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IMMERSIVE RESEARCH INTERNSHIP EXPERIENCE
HOST-PATHOGEN INTERACTIONS

Purification and Functional Characterization of PlyN74 Endolysin for Antibacterial Applications

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Introduction

- Antibiotic resistance is a growing problem in the medical field, which requires the development of non-antibiotic medicines to treat patients with bacterial infections.
- Bacteriophage endolysin proteins such as PlyN74 have been known to target *Bacillus cereus* as well as *Bacillus anthracis* (Anthrax).
- This study aims to characterize the effectiveness of PlyN74 at facilitating lysis of *Bacillus cereus* and how lysis can be affected by temperature.

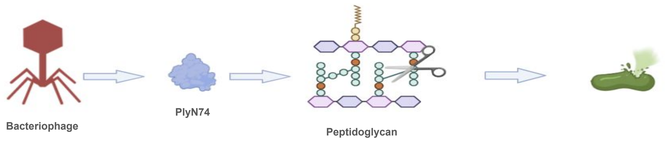


Figure 1. Phage endolysin proteins destroys bacterial peptidoglycan cell wall.

Materials and Methods

- *E. coli* with induced PlyN74 expression were lysed using **sonication** and **mechanical lysis** via glass beads in a bullet blender.
- PlyN74 was isolated from induced *E. coli* lysate using His-tagged Protein Purification.

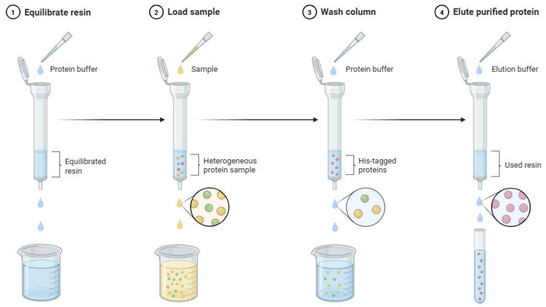
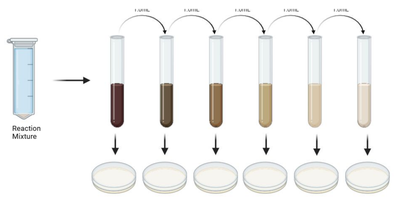


Figure 2. His-tagged Protein Purification methodology.
HisPur Ni-NTA Spin Columns were centrifuged to remove storage buffer. An equilibration buffer was added to the Spin Column and centrifuged. Induced *E. coli* lysate containing PlyN74 was loaded into the column, allowing the endolysin to bind to the His-tagged Protein. The column was then washed with wash buffer and the isolated protein was eluted.

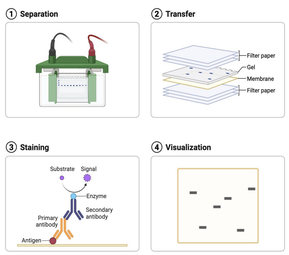
- A **BCA assay** was used to determine the concentration of endolysin in the purified endolysin sample.
- **Spectrophotometric** and **Log-Fold killing** assays were used to quantify how effective our endolysin is at lysing *B. cereus.*

Figure 3. Log-Fold Killing Assay.
Samples of the reaction mixture containing endolysin exposed bacteria were obtained at 0, 10, 20, 30, and 60 minutes. A 1:10 serial dilution was performed using each sample with each dilution being incubated on LB or BHI agar plates.



- A **Western blot** was performed to confirm the presence of PlyN74.

Figure 4. Western Blot.



Results

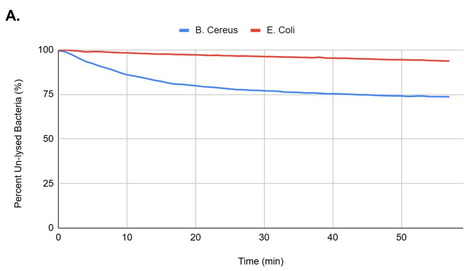


Figure 5. Spectrophotometric analysis of bacterial concentration following exposure to PlyN74.
Bacterial overnight cultures were centrifuged with the resulting pellet being resuspended in Phosphate-Buffered Saline (PBS). Samples of the resuspended solution were treated with purified PlyN74 at concentrations of 88 µg/mL, 8.8 µg/mL, and 0.88 µg/mL. The absorbance of each sample was recorded at OD600 every minute for an hour at approximately (A) room temperature (25 °C) and (B) body temperature (37 °C). PlyN74 demonstrated more activity at body temperature, lysing nearly 50% of *B. cereus* present in the sample. At room temperature, only 25% of *B. cereus* was lysed indicating that PlyN74 is more effective at 37 °C.

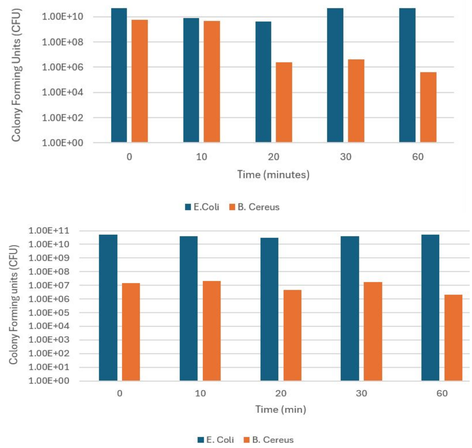


Figure 6. Log-Fold Killing Assay at 25 °C. Time-dependent bactericidal activity of the PlyN74 endolysin against *Escherichia coli* and *Bacillus cereus* at **25°C**. Bacterial suspensions were adjusted to 1 × 10^8 CFU/mL, mixed with purified PlyN74 protein (900 µL bacterial suspension + 100 µL protein), and incubated at room temperature. Samples were collected at **0, 10, 20, 30, and 60 minutes**, serially diluted, plated, and colony-forming units (CFU) were enumerated following overnight incubation. CFU values are plotted on a logarithmic scale. A progressive reduction in *B. cereus* viability was observed over time, whereas *E. coli* exhibited minimal changes in viable cell counts, indicating greater susceptibility of the Gram-positive organism to PlyN74.

Figure 7. Log-Fold Killing Assay at 37 °C. Time-dependent bactericidal activity of the PlyN74 endolysin against *Escherichia coli* and *Bacillus cereus* at **37°C**. Bacterial suspensions (1 × 10^8 CFU/mL) were incubated with purified PlyN74 protein, and aliquots were collected at **0, 10, 20, 30, and 60 minutes** for serial dilution and CFU enumeration. Colony counts are presented on a logarithmic scale. *B. cereus* demonstrated an overall decrease in viable cell counts throughout the incubation period, while *E. coli* remained largely unaffected, consistent with the expected specificity of PlyN74 toward Gram-positive bacteria.

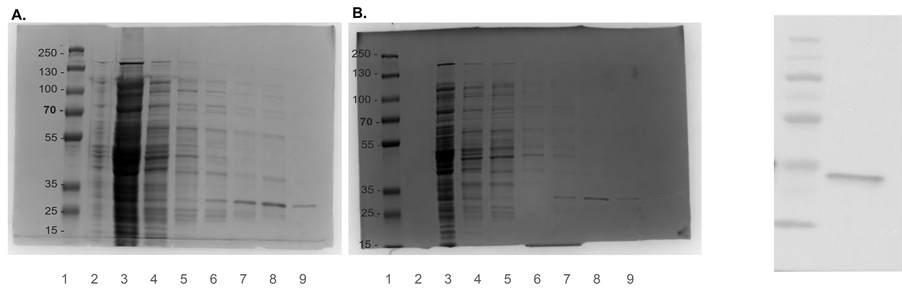


Figure 8. SDS-PAGE for PlyN74 using Sonication & Mechanical Lysis.
SDS-PAGE (Sodium Dodecyl Sulfate) electrophoresis was utilized to denature the protein, and evenly coat with a uniform negative charge, separating them based on their size as they migrate towards the positive electrode. PlyN74 has an actual weight of 31.4 kDa, and experimental weight between 25-35 kDa as seen through depiction of darker band. The elutions loaded into this gel pertained to the (A) sonication protein purification method as well as (B) mechanical lysis. Lanes of both gels are as follow: 1- Ladder, 2- Buffer, 3- Flow through, 4- Wash #1, 5- Wash #2, 6- Wash #3, 7- Elution #1, 8- Elution #2, 9- Elution #3

Figure 9. Western Blot of PlyN74.
An anti-His tag antibody was utilized to determine the presence of endolysins in purified protein samples. Dark bands represent the successful binding of antibodies to the endolysin.

Discussion and Future Directions

- Both our spectrophotometric and Log-fold killing assays showed that PlyN74 facilitated lysis of *B. cereus* while leaving *E. coli* virtually unaffected. The degree to which PlyN74 lyses *B. cereus* varies based on temperature, with the spectrophotometric assay showing a 25% increase in lysis at higher temperatures.
- If we had more time, we could further investigate the proteins ability to lyse *B. anthracis*. Additionally, we could test different temperatures, pH, or other conditions affect endolysin activity.
- Our findings show that temperature impacts how well endolysins can kill bacteria. This helps researchers understand how PlyN74 functions under certain conditions and when it is most effective to kill bacteria.

References and Acknowledgments

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- **Financial support for this project was provided by the First-Year Innovation Research Experience at UMD.**

- Etobayeva, I., Linden, S. B., Alem, F., Harb, L., Rizkalla, L., Mosier, P. D., Johnson, A. A., Temple, L., Hakami, R. M., & Nelson, D. C. (2018). Discovery and Biochemical Characterization of PlyP56, PlyN74, and PlyTB40—Bacillus Specific Endolysins. *Viruses*, *10*(5), 276. https://doi.org/10.3390/v10050276