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Curation and Characterization of a Collection of Mycobacteriophages

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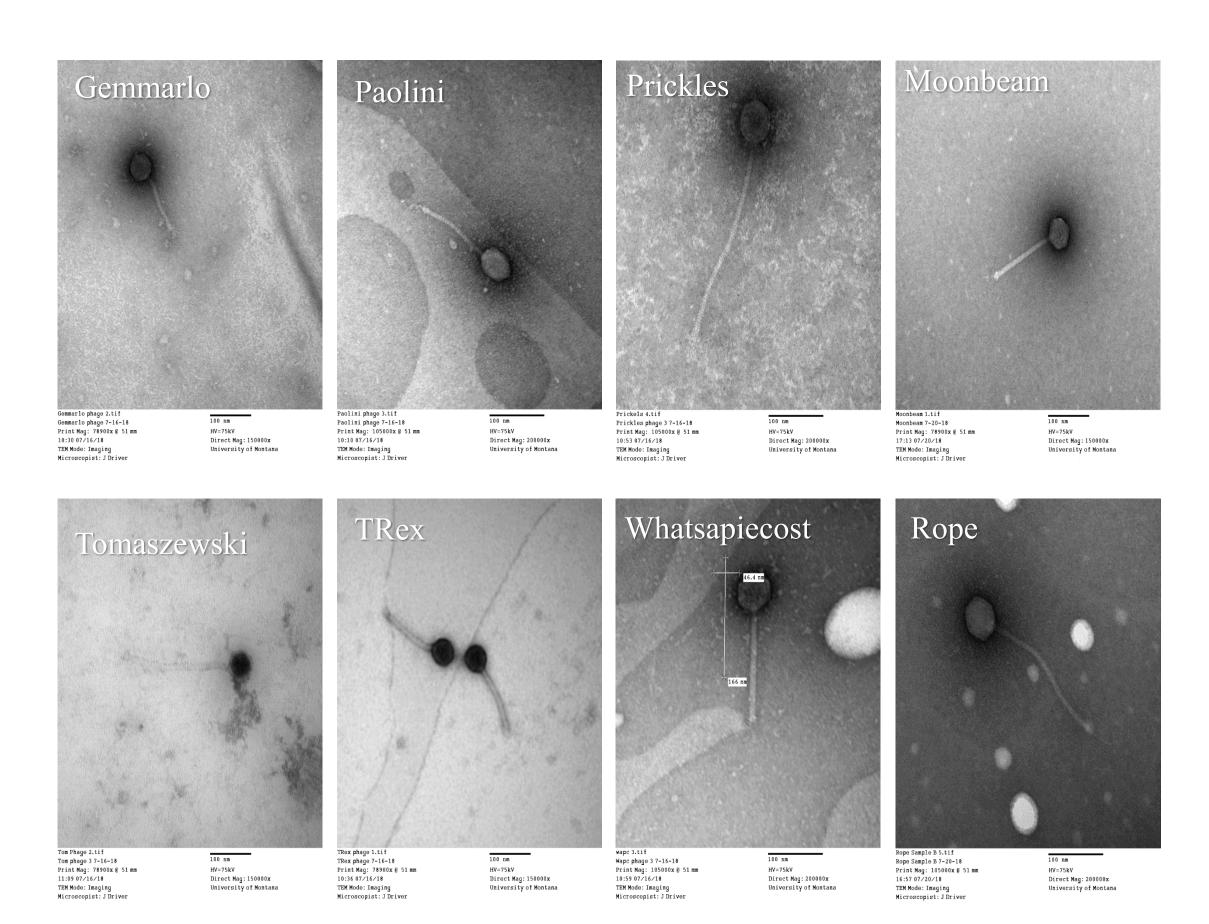
Curation and Characterization of a Collection of Mycobacteriophages

Hannah E. Sparks & Dr. Marisa L. Pedulla



Bacteriophage Background

- Bacteriophages are the most abundant biological entity on earth, with an estimated number of 10³¹.
- Bacteriophages specifically infect and kill bacteria.
- Phages that infect *M.smegmatis* could potentially be used as an alternative to antibiotics.



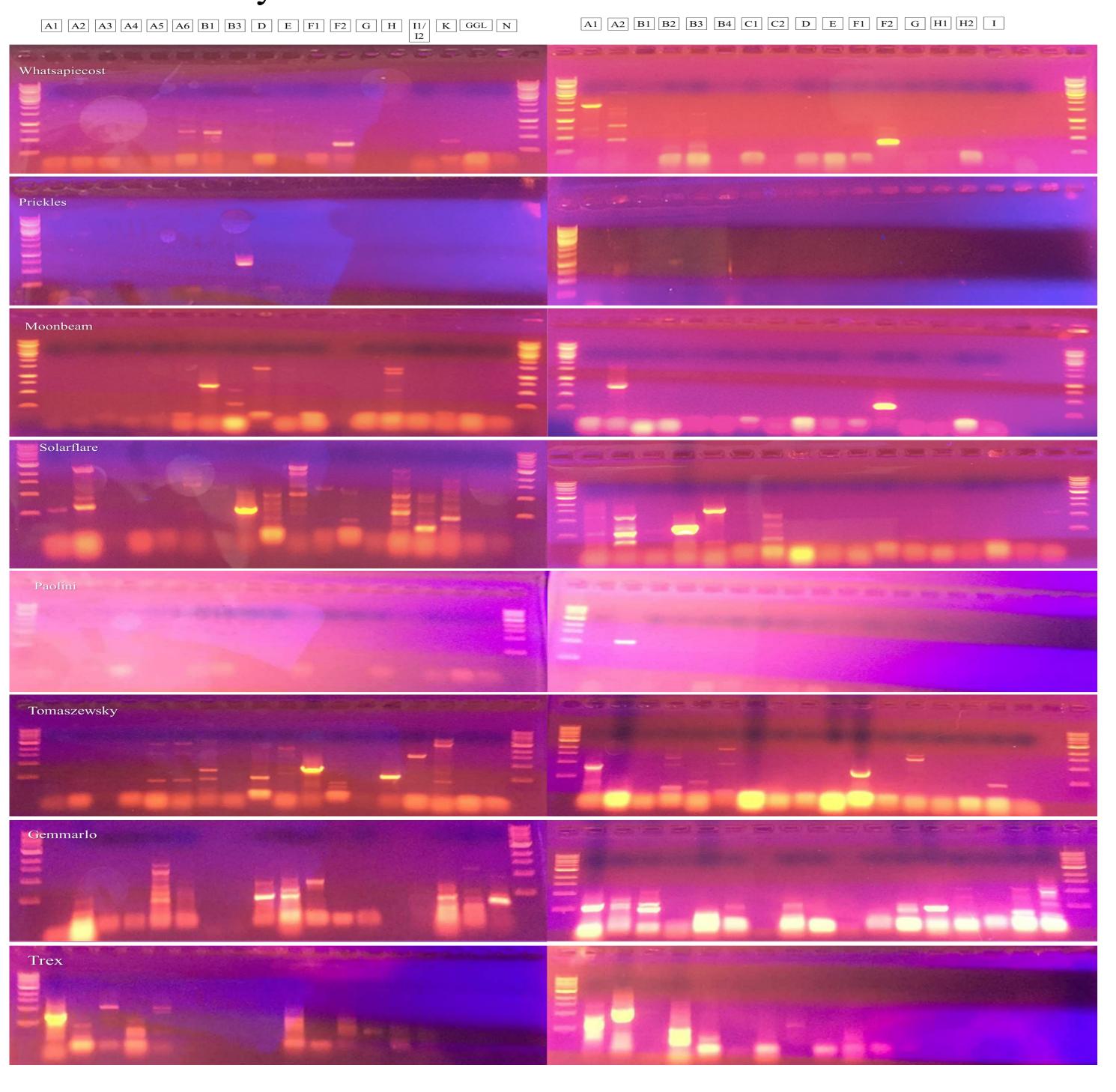
Methods

- 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



T-Rex Control T-Rex (BamHI) T-Rex (Clal) Paolini (Clal) Paolini (Clal) Paolini (Clal) Paolini (Clal) Cemmarlo Control Gemmarlo Control Gemmarlo (Clal) Prickles (BamHI) Prickles (BamHI) Prickles (Clal) Whatsapiecost Control Whatsapiecost (Clal) Whatsapiecost (Clal) Whatsapiecost (Clal)

Conclusions

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