FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Identification of Clostridium Phage Endolysins with Novel Multimeric Genetic Sequences

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Multimeric Structure of CD27L

- The endolysin CD27L is produced by Clostridium phage phiCD27.
- CD27L has two domains connected by a linker sequence.
  - Amidase_3 domain at the N-terminus, or the enzymatically active domain (EAD)
  - Cell wall binding domain (CBD) at the C-terminus
  - The gene sequence is EAD-linker-CBD (Mayer et al., 2011).
- CD27L can form a multimeric structure of one EAD and multiple CBDs in one structure and from one gene sequence.
  - The multimeric structure is achieved with two ribosome binding sites located at the 5' end and in the linker sequence before the CBD (Dunne et al., 2016).

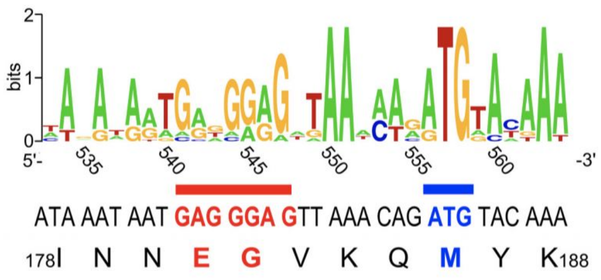

Figure 1. CD27L linker sequence The Shine-Dalgarno sequence (red) and start codon (blue) allow for separate translation from the EAD so multiple copies of CBD can be translated from one gene motif. (Dunne et al., 2016)

Methods for Genetic Sequence Analysis

Project Objective: Analyze the sequences of other Clostridium phage endolysins to find multimeric endolysins similar to CD27L. We are specifically looking for a ribosome binding site in the linker sequence with a start codon downstream.

- Use GenBank to find endolysin protein and DNA sequences
- Use PFam to identify length of amidase_3 EADs
- Manually search amino acid linker sequence for methionine and look for Shine Dalgarno upstream in nucleotide sequence.

Implications of Research

- Phage target bacteria and use an endolysin's enzymatic properties to lyse cells from within and release new replicated phages.
- Clostridium phage phiCD27 targets *Clostridium difficile*, a bacteria that causes the disease C. diff.
- Phage can be used to lyse harmful bacteria and treat bacterial infections.
  - Endolysin proteins alone can be used to fight infections.
  - Phage therapy is a promising alternative to antibiotic use as antibiotic resistance becomes a concerning factor.
  - Studying phage mechanisms, like enzymes involved in cell lysis, is vital for successful phage therapy.

Phage Endolysins with Multimeric Genetic Sequences

Key
Red: Ribosome Binding Site/Shine Dalgarno
Blue: Start Codon/Methionine
Phage Name: phiCD27
Linker Amino Acid Sequence: N N E G V K Q M
Linker Genetic Sequence: AAT AAT GAG GGA GTT AAA CAG ATG

Ribosome binding site identical to CD27L

Phage: phiC2
V L N K N I N N E G V K Q M
GTA TTA AAT AAA AAT ATA AAT AAT GAG GGA GTT AAA CAG ATG

Phage: phiCDHM11
N K N I G D E G V K E M
AAT AAA AAT ATA GGT GAT GAG GGA GTC AAA GAG ATG

Phage: phiCDHM13
N K N I G D E G V K E M
AAT AAA AAT ATA GGT GAT GAG GGA GTC AAA GAG ATG

Phage: phiCDHM14
N K N I G D E G V K E M
AAT AAA AAT ATA GGT GAT GAG GGA GTC AAA GAG ATG

Phage: phiCDHM19
N K N I N N E G V K Q M
AAT AAA AAT ATA AAT AAT GAG GGA GTT AAA CAG ATG

Phage: phiMMP01
N K N I N N E G V K Q M
AAT AAA AAT ATA AAT AAT GAG GGA GTT AAA CAG ATG

Ribosome binding site differing from CD27L

Phage: phiCD111
N K T I D N K E N S E D K K M
AAT AAG ACA ATA GAT AAT AAA GAA AAT AGT GAG GAT AAG AAA ATG

Phage: phiCD38-2
N K T I D N K E N S E D K K M
AAT AAG ACA ATA GAT AAT AAA GAA AAT AGT GAG GAT AAG AAA ATG

Phage: phiCD505
N K N I G N D G V K L M
AAT AAG AAT ATA GGA AAT GAT GGA GTT AAA CTG ATG

Phage: phiCD506
N K T I D N K E N G E G K I M
AAT AA ACA ATA GAT AAT AAA GAG AAT GGT GAG GGA AAA ATC ATG

Phage: phiCD6356
N K N I D N K E N G E D K K M
AAT AAA AAT ATA GAT AAT AAA GAA AAT GGT GAG GAT AAG AAA ATG

Phage: phiMMP02
N K N I G N D G V K L M
AAT AAG AAT ATA GGA AAT GAT GGA GTT AAA CTG ATG

Phage: phiMMP04
N K N I N N K E D S E G K I M
AAT AAA AAT ATC AAT AAC AAA GAA GAT AGC GAG GGA AAA ATC ATG

Phage: phiMMP03
N K N I G N D G V K L M
AAT AAG AAT ATA GGA AAT GAT GGA GTT AAA CTG ATG

Phage: QCD
I L N K T I D N K E N S E D K K M
ATT TTA AAT AAG ACA ATA GAT AAT AAA GAA AAT AGT GAG GAT AAG AAA ATG

These *C. difficile* phage were identified from a paper by Mondal et al. published in 2020. Specifically, the phage above are organized in Table 2 of the paper (Mondal et al., 2020). Phage were excluded if they lacked necessary information (genetic sequences or EAD domain length) in GenBank or PFam.

Conclusions and Future Directions

- Clostridium phage phiC2, phiCD111, phiCD27, phiCD38-2, phiCD505, phiCD506, phiCD6356, phiCDHM11, phiCDHM13, phiCDHM14, phiCDHM19, phiMMP01, phiMMP02, phiMMP03, phiMMP04, and QCD all have endolysin genetic sequences that foster a multimeric structure.
- Further studies could assess the likelihood of multimeric structure formation of these endolysins based on ribosome binding affinity, the existence of multimeric endolysins for other phage not evaluated in this study, and the reason for multimeric endolysins for *C. difficile* phage endolysins.

References and Acknowledgements

Dunne, M., Leicht, S., Krichel, B., Mertens, H. D. T., Thompson, A., Krijgsveld, J., Svergun, D. I., Gómez-Torres, N., Garde, S., Uetrecht, C., Narbad, A., Mayer, M. J., & Meijers, R. (2016). Crystal Structure of the CTP1L Endolysin Reveals How Its Activity Is Regulated by a Secondary Translation Product. *Journal of Biological Chemistry*, *291*(10), 4882–4893. https://doi.org/10.1074/jbc.M115.671172

Mayer, M. J., Garefalaki, V., Spoerl, R., Narbad, A., & Meijers, R. (2011). Structure-Based Modification of a Clostridium difficile-Targeting Endolysin Affects Activity and Host Range. *Journal of Bacteriology*, *193*(19), 5477–5486. https://doi.org/10.1128/JB.00439-11

Mondal, S. I., Draper, L. A., Ross, R. P., & Hill, C. (2020). Bacteriophage endolysins as a potential weapon to combat *Clostridioides difficile* infection. *Gut Microbes*, *12*(1), 1813533. https://doi.org/10.1080/19490976.2020.1813533

Financial support for this project was provided by the First-Year Innovation and Research Experience (FIRE) at the University of Maryland, College Park. Research support for this project was provided by Dr. Daniel Nelson of the Nelson Lab of the Department of Veterinary Medicine of the University of Maryland College Park.