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Investigation of Genomic Deletion of Mycobacteriophage Moonbeam

Lauren Schmitt
Montana Technological University

Hannah E. Sparks
Montana Technological University

Abbie George
Montana Technological University

Ozzie Indreland
Montana Technological University

Marisa L. Pedulla
Montana Technological University

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***Hypothesis*: There are two, genomically separate Mycobacteriophages in Moonbeam lysate that was used and sent DNA template to the University of Pittsburgh in 2020.**

***Experimental Aims*: Separate and characterize phages with deletion from non-deletion phages in Moonbeam lysate.**

Background & Significance

- **Characterization and isolation of Mycobacteriophages have significance in viral evolution studies and anti-microbial therapeutic research.**
- **A plaque is formed when a single phage successfully propagate among bacteria and have infected and lyses cells.**
- **Only a miniscule fraction of total phages have been discovered, and even less characterized.**
- **Moonbeam phage isolated in Helena, MT in 2017, as part of Montana Tech's NIH SEPA BRIC project.**
- **Sequencing in 2020 by the University of Pittsburgh HHMI SEA-PHAGES identified a subset of genomes with a deletion.**

Sequencing Notes	The sequencing reads indicate that in ~60% of the population of phages from which DNA was extracted, there was a 587 bp deletion. It runs from 25659 to 26245 (inclusive). Despite the majority of the population being this shorter version, the final fasta contains the deleted portion since otherwise it would be lost.

Phagesdb.org

Methods

- **A. Analyzed genomic content as part of bioinformatics class with three students using bioinformatics tools and online programs.**
- **B. Designed and ordered forward (A) and reverse (B) primers around the deletion.**
- **C. on Serially diluted Moonbeam phage lysate and infected *M. smegmatis*, then picked individual plaques.**
- **D. Performed PCR and Gel electrophoresis on samples and optimized PCR conditions.**

Results

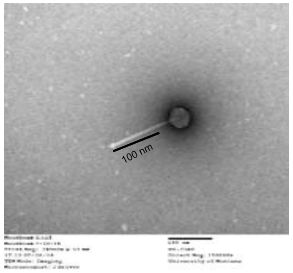

Figure 1: TEM of Moonbeam Mycobacteriophage. Bar represents 100 nm. (University of Montana Emtrix facility).

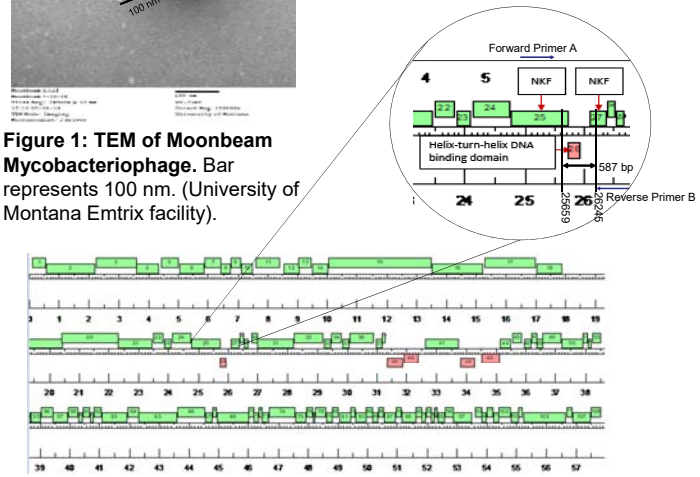

Figure 2: Gene map of Moonbeam phage genome. Zoomed in: Portion of the genome deleted from 25659 to 26245 and location of forward primer (A) and reverse primer (B). Refer to method A and B.

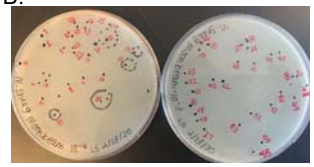

Figure 3: Infection of *M. smegmatis* with serially diluted Moonbeam lysate. Fifty five individual plaques were observed from the two plates above. Refer to method C.

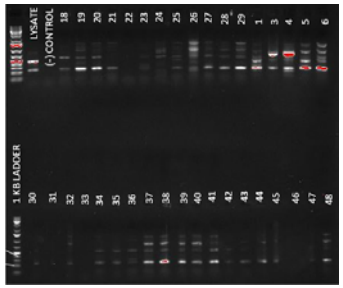

Figure 4: Agarose gel electrophoresis of thirty six PCR samples. Two bands observed in lysate lane and smaller band suggesting phage with smaller genome in lane containing sample 21. Refer to method D.

Citations: Phagesdb.org

Figure 5: Agarose gel electrophoresis of PCR products with temperature gradient. Deleted phages in sample #42 and full length Moonbeam in #47. Refer to method D.

Conclusions

- **Hypothesis was supported by 2 bands of predicted sizes 226 and 813 in the lysate PCR products (figure 4 and figure 5). Forward and reverse primers were successful in annealing around the deletion.**
- **Annotated genome completed and will be sent to Phagesdb and Genbank.**
- **Optimized annealing temperature to 63˚ C.**
- **Individual plaque #21 and #42 contain deleted phages while plaque #47 contains full length Moonbeam phages.**
- **Met experimental aim to separate deleted phages and full length Moonbeam phages.**

Future work

- **Characterize and compare full length and deleted phages to attempt to elucidate the role of gene #26 and portions of gene #25 and #27.**

Acknowledgments

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***Student Profile**

I am a senior Biological science major from Helena. MT. Upon graduation, my goal is to work in a medical lab in Butte, MT.
lschmitt@mtech.edu