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Curation and Characterization of a Collection of Mycobacteriophages

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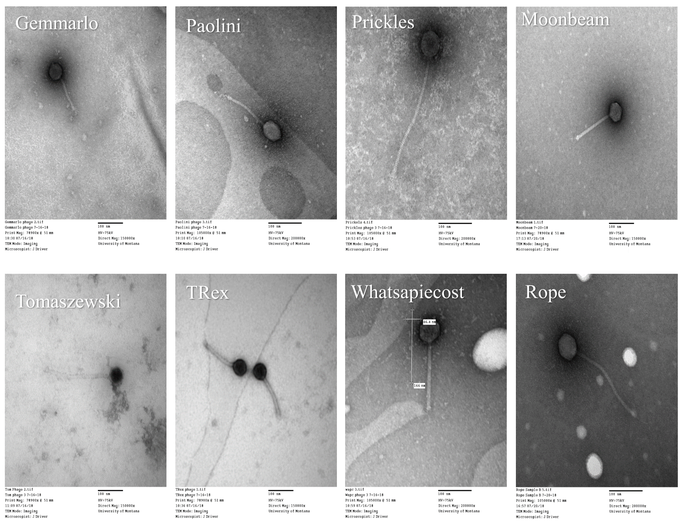
Bacteriophage Background

- Bacteriophages are the most abundant biological entity on earth, with an estimated number of 10^{31}.
- Bacteriophages specifically infect and kill bacteria.
- Phages that infect *M.smegmatis* could potentially be used as an alternative to antibiotics.



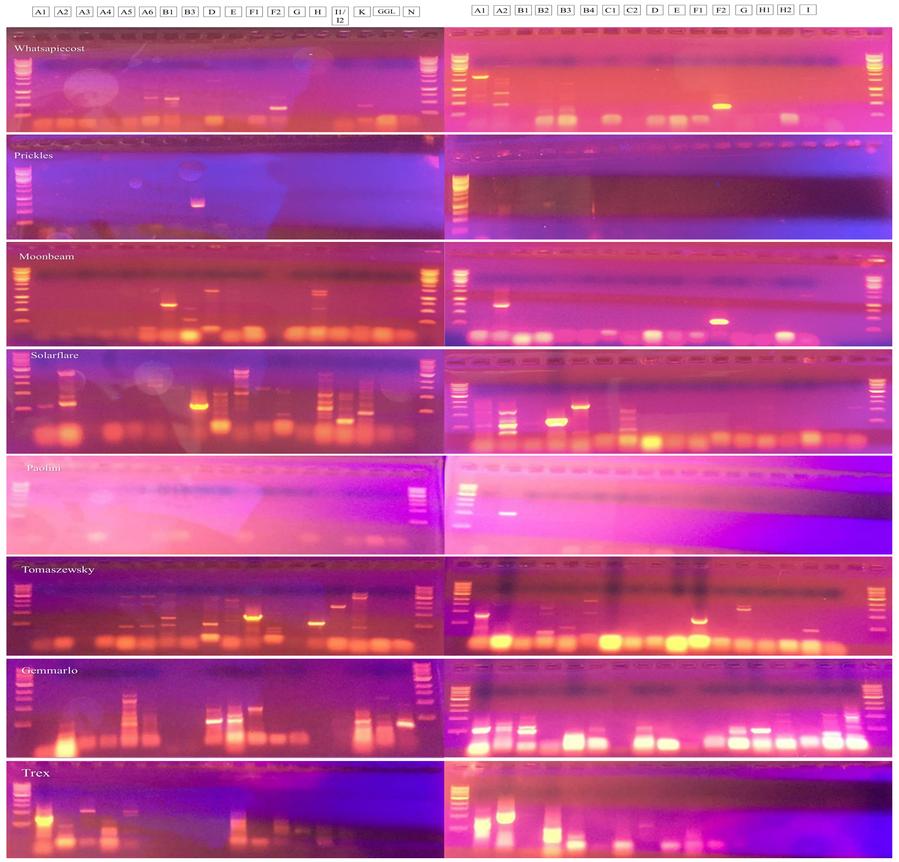
Methods

- Isolation of single plaques from phages stored at various purification stages at Montana Tech.
- Creation of a high titer sample.
- DNA Extraction
  - Restriction Enzyme Digests & Gel Electrophoresis
  - Send sample for sequence analysis at the University of Montana
  - Polymerase Chain Reaction (PCR) for cluster analysis
- Update Phage Information
  - Archive samples at the University of Pittsburgh
  - Upload information to phagesdb
  - Update spreadsheet of Montana Tech's phages
  - Transmission Electron Microscopy.

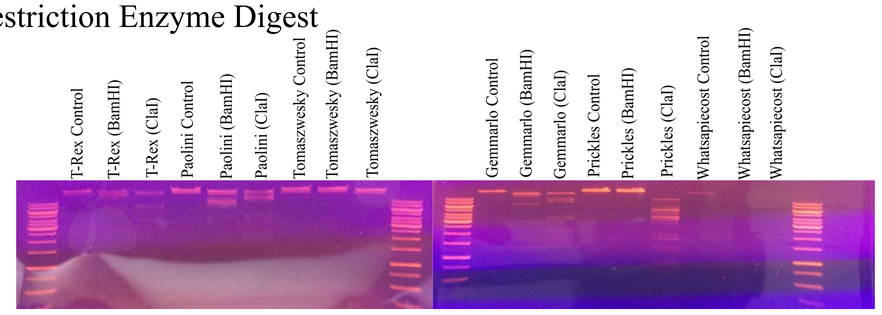
Results

Phage Name	Concentration from DNA extraction (ng/uL)	Lysate Concentration (pfu/mL)
Gemmarlo	14.2	3.7×10^{10}
Moonbeam	55.8	1.67×10^{10}
Paolini	34.3	1.67×10^{10}
Prickles	31.2	6.67×10^{9}
Solarflare	72.9	In Progress
Tomaszewsky	65.7	2.33×10^{10}
TRex	40.4	1.67×10^{9}
Whatsapiecost	13.4	6.0×10^{10}

PCR Cluster Analysis



Restriction Enzyme Digest



Conclusions

Phage Name	Putative Clusters
Whatsapiecost	A1, A2, A6, B1, **F2**, K
Prickles	**D**
Moonbeam	B1, B3, D, I1/I2, A1, **F2**, I
Solarflare	A1, A2, B1, **B3**, **D, E,** F1, F2, G, I1/ I2, K, B4, C2
Paolini	A2
Tomaszewsky	A5, A6, B1, D, E, **F1,** F2, G, H, I1/I2, A1, B2, B3,**E,** I
Gemmarlo	A2, A4, **D, B3,** E, I1/I2, K, **GGL, A1,** B1, B2, **F2,** F1, G, H2, I
TRex	**A1, A2**, A3, A4, A5E, F2, G, **B3,** C2

- Transmission electron microscopy indicated Siphoviridae morphology for all eight phages.
- Restriction enzyme digests indicated multiple cut sites for all phage genomes, giving each phage a unique fingerprint.
- Updating information of phages is important for future research.

Future Work

- Annotate genomic sequences when they are returned.
- Repeat methods for more phages stored at Montana Tech.
- Further discovery through bioinformatics or wet lab procedures.

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