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Unveiling the antagonistic activity of '*Candidatus Pseudomonas auctus*' JDE115 against *Agroathelia rolfsii*: a soybean nodule endophyte with biocontrol potential. (In preparation)

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We use this protocol and it's working

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Disclaimer

This protocol is provided for research and educational purposes only. While the procedures described have been successfully used in our laboratory, results may vary depending on experimental conditions, microbial strains, and available equipment. Users are responsible for ensuring compliance with their institutional biosafety, chemical safety, and ethical regulations before implementation. The authors and affiliated institutions assume no liability for any misuse or misapplication of this protocol.

Abstract

This protocol describes the preparation and scanning electron microscopy (SEM) analysis of *Agroathelia rolfsii* sclerotia to evaluate morphological alterations induced by the soybean nodule endophyte '*Candidatus Pseudomonas auctus*' JDE115. Sclerotia are fixed in glutaraldehyde, dehydrated through an ethanol series, freeze-dried, sputter-coated with gold, and imaged under high vacuum using a Jeol NeoScope JCM-5000 SEM. Expected results include clear visualization of surface disruptions such as cracking, pitting, or deformation in treated sclerotia compared to intact control samples. This method enables reproducible characterization of pathogen suppression mechanisms mediated by microbial biocontrol agents.

Attachments



[SEM Analysis of Scle...](#)

2.2MB

Image Attribution

Fig. 5. Scanning electron microscopy (SEM) images showing structural integrity of *Agroathelia rolfsii* (Curzi) Redhead \u0026 S.T. Mull. (2021) sclerotia under different treatment conditions. From left to right: (a) Sclerotia exposed to '*Candidatus P. auctus*' JDE115 Ali et al. 2025 for 7 days, (b) Untreated stock sclerotia (negative control) representing natural morphology, and (c) Sclerotia incubated for 7 days on PDA without JDE115 (positive control for fungal development). All samples were processed under identical preparation conditions and imaged at 10 kV using SEM at 54× magnification.

Fig. 6. High-resolution scanning electron microscope (SEM) images of *Agroathelia rolfsii* (Curzi) Redhead \u0026 S.T. Mull. (2021) sclerotia under different treatment conditions at 300× magnification. From left to right: (a) Sclerotia treated with '*Candidatus P. auctus*' JDE115 Ali et al. 2025, (b) Stock sclerotia (negative control), and (c) Sclerotia incubated for 7 days without JDE115 (positive control). The images highlight ultrastructural differences in surface integrity and hyphal network organization, with notable disruption observed in sclerotia treated with JDE115.

Guidelines

Three sclerotial sample types were prepared for scanning electron microscopy (SEM): (a) sclerotia incubated on PDA plates with JDE115 for 7 days (treatment), (b) untreated sclerotia from the original stock (negative control), and (c) sclerotia incubated on PDA for 7 days without JDE115 (positive control). After incubation, sclerotia were gently harvested using sterile forceps and rinsed with phosphate-buffered saline (PBS) to remove residual media. Samples were fixed overnight at 4°C in 4% glutaraldehyde prepared in PBS (pH 7.2). Following fixation, they were rinsed three times with PBS and dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, and 100%; 10 minutes at each step). Dehydrated samples were freeze-dried to preserve surface morphology. Dried sclerotia were mounted onto aluminum stubs using double-sided carbon tape and sputter-coated with a thin layer of gold using an EMITECH® SC7620 Mini Sputter Coater. SEM imaging was performed under high vacuum using a Jeol® NeoScope® JCM-5000 scanning electron microscope at 10 kV accelerating voltage and an appropriate working distance for capturing surface features at 54× magnification (Fig. 5) and at 300× for high-resolution images (Fig. 6).

Materials

- Sclerotia:
 - a) incubated with JDE115 on PDA for 7 d (treatment)
 - b) stock sclerotia, not incubated (negative control)
 - c) sclerotia incubated on PDA 7 d without JDE115 (positive control)
- PBS, pH 7.2 (10 mM phosphate, 137 mM NaCl, 2.7 mM KCl)
- Glutaraldehyde, 25% stock (prepare 4% in PBS fresh)
- Ethanol series: 30%, 50%, 70%, 90%, 100% (molecular biology grade)
- Double-sided carbon adhesive tabs
- Aluminum SEM stubs
- Gold target for sputter coating

Safety warnings

- ❗
 - Handle 4% glutaraldehyde only in a fume hood with appropriate PPE (gloves, lab coat, safety glasses), as it is toxic and irritating.
 - *Agroathelia rolfii* is a plant pathogen; prevent accidental spread by sterilizing all waste and following institutional biosafety protocols.
 - Ensure proper training before operating SEM and sputter coater equipment.
 - Avoid sample drying at intermediate steps, as this may cause artifacts or loss of morphology.

Ethics statement

This study did not involve humans or vertebrate animals. All work was conducted with microbial cultures under the biosafety regulations of Virginia Tech and in accordance with institutional guidelines for safe handling and disposal of plant pathogens and chemical fixatives. No additional ethics approval was required.

Before start

Prepare three sample categories: (a) sclerotia inoculated/incubated on PDA with JDE115 for 7 days, (b) untreated stock sclerotia (negative control), and (c) sclerotia incubated on PDA for 7 days without JDE115 (positive control). Prepare fixative: 4% glutaraldehyde in PBS (pH 7.2). Prepare graded ethanol series (30%, 50%, 70%, 90%, 100%) and materials/equipment for freeze-drying, mounting (aluminum stubs, double-sided carbon tape), sputter coating (EMITECH® SC7620), and SEM imaging (Jeol® NeoScope® JCM-5000).



A. Harvest and pre-rinse (~10 min)

- 1
 - Using sterile forceps, gently collect sclerotia from plates. Avoid scraping agar onto the sample.
 - Transfer into 1.5 or 2.0 mL tubes or glass vials.
 - Rinse with PBS to remove residual media: add 1 mL PBS, invert 5–10 times, settle, and decant. Repeat **once**.

Critical: Keep samples submerged. Do not let them dry at any point before the drying step.

Primary fixation (overnight)

- 2
 - Add **4% glutaraldehyde in PBS** at $\geq 10\times$ sample volume (for example, 1 mL per 5–10 sclerotia).
 - Fix **overnight at 4 °C** (12–16 h).

Pause point: Fixed samples can remain at 4 °C up to 24 h.



PBS rinses (~15 min)

- 3
 - Decant fixative to proper chemical waste.
 - Rinse **3x with PBS**, 5 min each, gentle inversion.

QC: After the final rinse, odor of glutaraldehyde should be minimal.



Graded ethanol dehydration (~60–70 min)

- 4
 - Replace PBS with **30% ethanol**, incubate **10 min**.
 - Replace with **50% ethanol**, **10 min**.
 - Replace with **70% ethanol**, **10 min**.
 - Replace with **90% ethanol**, **10 min**.
 - Replace with **100% ethanol**, **10 min**, then **refresh 100% ethanol** for an additional **10–15 min**.

Critical: Ensure complete replacement at each step. Incomplete dehydration leads to surface collapse artifacts.



Drying (2–12 h, method as available)

- 5
 - Preferred:** Transfer to a freeze dryer and dry until samples are crisp and non-tacky (typical 2–12 h, depends on load).
 - Alternative:** If a freeze dryer is not available, air dry in a vacuum desiccator with fresh desiccant for several hours to overnight.
 - QC:** No visible wet sheen; sclerotia feel firm. If soft or glossy, extend drying.



Sputter coating (~5 min)

- 6
 - Apply **carbon adhesive tab** to each aluminum stub.
 - Using fine forceps, place **3–6 sclerotia per stub** with natural outer surface facing upward. Create both intact and fractured faces if cross-sectional texture is of interest.

Tip: Gently press to ensure full contact with the adhesive to reduce charging.

SEM imaging (30–90 min per batch)

- 7
 - Load stubs into **Jeol NeoScope JCM-5000** under high vacuum.
 - Start with **10 kV accelerating voltage** and a working distance suitable for low charging and good depth of field.
 - Suggested magnifications: **50×–100×** for whole sclerotium overview, **200×–500×** for surface topology, **800×–1500×** for micro-features such as cracking, pitting, and hyphal remnants.
 - Optimize focus, astigmatism, and brightness/contrast at each field. If charging occurs, reduce kV, increase working distance slightly, or confirm coating continuity.
 - Capture **at least 5–10 fields per sample** and **≥3 stubs per condition** to cover within-sample variation.

QC: Include scale bars. Record kV, WD, magnification, detector, and date in image metadata.

Protocol references

Bozzola JJ, Russell LD (1999) *Electron Microscopy: Principles and Techniques for Biologists*, 2nd edn. Jones & Bartlett Publishers

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