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The OTO Specimen Preparation Method for Optimal Scanning Electron Microscopy Imaging of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa, a ubiquitous gram-negative rod-shaped bacterium, has been intensively studied as an opportunistic human pathogen. It is one of the most common pathogens for nosocomial infection in immunocompromised individuals, e.g. cystic fibrosis patients [1]. Scanning electron microscopy (SEM) is a useful tool for obtaining detailed surface topography of microorganisms. For instance, it has been used for studying the ultrastructural basis of the resistance of *P. aeruginosa* to antiseptics, disinfectants and antibiotics [2]. Several methods for SEM sample preparation have been developed in order to enhance contrast, reduce structural damage and preserve cell structure in the native state. These techniques include glutaraldehyde fixation, negative staining, cryo-techniques, critical point drying, coating specimens with gold or osmium, and OTO staining. OTO staining has mostly been used in the preparation of biological tissues to provide bulk conductivity, which enables enhanced contrast [3]. Here, we report a specimen preparation protocol for optimal SEM imaging of *P. aeruginosa* using the OTO staining method.

In our optimized protocols, *P. aeruginosa* in the mid-exponential growth phase was washed and diluted with 0.9% sodium chloride to a cell density of 10^8 cfu/ml. It was then fixed with 2.5% glutaraldehyde in PBS and deposited onto a 0.4 μm-pore-size polycarbonate membrane. After fixation for 1 hour at room temperature and overnight at 4°C, it was post-fixed with 1% OsO_4 for 1 hour, followed by 1% thiocarbohydrazide (TCH) for 5 min, and 1% OsO_4 again for 5 min, with thorough washing between each step. The sample was dehydrated with graded ethanol series, and then impregnated with 50% hexamethyldisilazane (HMDS) in ethanol, and finally 100% HMDS. The air-dried sample was sputtered with gold before imaging. As a comparison, we have also processed the sample without the steps of 1% TCH followed by 1% OsO_4.

Compared to the specimen prepared with the conventional method (Fig.1), the OTO method revealed significantly higher resolution ((Fig.2), which allows the observation of bacterial surface details. Our protocol also helps to preserve the cells during sample processing, as cell shrinkage was only observed in the conventional method. The improved image quality allows detection of subtle effects of any treatments to the bacterial surface morphology.

References:
[1] J.M. Plotnikova, L.G. Rahme and F. M. Ausubel, Plant Physiol. 124 (2000), 1767-1774.
[2] U. Tattawasart, *et al*. J. of Antimicrobial Chemotherapy. 45 (2000), 145-152.
[3] S. V. Buravkov, V. P. Chernikov, and L. B. Buravkova. Bulletin of Experimental Biology and Medicine. 151 (2011), 378-382.

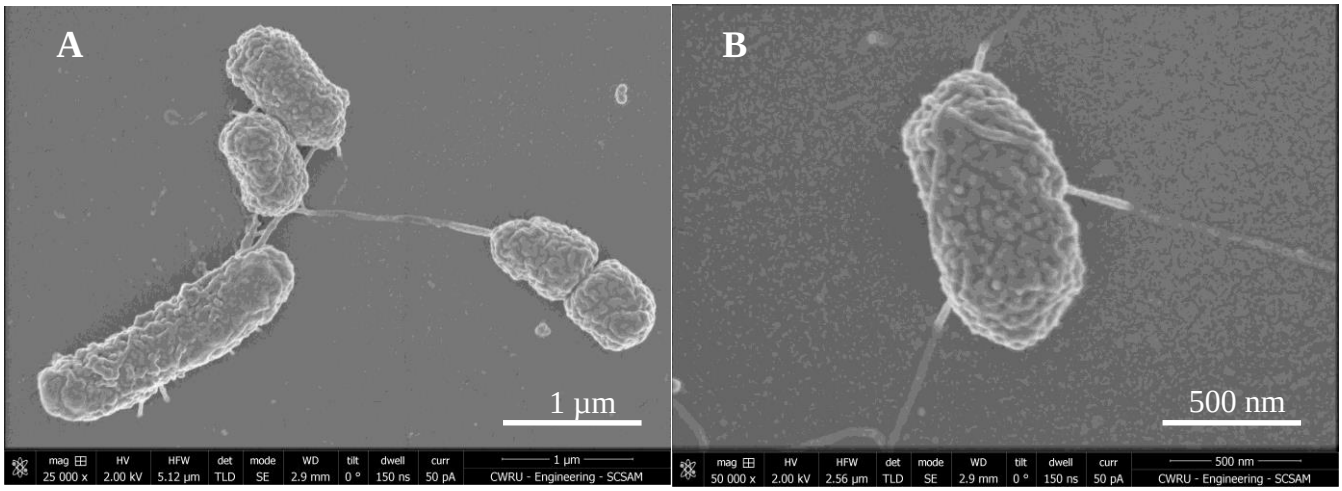

Figure 1. SEM images of *P. aeruginosa* using the conventional method for sample preparation at magnifications of 25,000x (A) and 50,000x (B). The SEM images were taken with FEI Helios Nanolab 650 using 2 KV, 50 pA with TLD mode 2. Loss of surface components and cell shrinkage were observed.

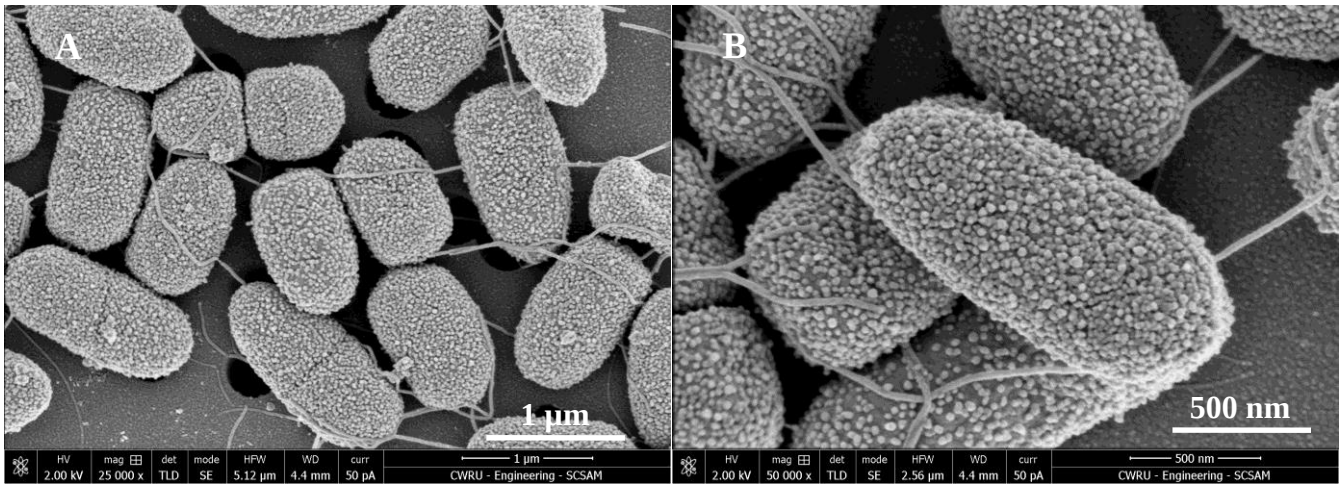

Figure 2. SEM images of *P. aeruginosa* using the OTO method for sample preparation at magnifications of 25,000x (A) and 50,000x (B). The SEM images were taken with FEI Helios Nanolab 650 using 2 KV, 50 pA with TLD mode 2. Bacterial surface components were well preserved and cell shrinkage was not observed.