and cold temperature transfer was facilitated by driving the edge of the planchet into a sheet of indium. In both cryoFESEMs, samples were examined at 10 keV, and backscatter electron (BSE) images were collected using an Autrata modified YAG detector.

Use of the double-cryocoating method greatly facilitated the investigation of microbial surfaces. Single coating with platinum only was susceptible to radiolysis and resulted in a "cracking" of the microbial surface. Examination of extracellular surface of *P. mirabilis* in the below-lens cryoFESEM clearly revealed an anastomotic network interpreted as capsular structure (Figure 1). In bacterial flagellar filaments, a periodic substructure was also detected. High resolution BSE images taken in an in-lens cryoFESEM showed similar results in that the bacterial surface was covered with a network of capsular material. The cryoimages from in-lens cryoFESEM (Figure 2) were somewhat sharper than those from the below-the-lens cryoFESEM, and may be a result of the shorter focal length attainable in the former (2.5 mm versus 6 mm, respectively). Thus, high resolution BSE imaging can be accomplished in both in-lens and below-the-lens cryoFESEM, and due to design differences, the former may offer better results.

1. A. Beckett and N.D. Read, In "Ultrastructural Techniques for Microorganisms, Plenum Press, NY, 1986
2. P.E. Walther et al., J. Microsc. 179 (1995) 229.

https://doi.org/10.1017/S143192760344004X Published online by Cambridge University Press

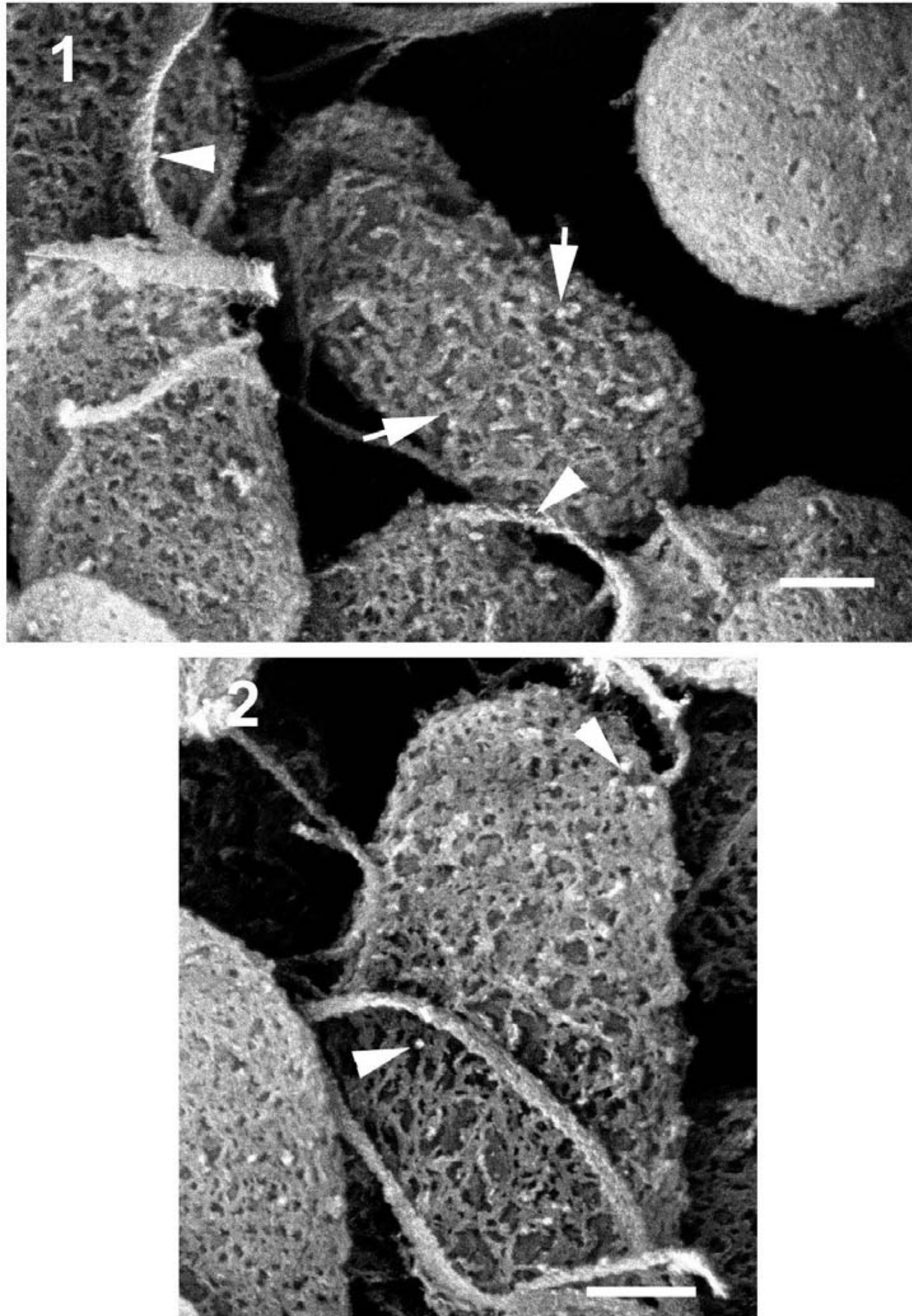

Figure 1. High resolution BSE cryoSEM of *P. mirabilis* taken in a below-the-lens ESEM. Observe network of capsular material covering cell and periodic pattern in the flagella (arrowheads). 10 nm colloidal gold (arrows), Magnification bar, 250 nm.
Figure 2. High resolution cryoSEM of *P. mirabilis* taken in an in-lens FESEM. Compare capsular material and flagellar structure with Figure 1. 10 nm colloidal gold (arrows), Magnification bar, 250 mm.

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