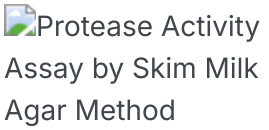
Sep 12, 2025

Protease Activity Assay by Skim Milk Agar Method



DOI
https://dx.doi.org/10.17504/protocols.io.kqdg318ppl25/v1

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Protocol Citation: Md Sahadat Ali, Jonathan D. Eisenback, Fatima Tuz Zohora Mony 2025. Protease Activity Assay by Skim Milk Agar Method. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kqdg318ppl25/v1

Manuscript citation:
Ali, M. S., Mony, F. T. Z., Evans, M., Rideout, S., Haak, D., & Eisenback, J. D. (2025). 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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Protocol status: Working
We use this protocol and it's working

Created: September 12, 2025

Last Modified: September 12, 2025

Protocol Integer ID: 227044

Keywords: Protease, casein hydrolysis, skim milk agar, Pseudomonas auctus JDE115, enzymatic activity, protease activity assay by skim milk agar method, detection of protease activity, protease activity assay, skim milk agar plate assay, protease activity, qualitative measure of proteolytic activity, proteolytic activity, skim milk agar method, biocontrol bacteria, clear zones around bacterial colony, bacterial colony

Disclaimer

This protocol is provided for research and educational purposes only. While the described methods have been validated in our laboratory, results may vary depending on microbial strains, reagents, and laboratory conditions. Users are responsible for following institutional biosafety and chemical safety regulations. The authors and Virginia Tech assume no responsibility for misuse or misapplication of this protocol.

Abstract

This protocol describes the detection of protease activity in 'Biocontrol bacteria' using a skim milk agar plate assay adapted from Khalil et al. (2014). Casein hydrolysis is visualized as clear zones around bacterial colonies, providing a qualitative measure of proteolytic activity.

Attachments

DOCX

Protease Activity As...

2.4MB

Image Attribution

All images (e.g., skim milk agar plates showing protease activity) were generated by the authors at Virginia Tech. Images are original and may be used for academic and research purposes with proper citation of this protocol.

Guidelines

- Prepare all reagents fresh where indicated, particularly $FeCl_3$ and culture media.
- Maintain aseptic technique throughout to prevent contamination.
- Include both positive and negative controls for reliable interpretation of enzymatic activity.
- Perform each assay in replicates and repeat independently to ensure reproducibility.

Materials

- Skim Milk Powder
- Agar
- Weighboat
- Scale
- 250mL Graduated Cylinder
- 2 500mL Beakers
- 2 Stir Bars
- Magnetic Stir Rod
- Stir Plate
- Deionized Water

Safety warnings

- Handle microbial cultures under BSL-2 practices to prevent accidental exposure or cross-contamination.
- Autoclave all biological waste (plates, cultures) before disposal.
- Wear gloves, lab coat, and eye protection when preparing and handling chemicals ($FeCl_3$, glutaraldehyde, Congo red, etc.).
- Avoid opening plates unnecessarily during incubation to reduce contamination risk.

Ethics statement

This work involves microbial cultures only and does not require human or animal ethics approval. All experiments were conducted in compliance with Virginia Tech institutional biosafety regulations and chemical safety guidelines.

Before start

- Sterilize all glassware and media in advance.
- Calibrate pipettes and spectrophotometer if measuring OD_{600} for inoculum preparation.
- Ensure incubator is set to 28 °C and functioning properly.
- Label all plates and controls before inoculation to avoid mix-ups.

Preparation of Skim Milk Agar

1
- Dissolve **25 g skim milk powder** in 200 mL deionized water with stirring.
- Dissolve **5 g agar** in 200 mL deionized water separately.
- Combine the two solutions in a 1 L flask, rinse beakers with small volumes of water, and adjust the final volume to **500 mL**.
- Mix thoroughly and sterilize at **121 °C for 15 min**.
- Cool to ~50 °C, pour into sterile Petri dishes, and allow to solidify.

Inoculation

2
- Prepare bacterial suspensions of JDE115 as described previously.
- Spot **10 µL** of each dilution onto sectors of the skim milk agar plates.
- Apply **LB broth** as negative control.
- Apply **undiluted Pseudomonas sp.** as positive control.

Incubation and Assessment

3
- Incubate plates at **28 °C for 24 h**.
- Assess protease activity by **measuring clear hydrolysis zones** around colonies.
- Record zone diameter (colony + halo vs colony alone).

Protocol references

Khalil KA, Mustafa S, Mohammad R, et al (2014) Optimization of Milk-Based Medium for Efficient Cultivation of *Bifidobacterium pseudocatenulatum* G4 Using Face-Centered Central Composite-Response Surface Methodology. BioMed Res Int 2014:1–10. https://doi.org/10.1155/2014/787989

Acknowledgements

We acknowledge the Nematode Diagnostic Lab at Virginia Tech for providing facilities and resources essential for conducting this work.