Sep 19, 2025

Scanning Electron Microscopy Observations of *Pseudomonas* sp.

DOI
https://dx.doi.org/10.17504/protocols.io.6qpvrkxnplmk/v1

Md Sahadat Ali[1], Fatima Tuz Zohora Mony[1], Jonathan D. Eisenback[1]

[1]Virginia Tech

Md Sahadat Ali
Virginia Tech

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.6qpvrkxnplmk/v1

Protocol Citation: Md Sahadat Ali, Fatima Tuz Zohora Mony, Jonathan D. Eisenback 2025. Scanning Electron Microscopy Observations of Pseudomonas sp.. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvrkxnplmk/v1

Protocol status: Working
We use this protocol and it's working

Created: March 24, 2025

Last Modified: September 19, 2025

Protocol Integer ID: 124882

Keywords: Scanning electron microscopy, glutaraldehyde fixation, ethanol dehydration, propane freezing, freeze-drying, Pseudomonas sp., bacterial morphology, cryopreservation, ultrastructure imaging, electron microscopy observations of pseudomonas sp, pseudomonas sp, pseudomona, scanning electron microscopy, electron microscopy, preservation of bacterial ultrastructure, scanning electron microscopy observation, bacterial ultrastructure, gold sputter coating, resolution sem imaging, structural clarity during imaging

Disclaimer

This protocol is intended for research use only. It should be conducted by trained personnel in appropriately equipped laboratories following institutional biosafety and chemical handling guidelines. Use of cryogenic substances such as liquid propane and liquid nitrogen requires extreme caution and proper personal protective equipment (PPE), including cryo gloves and face protection. The authors and affiliated institutions are not responsible for any damage or injury resulting from improper use of this protocol or failure to follow safety procedures.

Abstract

This protocol outlines a detailed method for preparing *Pseudomonas* sp. for scanning electron microscopy (SEM). A combination of glutaraldehyde fixation, ethanol dehydration, dual-step cryopreservation (propane and liquid nitrogen), and freeze-drying ensures the preservation of bacterial ultrastructure. Gold sputter coating enhances conductivity, allowing high-resolution SEM imaging. This method is optimized to avoid morphological distortions and ensure structural clarity during imaging.

Guidelines

- Ensure all steps involving biohazardous material, including bacterial cultures and glutaraldehyde fixation, are performed in a certified biosafety cabinet (BSC).
- Always wear appropriate personal protective equipment (PPE) including lab coat, gloves, and safety goggles.
- Handle glutaraldehyde, ethanol, liquid nitrogen, and liquid propane in well-ventilated areas or chemical fume hoods due to their toxic, flammable, or cryogenic nature.
- All fixatives and waste solvents must be disposed of according to institutional hazardous waste protocols.
- Ensure samples are never exposed to air during dehydration and freezing steps to avoid shrinkage or structural distortion.
- Operate the sputter coater and SEM only after receiving proper training and under supervision if necessary.
- Always verify that the freeze-dryer and SEM chamber are pre-cooled and cleaned before use to ensure sample integrity.

Materials

Materials

- *Pseudomonas* sp. culture
- LB lite liquid and solid media
- Sterile scalpel
- Glass petri dishes
- 4% Glutaraldehyde solution in 0.1M sodium cacodylate buffer (pH 7.4)
- 1× Phosphate-buffered saline (PBS)
- Ethanol (5%, 10%, 25%, 50%, 75%, 90%, 95%, 100%)
- Liquid propane and liquid nitrogen
- Metal freezing chamber (pre-chilled with LN_2)
- Harvest Right® freeze-dryer
- Double-sided carbon tape
- Aluminum SEM stubs
- EMITech® SC7620 Mini Sputter Coater
- Jeol® NeoScope® JCM 5000 SEM
- Conductive gold target
- Cryo tools, forceps, vacuum containers
- Protective gloves, cryo goggles

Safety warnings

- Always wear cryo-protective gloves and goggles when handling propane and LN_2.
- Never seal vials tightly during cryo-transfer to avoid pressure buildup.
- Maintain ethanol immersion until freezing to avoid dehydration artifacts.

Ethics statement

This protocol does not involve animals or human subjects. All procedures were conducted using bacterial cultures under biosafety level 1 conditions. If adapted for animal/human tissues, appropriate IACUC or ethics approval must be obtained.

Before start

- Ensure that all reagents, including 4% glutaraldehyde at 4°C, graded ethanol solutions, and 1× PBS, are freshly prepared and properly labeled.
- Pre-chill the liquid nitrogen dewar, propane chamber, and freeze-dryer at least 30 minutes prior to use.
- Double-check that conductive carbon tape, and aluminum stubs are available and contamination-free.
- Confirm access to the Jeol® NeoScope® JCM 5000 SEM, and ensure that gold sputter coater is functional with sufficient gold target material.
- Review the entire protocol and conduct a dry run or mock setup to avoid workflow interruptions once biological samples are handled.
- Label all sample containers clearly before starting and maintain a detailed sample tracking sheet throughout the procedure.

Procedure

1 **Bacterial Culture and Colony Preparation**
- Inoculate *Pseudomonas auctus* JDE115 into LB lite broth.
- Incubate at 28°C for 24 hours.
- Serially dilute and plate on LB lite agar.
- Incubate plates for 48 hours at 28°C until ~6 mm colonies appear.

2 **Sample Fixation**
- Excise bacterial colonies with sterile scalpel.
- Transfer to glass petri dishes.
- Submerge in 4% glutaraldehyde in 0.1M sodium cacodylate buffer at 4°C overnight.
- Rinse thoroughly with 1× PBS.

3 **Ethanol Dehydration Series**
- Dehydrate sequentially in 5%, 10%, 25%, 50%, 75%, 90%, and 95% ethanol (15 min each).
- Incubate in 100% ethanol for 3 × 15 minutes.
- Maintain full ethanol immersion throughout.

4 **Cryopreservation and Freeze-Drying**
- Submerge dehydrated samples in liquid propane (held in LN_2-chilled metal holder) for ultra-rapid freezing.
- Immediately transfer to liquid nitrogen for solidification.
- Transfer frozen samples into a pre-cooled freeze-dryer (Harvest Right®) and lyophilize for 2 hours under vacuum.

Note: This dual cryopreservation minimizes ice crystal formation and preserves native ultrastructure.

5 **Gold Sputter Coating**
- Mount dried samples onto aluminum SEM stubs with double-sided carbon tape.
- Sputter-coat with 90 Å of gold using EMITech® SC7620 coater.

6 **SEM Imaging**
- Visualize using a Jeol® NeoScope® JCM 5000 SEM at 10kV.
- Capture images at multiple magnifications to assess morphology and flagellar structure.

Protocol references

Kaláb, M., Yang, A.-F., & Chabot, D. (2008). Conventional scanning electron microscopy of bacteria. *Infocus Magazine*, *10*, 42–61.