FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Lytic Activity of Endolysins PlyP56 and PlyG

Milana Gurvich, Anika Dahiya, Jessica O'Hara, Daniel Nelson

Materials and Methods

- PlyP56 expression was induced overnight with L-arabinose under shaking conditions, while PlyG expression was induced using IPTG, which were both incorporated into an expression vector with a 6x-His tag.
- Sonication was used to lyse cells and release the desired protein.
- Both endolysins were purified using a Ni-NTA resin column that targets the proteins' 6x-His-tag.
- Protein purity and identity were verified by SDS-PAGE and a Western blot.
- A 96-well microplate was prepared containing experimental conditions, along with appropriate positive and negative controls.
- Bacteriolytic activity was assessed using a plate reader to monitor changes in OD600 over time.

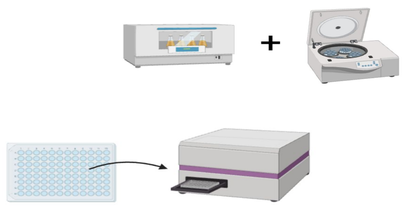
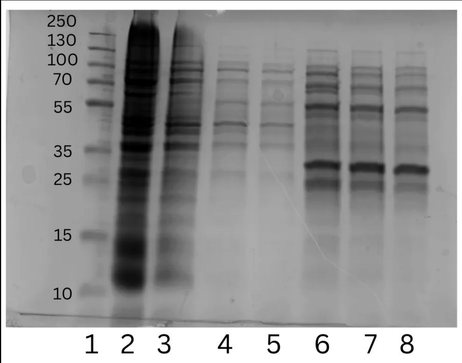


Figure 1. Induction, Purification, and Assay Visual (Biorender, 2026)

Expression and Purification



a. SDS-Page
Lanes:
1: Ladder
2: Running Buffer
3: Wash 1
4: Wash 2
5: Wash 3
6: Elution 1
7: Elution 2
8: Elution 3

Figure 4. Protein Gels of PlyP56. The method of purification used included sonication and HisPur Ni-NTA resin. The protein is 28.5 kDa (Etobayeva et al., 2018), thus this purification method was proven to be successful.

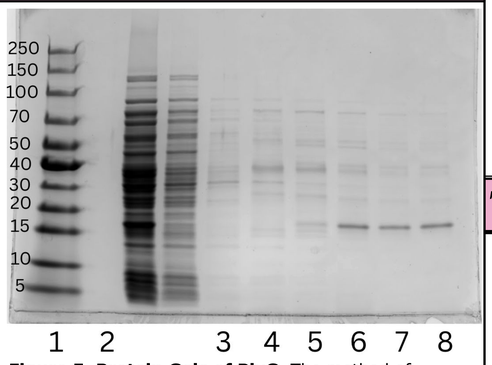
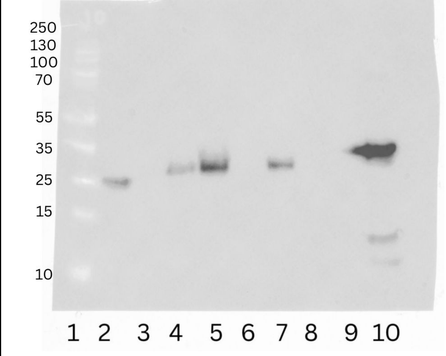


Figure 5. Protein Gels of PlyG. The method of purification used included sonication and HisPur Ni-NTA resin. The protein is 26.27 kDa.



b. Western Blot.
Lanes:
1: Ladder
2: PlyG
3: Buffer
4: PlyP56 old
5: PlyP56 new
6: Buffer
7: PlyN74
8: Buffer
9: LysM
10: SH3

Figure 6. Western Blot of PlyP56 and PlyG. This western blot used an anti-His tag antibody. Each of the endolysins contain a 6xHis tag, which the antibody specifically targets. The presence of a dark band indicates successful binding of the antibody to the endolysin, signifying its presence and purification.

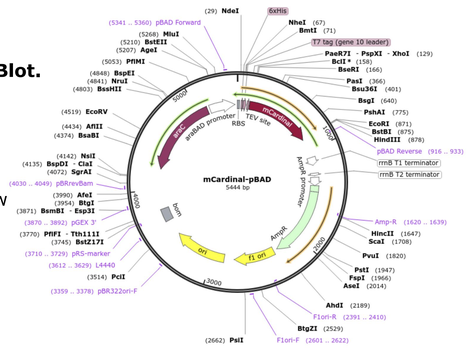


Figure 7. pBAD Expression Vector (Chu et al., 2014).

PlyP56 and PlyG Endolysin Background

- Antibiotics are typically used as treatments against bacterial infections, however, bacteria have evolved to be antibiotic resistant.
- Antibiotic resistance has led to millions of deaths and has placed a significant burden on the US healthcare system, costing billions of dollars on healthcare systems (Habboush and Guzman, 2023).
- Phages specifically target bacteria and can be used as an alternative treatment to antibiotics (Habboush and Guzman, 2023).
- PlyP56 is an endopeptidase which is produced at the end of a phage lytic cycle and degrades cross links within the bacterial cell wall using osmotic lysis (Etobayeva et al., 2018).
- PlyG is an amidase lysin which is a type of hydrolase that cuts a specific peptide bond within the bacterial peptidoglycan cell wall (Schuch et al., 2002)
- Classified by which peptidoglycan bond they cut (Vander Elst, 2024).
- PlyP56 targets *Bacillus cereus*, while PlyG targets *Bacillus anthracis*, which are both similar bacterium.
- Both endolysins contain an N-terminal catalytic domain that degrades peptidoglycan and a C-terminal cell wall binding domain that is specific to *Bacillus* bacteria.

Figure 2. Bacterial Cell Wall (Biorender, 2026).

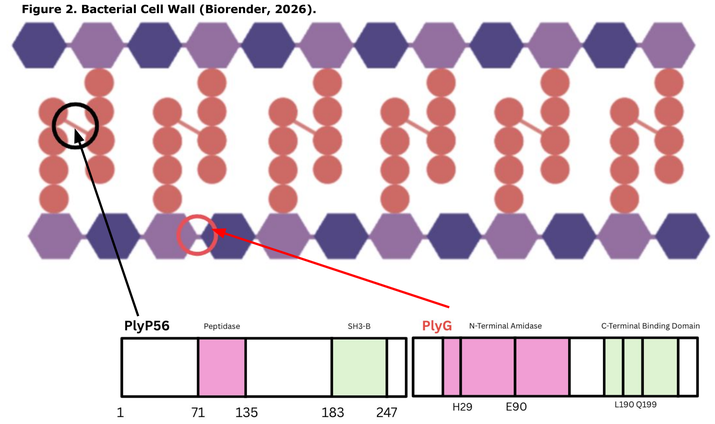


Figure 3. Protein Domains of PlyP56 and PlyG (Etobayeva et al., 2018) and (Kikkawa et al., 2008).

Testing Lytic Activity

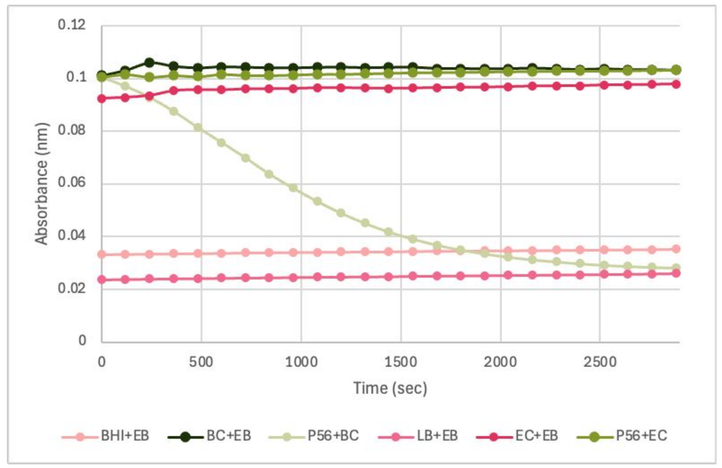


Figure 8. Change in Bacterial Absorbance Over Time Following Endolysin PlyP56 Treatment. This graph represents the results obtained from the plate-reader lysis assay. The results demonstrated a decrease in absorbance in the *B. cereus* and PlyP56 mixture, signifying lytic activity.

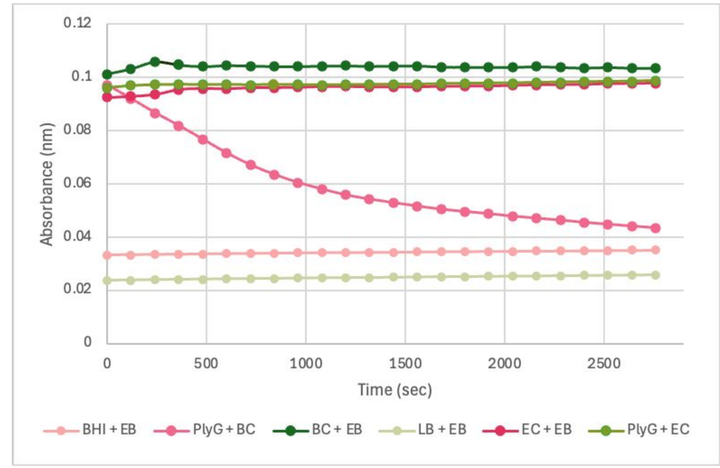


Figure 9. Change in Bacterial Absorbance Over Time Following Endolysin PlyG Treatment. This graph represents the results obtained from the plate-reader lysis assay. The results demonstrated a decrease in absorbance in the *B. cereus* and PlyG mixture, signifying lytic activity.

Key:
- BHI: Brain Heart Infusion Media
- EB: Elution Buffer
- BC: *B. Cereus*
- LB: Luria-Bertani Broth
- EC: *E. coli*

Discussion

- Both endolysins demonstrated lytic activity against *B. cereus* and no lytic activity against *E. coli*, demonstrating specificity for activity against the *Bacillus* genus of bacteria.
- Phage-encoded endolysins could potentially be used as antimicrobial treatments in the future.

Future Directions

- Testing the combined activity of PlyP56 and PlyG endolysins to determine whether they produce an additive lytic effect.
- Performing quantifying methods of measuring lytic activity, such as log-fold killing assays.
- Comparing lytic activity against other endolysins, such as SH3 and LysM.

Figure 10. Bacterial Lytic Cycle

References & Acknowledgments

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