Hits from Host to Host: Isolation, Characterization, and Comparative Genomics of Five New Gordonia phages

Alanna M Bram, Amanda J Weber, Katelyn R Chester, Kimberly E Doerr, Amanda A Drigans, Christopher O Dusek, Brenna K Franke, Allison K Granberg, Shiza S Idrees, Katie E Klinkhammer, Danielle M Kocina, Marissa L Leopold, Zachary A Lund, Theresa N Lusk, Kaitlyn J Matczynski, Abbey L Novotny, Kayla E Oberding, Allison N Potocnik, Chase A Quandt, Marissa Y Schmitz, Danielle E Schultz, Tiffany A Stocks, Willow M Stuart, Christian Thaoxaochay, Cami P Thomas, Tara N Toland, Samantha A Topel, Emily R Warren, Max R Wetzel, Rebecca J Welman, Katlyn M Williams, Olivia H Yue, Cody J Ziegler; J. Alfred Bonilla and Karen K. Klyczek, Instructors

Biology Department, University of Wisconsin-River Falls, River Falls, WI 54022

Isolation of *Gordonia* phage

Relatively few phages infecting hosts other than *Mycobacterium smegmatis* have been isolated and characterized (1). UWRF phage hunters set out to isolate phage infecting another member of the phylum Actinobacteria, *Gordonia terrae*. *Gordonia* species are found in soil and wastewater, and are associated with foaming in wastewater treatment. The same procedures used for isolating *Arthrobacter* and *Rhodococcus* phage in previous years were used to test 44 soil samples:

- LB broth was used for bacteria culture and top agar, no antibiotics
- $CaCl_2$ was added at 4.5 mM in culture broth and 2.25 mM in top agar
- Enrichments were cultured at 30ºC for 2-5 days

Results: Only five student samples yielded plaques, along with 3 instructor samples piloted prior to class. Several phages were isolated from these samples. We selected phages for sequencing that were likely to be different based on plaque and EM morphologies (Fig. 1), restriction enzyme patterns (not shown), and soil source. Five unique phage sequences were obtained (Table 1).

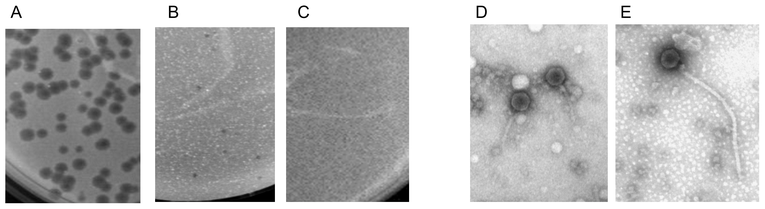


Fig. 1. Plaque morphology and electron microscopy. There was a variety of plaque morphologies observed. JSwag (A), had clear plaques of variable size (1-3 mm), sometimes with halos; Remus and Strosahl had a similar appearance. Jumbo (B) had smaller clear plaques (0.5 mm) and Bantam (C) had very small (<0.5 mm), turbid plaques. All of these new phages are Siphoviridae; Remus (D), JSwag and Strosahl had tails 100-125 nm in length, while Jumbo (E) and Bantam had longer tails, 450-475 nm.

Table 1. Sequencing Results

Phage	Soil Source	Discoverers	Genome Size and ends	GC Content	Cluster
JSwag	Garden Soil Waterford, WI	Alexandria Wojtak and Katelyn Chester	52,726 bp 3' overhang	61.9%	A15
Remus	Compost Hudson, WI	Shiza Idrees and Samantha Topel	52,738 bp 3' overhang	62%	A15
Strosahl	Compost Hudson, WI	Kerstin Strosahl	52,738 bp 3' overhang	62%	A15
Jumbo	Compost Hudson, WI	Amanda Weber	78,302 bp Direct repeat	54.5%	Singleton
Bantam	River bank soil St Croix River Hudson, WI	Max Wetzel and Marissa Leopold	92,580 bp 3' overhang	64.7%	Singleton

Bantam: Comparative Genomics

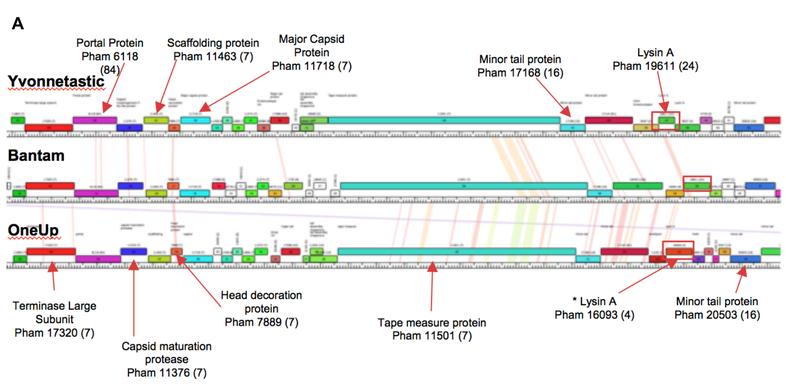


Fig. 2. Synteny and homology between Bantam and other *Gordonia* phages, Yvonnetastic (Singleton) and OneUp (cluster CQ). **A**. Shared phams encoding structural genes. **B**. Shared genes involved in replication and regulation illustrate mosaic nature of these genomes.

Bantam summary:

- 108 / 172 orfs are orphams
- No tRNAs detected
- Shared phams: structural genes, integrase, lysinA, exonucleases
- 27 phams shared with *Gordonia* cluster CQ, singleton Yvonnetastic
- 32 phams shared with phages in other hosts: *Mycobacterium* (23), *Rhodococcus* (10), *Arthrobacter* (3), *Corynebacterium* (2), *Streptomyces* (2)

Jumbo: Comparative Genomics

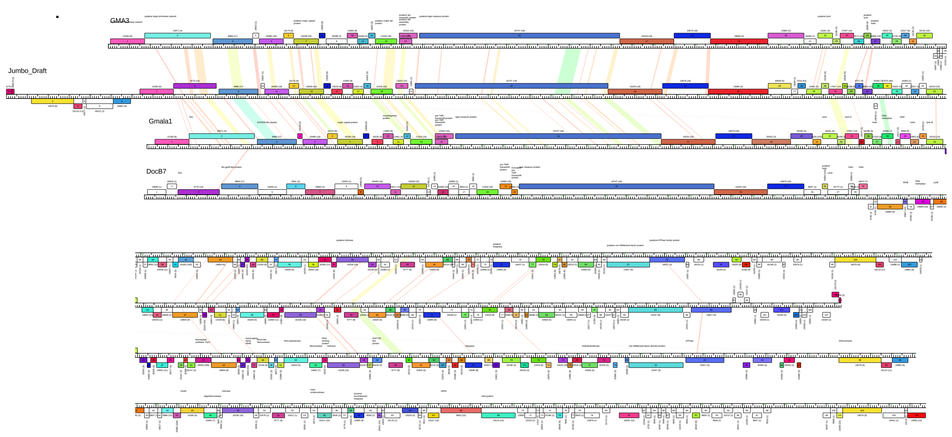


Fig. 3. Homology between Jumbo and *Gordonia* singleton GMA3 (1), cluster DF phage Gmala1 (3), and *Rhodococcus* singleton DocB7 (4), all isolated outside of the SEA-PHAGES program.

Jumbo summary:

- 42/107 orfs are orphams
- No tRNAs detected
- Shared phams: structural genes, integrase, helicase, exonuclease, endolysins, holins
- Phams shared with singleton GMA3, *Gordonia* cluster DF, *Rhodococcus* singleton DocB7
- 21 phams shared with phages in other hosts: *Rhodococcus* (15), *Corynebacterium* (2), *Streptomyces* (4)

Cluster A15 phages

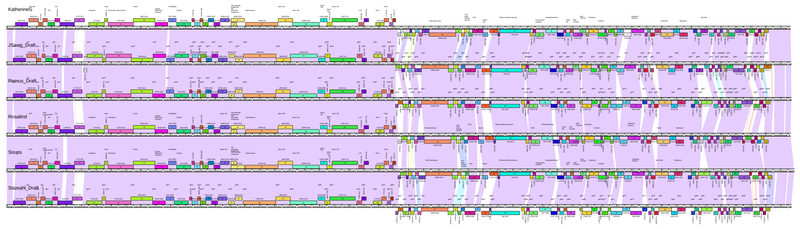


Fig. 5. Map of cluster A15 phages, showing extensive homology

Jswag, Remus, Strosahl summary:

- 96% average nucleotide identity
- 3 tRNA genes
- No orphams
- 33/102 phams unique to A15 phages
  - 24/33 in all A15 phages
- 58 phams shared with other cluster A phages
  - Most are in A2 subcluster
- 14 phams shared with other *Gordonia* clusters
- 21 phams shared with other hosts: *Rhodococcus* (15), *Streptomyces* (4), *Corynebacterium* (2)

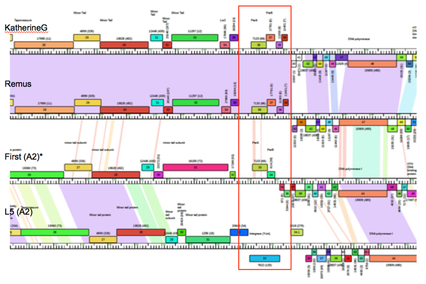
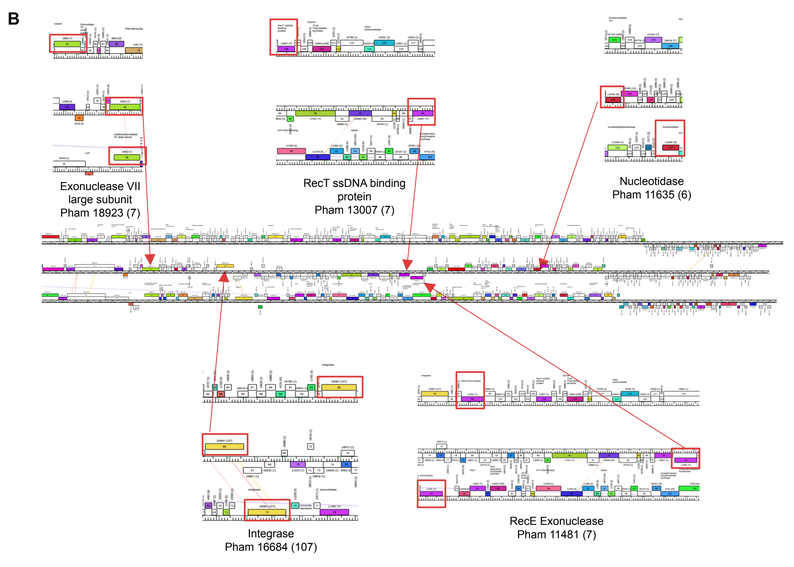


Fig. 6. Immunity regions of *Gordonia* cluster A15 and *Mycobacterium* cluster A2 phages. Note the presence of ParA and ParB genes present in A15 phages instead of integrase, suggesting a partitioning method of prophage maintenance (Stella et al 2013)



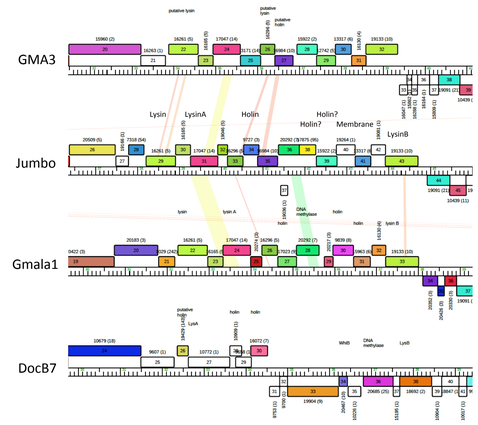
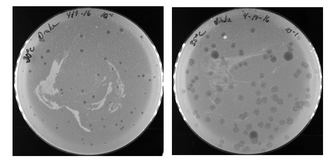


Fig. 4. Lysis cassettes in Jumbo and related phages, which were isolated as potential biocontrol agents for reducing foaming in wastewater sludge by lysing bacteria.

Fig. 7. Lysogeny in A15 phages. **A.** Culture at 35º C yielded turbid plaques. **B**. Cells from turbid plaques of JSwag, Remus, and Figaro (A15 by PCR) were streaked, transferred to liquid culture, and used in spot tests with lysates of these same phages. Lysogens were immune to all A15 phages tested, but not to Jumbo.

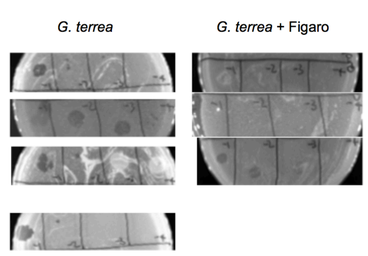
A.



Phage Drake (A15 by PCR)

B.

Lysate:	Immunity	
	Jswag lysogen	Figaro lysogen
Jswag	Yes	Yes
Figaro	Yes	Yes
Remus	Yes	Yes
Jumbo	No	No



Summary of Results

- Bantam is a singleton, with little nucleotide similarity to other phages but shared phams with other *Gordonia* phages (Fig. 2)
- Jumbo is also a singleton with similarities to *Gordonia* singleton GMA3 (isolated in Australia), *Rhodococcus* phage DocB7 (isolated in Texas), and *Gordonia* cluster DF (isolated in Texas) (Fig. 3)
- Jumbo and related phages may have multiple lysin and holin genes that could increase efficiency of cell lysis (Fig. 4)
- Remus, JSwag, Strosahl are cluster A15 phages, very similar to others in that cluster (Fig. 5). Several other unsequenced class phages were A15 according to PCR results (not shown).
- JSwag and Remus are temperate, lysogens were isolated at 35º C (Fig. 7A)
  - Partitioning system, similar to some A2 phages (Fig. 6)
  - Immunity to other A15 phages (Fig. 7B)

Conclusions

- Overall rates of phage isolation in Gordonia were fairly low. However, 5 new phages were identified, demonstrating the potential for successfully using this host
- All of the phages shared phams with phages from different hosts, suggesting old evolutionary connections
- The evolutionary histories of Jumbo, Bantam, and the A15 phages are different, based on GC content and shared phams
- One or more of these phages may have significant environmental applications, including treatment of wastewater sludge

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Acknowledgements

We thank Rick Ellingworth and the Biology Department student lab assistants for preparing materials, the Hatfull lab, University of Pittsburgh, and HHMI SEA PHAGES program for supporting this work, and the entire SEA community, for sharing experiences and advice.