Isolation and genome annotation of mycobacteriophage Jabith, a cluster A11 phage which increases biofilm growth of *Mycobacterium smegmatis*

DOANE UNIVERSITY

Nadezdha Hughes, **Connor Long**, AJ Allen, Kaycee Bartels, Jared Bithell, Jared Foote, Dalton Fellows, Barb Clement and Erin Doyle
Biology Department, Doane University, Crete, NE 68333

Introduction

- Biofilms are communities of bacteria and other microorganisms that stick to surfaces and each other by means of a self-produced extracellular matrix composed of proteins, polysaccharides, and extracellular DNA (extracellular polymeric substance or EPS).
- Biofilms can grow on a variety of surfaces, including medical devices and implants
- Biofilms are often antibiotic resistant due to changes in metabolism and protective EPS coating
- Students at Doane University attempted to isolate mycobacteriophage and test their ability to lyse *Mycobacterium smegmatis* when grown in a biofilm.
- In 2015-2016, twenty-seven phage were isolated from soil samples on Doane's campus using *M. smegmatis* mc^2155 and tested for their ability to lyse *M. smeg* growing in a biofilm

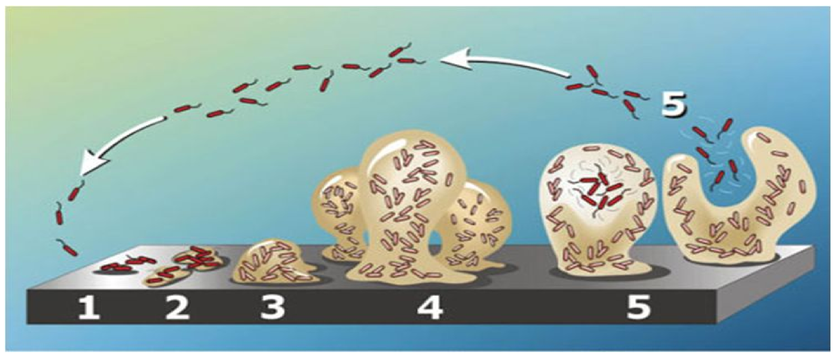
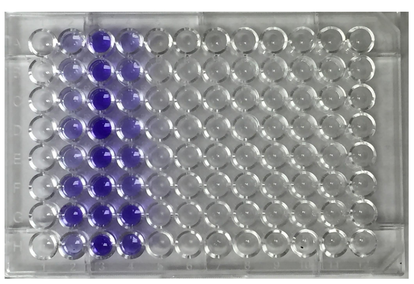


Figure 1. The 5 stages of biofilm growth. 1) Initial, reversible attachment. 2) Irreversible attachment and initial EPS production. 3) Early development of biofilm architecture. 4) Mature, 3-D biofilm. 5) Dispersal.

Methods

- Grow liquid culture of *M. smegmatis* at 37 C° (until mid-log phase)
- Set up plate as shown in Figure 2
- Incubate at 37 C° for 24 hours
- After 24 hours, remaining liquid removed and wells washed 2x with H_2O
- 0.1% crystal violet added to each well (stains cell walls purple)
- Excess CV solution removed and stained cells washed with H_2O
- Plates destained with ethanol
- Stained ethanol transferred to a new plate
- Absorbance of new plate measured at 550 nm



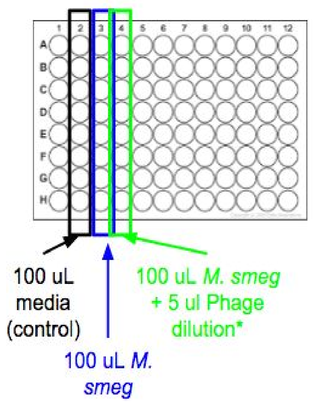


Figure 2. Initial set-up of biofilm plate. The phage dilution used depends on the concentration of the MTL or HTL. The phage dilution used depends on MTL or HTL concentration.

Figure 3. Sample plate showing crystal violet staining. Darker violet indicates more biofilm growth.
Column 1-control
Column 2-bacteria
Column 3-bacteria and phage

Discussion and Conclusions

- Mycobacteriophage Jabith and 4 other phage unexpectedly increased *M. smegmatis* mc^2 155 growth in the biofilm
- An additional 4 phage decreased biofilm growth
- No conclusions drawn about Jabith genome and effects on biofilm
- Linking genetic elements to effects of phage on biofilm growth will require sequencing additional phage that have been tested on biofilms
  - Biofilm assay could be implemented into SEA-PHAGES curriculum at other institutions
  - Requires approx. 1.5 hours over 2 days

Results

27 phage were isolated using standard procedures and tested in the biofilm assay for their effects on *M. smeg* biofilms

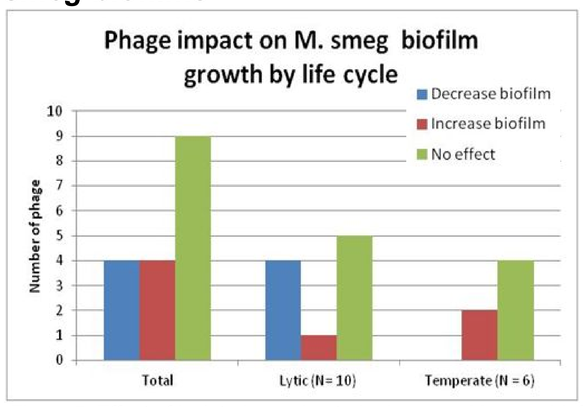


Figure 5. Effect of isolated mycobacteriophage on *M. smeg* biofilm growth. N = 17 because phage had inconclusive results due to low titer or no signivicabt difference between media control and *M. smeg* only.

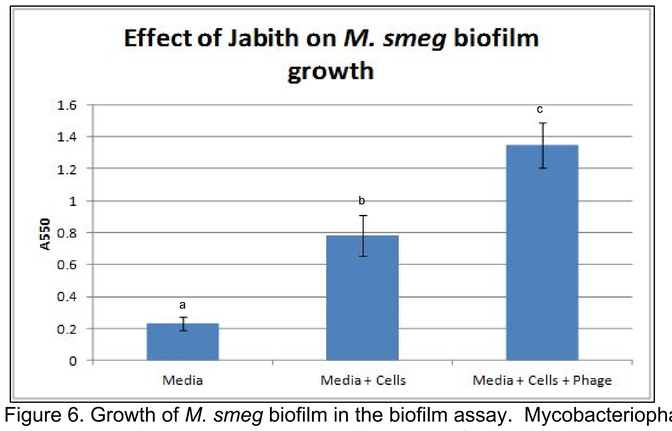


Figure 6. Growth of *M. smeg* biofilm in the biofilm assay. Mycobacteriophage Jabith caused a significant increase in the biofilm growth ($p<0.05$). A550 measures absorbance and indicates the relative amount of biofilm growth.

4 phage actually increased the growth of *M. smegmatis* in a biofilm. One of those phage was Jabith.

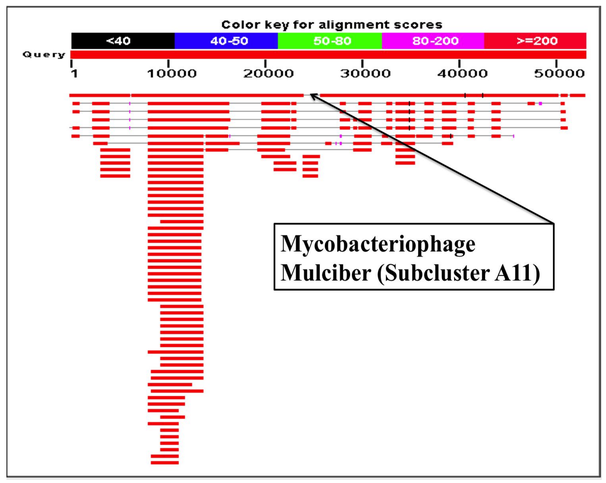


Figure 9. BLASTn of Jabith from NCBI shows alignment scores and sequence similarity. In BLASTp, Jabith showed strongest similarities to Mulciber, also an A11 phage. Also showed significant similarity to another A11 phage, Et2Brutus.

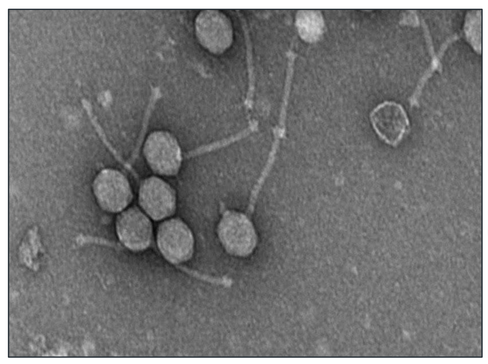


Figure 7. TEM of mycobacteriophage Jabith. Assigned morphology: Siphoviridae due to its long, flexible tail.

Figure 8. Plate of *Mycobacterium smegmatis* with Jabith. Cloudy plaques suggest Jabith is a temperate phage.

Out of 99 predicted ORFs in Jabith's sequenced genome, 88 were retained in the final annotation. 38 were assigned function.

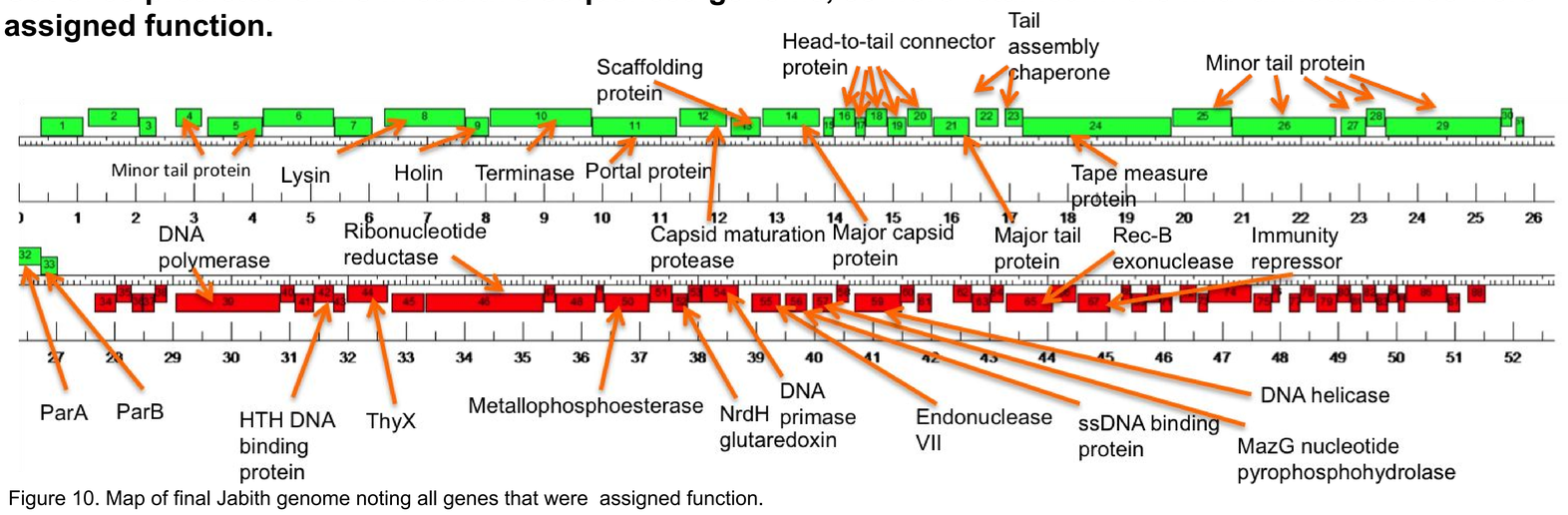


Figure 10. Map of final Jabith genome noting all genes that were assigned function.

Works Cited:

Donlan, RM. Biofilms: Microbial Life on Surfaces. Emerging Infectious Diseases, 2002.
Karatan E and Watnick P. Signals, Regulatory Networks, and Materials that Build and Break Bacterial Biofilms. Microbiology and Molecular Biology Reviews, 2009.

Acknowledgements:

Doane University 2015-2016 phage hunters
TAs Brielle Debusk, Melissa Shadoin, Zak Stevenson
Biofilm assay: personal communication; Susanne Häußler lab, Helmholtz Institute for Infectious Disease Research, Hannover, Germany.
UNL TEM lab
HHMI SEA-PHAGES program