Protocol: Observation of Bacteria Flagellum (Gold Labeling)

Required reagents

1) PBS (phosphate buffered saline)
2) 4% paraformaldehyde/1% glutaraldehyde in PBS
3) 0.01% Poly-L-lysine solution
4) DDW (double distilled water)
5) Positively charged nanogold (Nanoprobes, #2022)
6) Gold Enhance EM Formulation (Nanoprobes, #2113)
7) Dextrose in Water (10 mg/ml)

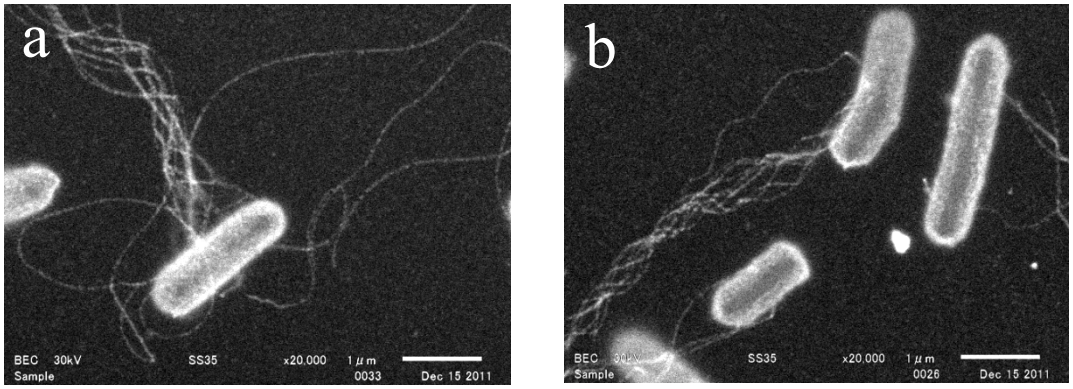
ASEM dish film coating

1. Place 0.01% Poly-L-lysine solution in the ASEM dish. Leave for 30 min at room temperature.
2. Wash three times with DDW.

Sample preparation

1. Centrifuge 1000 µl of the bacteria suspension at 3000 rpm for 5 minutes. Remove the supernatant and add 1000 µl PBS. (Repeat three times.)
2. Remove the supernatant and fix with 200 µl 4% paraformaldehyde/1% glutaraldehyde for 10 minutes.
3. Centrifuge at 3000 rpm for 5 minutes. Remove the supernatant and add 1000 µl DDW. (Repeat three times.)
4. Remove the supernatant. Suspend bacterial cells in 100 µl DDW.
5. Apply 20 µl bacteria suspension to the ASEM dish membrane and incubate for 30 min at room temperature.
6. Wash three times with DDW.
7. Apply 3 µM positively charged Nanogold in DDW and incubate 20 min.
8. DDW wash; 5 min x 3.
9. Gold enhancement: Nanoprobes, #2113 protocol.* Development time: 10 minutes.
10. Exchange the supernatant for dextrose (10 mg/ml) prior to ASEM observation.
11. Recommended ClairScope conditions: spot size = 20 - 35, acceleration voltage = 30 kV (Fig. 1).

*Nanoprobes #2113 protocol: steps #3 & 4 (PBS wash with 50 mM glycine and PBS-BSA wash) were not used.



x20,000 scale bar = 1 µm x20,000 scale bar = 1 µm

The bacteria specimen was kindly supplied by Dr. Seiichi Haga, Meijyo University, Japan

Fig. 1. ASEM images of *Salmonella enterica* NBRC 13245