7th annual sea-phages symposium

hhmi | Howard Hughes Medical Institute

Program & Abstracts

June 12 - 14, 2015

hhmi | Howard Hughes Medical Institute

Science Education Department
Undergraduate and Graduate Programs
4000 Jones Bridge Road
Chevy Chase, MD 20815-6789
www.hhmi.org

Images:

FRONT & BACK COVERS

"Phage" by Dr. Graham Beards - en:Image:Phage.jpg. Retrieved 4/21/15. Licensed under CC BY-SA 3.0 via Wikimedia Commons - http://commons.wikimedia.org/wiki/File:Phage.jpg#/media/File:Phage.jpg

INSIDE COVER

Phage Isolation Locations by GPS Coordinates, Retrieved 4/21/14 from http://phagesdb.org/GPSmap.

June 12, 2015

Welcome to the 7th annual SEA-PHAGES symposium!

Over the past seven years, the Science Education Alliance – Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program has grown steadily, and this year there are more than 2,600 students at 74 institutions actively engaged in phage discovery and genomics. The program is vibrant and dynamic because of the contributions of all the students, faculty, and teaching assistants, and this year there have been many notable advances, both programmatic and scientific. We are delighted to welcome you to another great symposium highlighting your accomplishments.

The overall quality of the abstracts is outstanding, and selecting from among them for oral presentations was especially challenging. We hope you will make it a priority to see as many posters as possible and discuss the findings with the presenters.

Our keynote speaker this year is Eric Betzig from right here at the Janelia Research Campus. Eric shared the Nobel Prize for Chemistry in 2014 for the development of advanced methods that exceed the theoretical resolution limits of light microscopy. We very much look forward to his keynote presentation.

The alliance continues to grow, and please join us in welcoming faculty from seventeen new schools:

Albion College, Drexel University, Durham Technical Community College, Kansas State University, Lafayette College, LeTourneau University, Massey University, University of California-Los Angeles, University of Detroit Mercy, University of Minnesota-Morris, University of Southern Mississippi, University of the Sciences in Philadelphia, University of West Florida, Virginia Polytechnic Institute and State University, Wayne County Community College District, Western Carolina University, and Worcester Polytechnic Institute. We hope you enjoy meeting your new colleagues.

Our team at HHMI, James Madison University, and the University of Pittsburgh relies on your feedback about the symposium and the SEA-PHAGES program in general, and your input is extremely valuable. We look forward to hearing your thoughts.

Sincerely,

David J. Asai
Senior Director
Department of Science Education
HHMI

Graham F. Hatfull
Department of Biological Sciences
University of Pittsburgh

welcome

save the dates

8th Annual SEA-PHAGES Symposium

Ashburn, VA

June 10-12, 2016

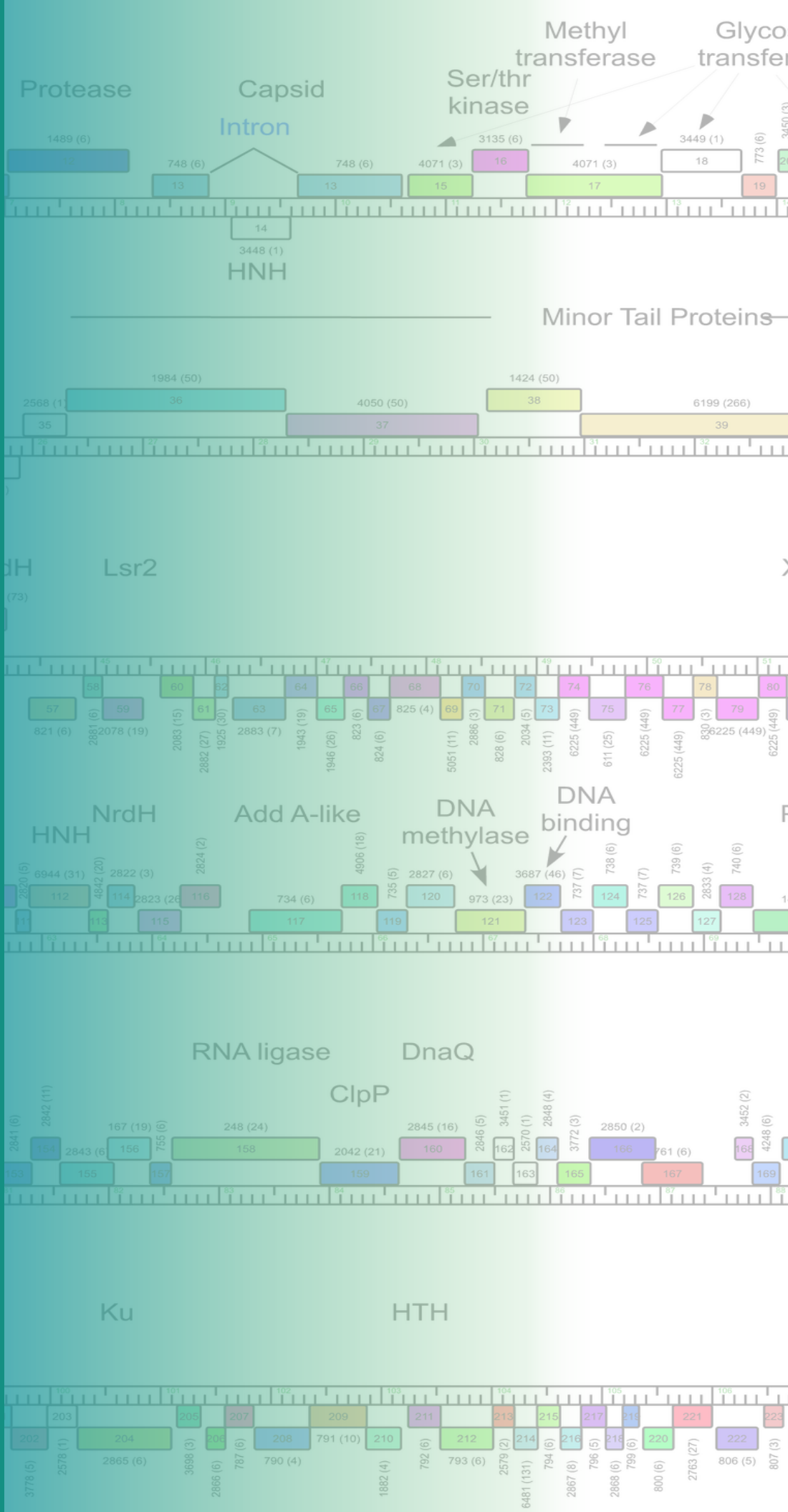
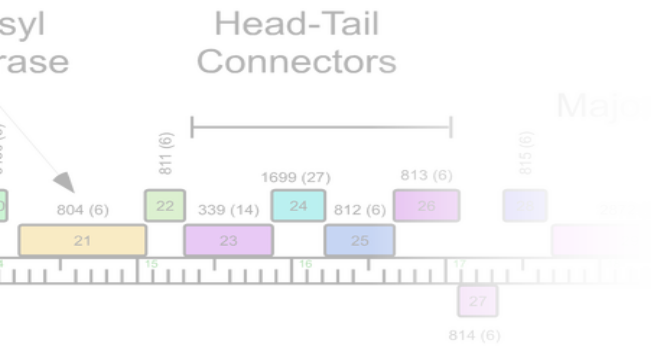
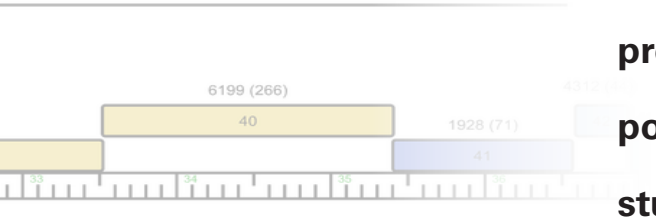
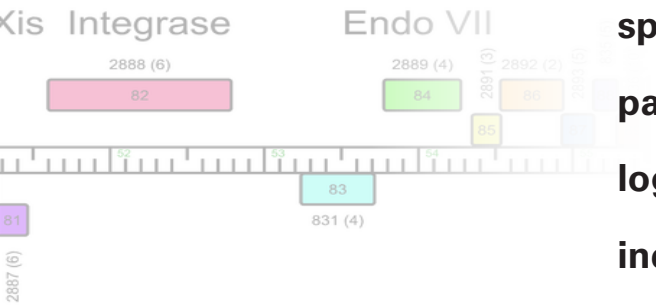
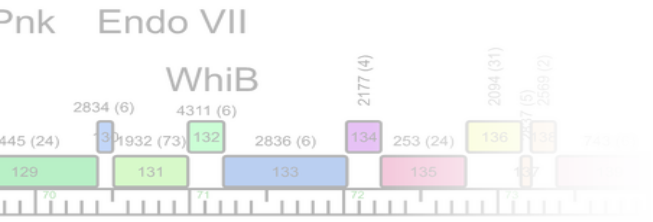


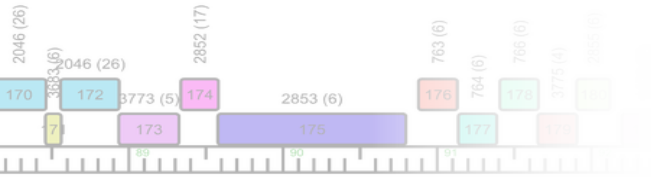
Image: Genome map of Mycobacteriophage LittleE, Retreived 4/21/2014 from http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0069273

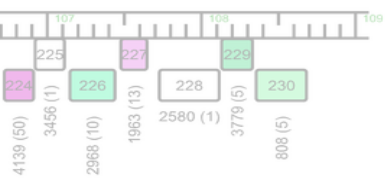












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7th Annual SEA-PHAGES Symposium

HHMI Janelia Research Campus :: Ashburn, VA
June 12-14, 2015

Friday :: June 12

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Poster Set-up :: *Lobby*

Student Presenter Practice Period :: *Seminar Room*

5:00 – 6:00p **Student Debriefing** :: *Seminar Room*

5:00 – 6:30p **Faculty Meeting** :: *Auditorium*

6:00 – 7:30p **Dinner** :: *Dining Room*

7:30 – 7:45p **Welcome and Remarks** :: *Auditorium*
David Asai :: HHMI Science Education

7:45 – 9:00p **Keynote Speaker I** :: *Auditorium*
Eric Betzig :: Janelia Research Campus
Imaging Life at High Spatiotemporal Resolution

***Introduction by**:*
Kayla Long and Courtney Smith :: Baylor University

9:00 – 9:30p **Social** :: *Lobby*

Saturday :: June 13

7:00 – 8:30a **Breakfast** :: *Servery*

Poster Set-up :: *Lobby*

8:30 – 8:45a **Introductory Remarks** :: *Auditorium*
David Asai :: HHMI Science Education

8:45 – 10:00a **Keynote Speaker II** :: *Auditorium*
Graham Hatfull :: University of Pittsburgh
Lost at SEA: Navigating the Actinophagosphere

***Introduction by**:*
Johnathan Schiebel and Megan Ulbrich :: University of Pittsburgh

10:00 – 10:15a **Break** :: *Lobby*

10:15a – 12:00p **Poster Session I** (Odd-numbered Posters) :: *Lobby*

12:00 – 12:30p **Group Photo** :: *TBD*

program

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12:30 – 2:00p **Lunch** :: *Servery*

2:00 – 3:30p **Student Talks I** :: *Auditorium*

Moderators:
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Brianna Morgan :: University of Colorado Boulder

Izzy Owen :: Seton Hill University
Isolation and genomic characterization of Mycobacteriophages Wilbur and Romney2012

Sarah Belay and Sarah Modlin :: College of William & Mary
From Compost to Clusters: Rhodococcus *Phages from Coastal Virginia*

Amanda Sciola and Kamil Wielechowski :: Southern Connecticut State University
Genomic analysis of four mycobacteriophages from Southern Connecticut: Two A3's, EpicPhail & Veracruz, and two new Cluster N phages, Xeno and Phrann

Kaivalya Dandamundi and Shelby Edling :: Virginia Commonwealth University
OutPHAGEous Discoveries

Sophie Jurgensen :: James Madison University
Bioinformatic and Virological Evidence Suggests a Novel Scheme for Lysogeny in a Mycobacteriophage

Julia Gross :: Brown University
Characterization of Palindrome Usage in Mycobacteriophage Genomes

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7:00 – 8:30p **Student Talks II** :: *Auditorium*

Moderators:
Pamela Fawns and Taylor Osborne :: Nebraska Wesleyan University

Erin Lockwood and Nijewel Holliday :: Lincoln University
Finding Panchino - a novel Cluster N Mycobacteriophage isolated at Lincoln University, Pennsylvania

Brigett Carvajal :: Queensborough Community College
Genomic Analysis of Mycobacteriophage Smeadley

Sara Lohbauer and Brittany Sisson :: Florida Gulf Coast University
A Comparative Bioinformatic Analysis of Four Novel Rhodococcus *Bacteriophages*

Alexandra Crum and Trang Nguyen :: College of St. Scholastica
Proteomic and Genomic Analysis of Novel Cluster A, B and P Mycobacteriophages

Gina Brockman and Elise Lemanski :: The Ohio State University
Four years of mycobacteriophage isolation at Ohio State

Madeline Kosch and Kaylah Schuette :: University of Wisconsin-River Falls
From Australia to Wisconsin: Isolation and characterization of several members of a related group of Rhodococcus *phage*

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Sunday :: June 14

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8:30 – 10:00a **Student Talks III** :: *Auditorium*

Moderators:
Kelly Garrigan :: Saint Joseph's University
Connor McKenney :: North Carolina State University

Cedric Penn Jr. :: Morehouse College
The discovery and annotation of mycobacteriophages BigPhil and Sotrice96

Alyssa Benjamin :: Bucknell University
Analysis of Additional Arthrobacter *Phage with Small Genomes (Cluster D) and the Singleton Kitkat*

Johnathon Schiebel and Megan Ulbrich :: University of Pittsburgh
Gee Whiz: Emerging diversity of the Cluster G mycobacteriophages

Kyle Cushman and Haley Fischman :: Hope College
Lysis Cassette Mosaicism and Potential Expanded Host Range Evident in the Genomes of Glass and Bella96

Fernanda Alonzo and Gillian Holder :: University of Louisiana at Monroe
Annotation of Rhodococcus erythropolis *Phage Singletons Trina and Chewy VIII*

Nolan Games and Buzz Hardin :: Ouachita Baptist University
Genomic Analysis of Cluster T Mycobacteriophage

10:00 – 10:30a **Break** :: *Lobby*

10:30 – 10:40a **Awards Ceremony** :: *Auditorium*

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10:40 – 11:40a **Faculty Presentations** :: *Auditorium*

Moderators:
Sara Lohbauer :: Florida Gulf Coast University
John Ramirez :: Del Mar College

Randall DeJong :: Calvin College
Phamerator Database Manager: A web-based application for building customized Phamerator databases

Julianne Grose :: Brigham Young University
Investigating the Relationships of Bacteriophages with a Class Reveals Obvious Borders Between Bacterial Orders

Nathan Reyna :: Ouachita Baptist University
Marianne Poxleitner :: Gonzaga University
Cluster O promoter investigations at Gonzaga and Ouachita Baptist Universities

Kari Clase :: Purdue University
Exploring the Impact of Bacterial Growth on Mycobacteriophage Protein Expression by Mass Spectrometry

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Graham Hatfull

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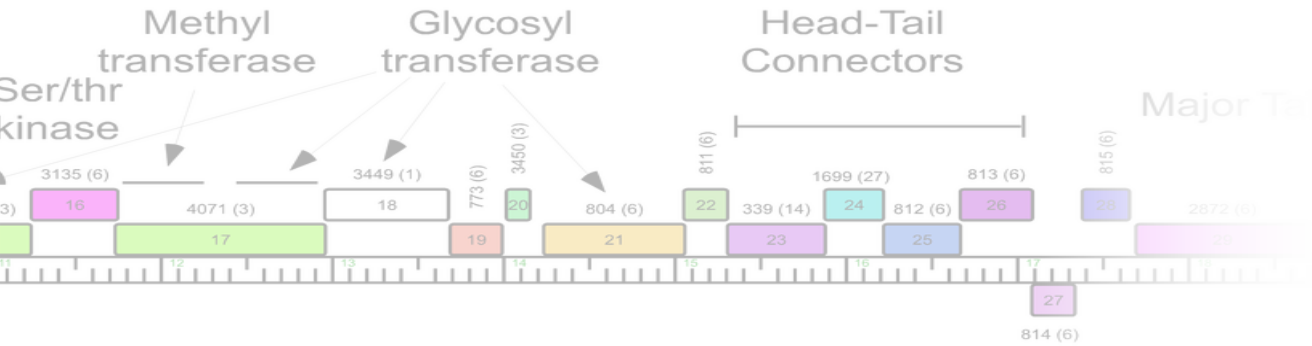
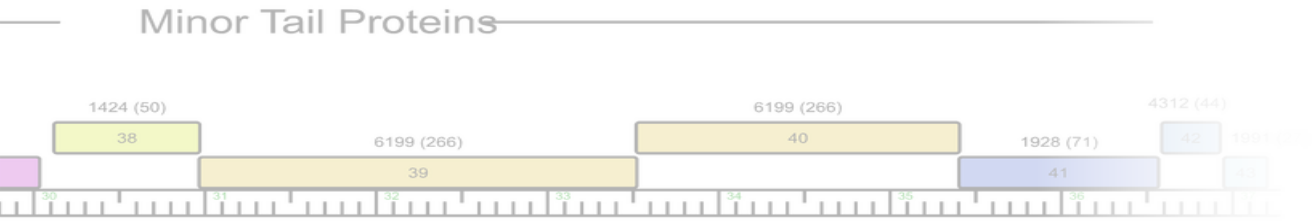
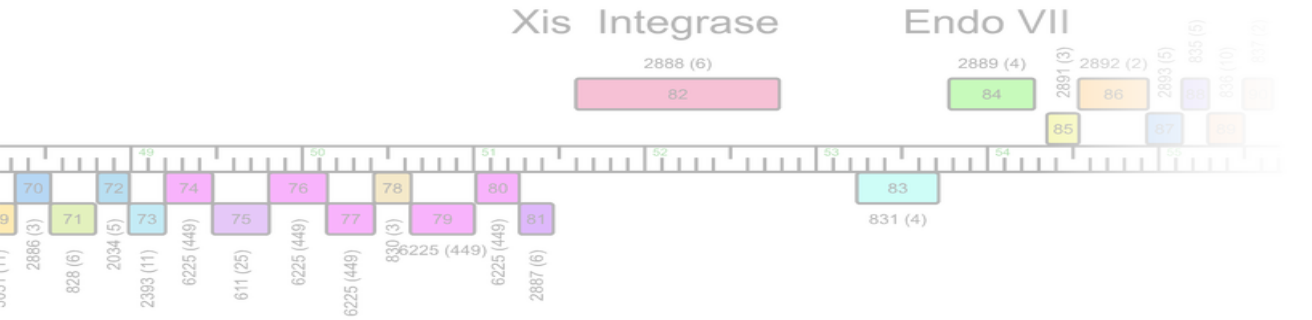
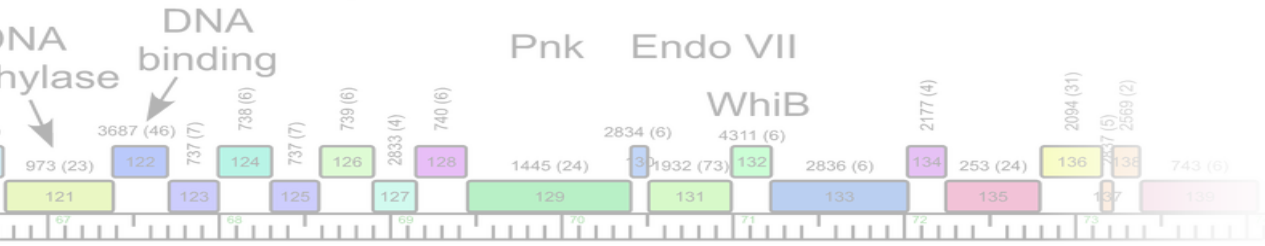
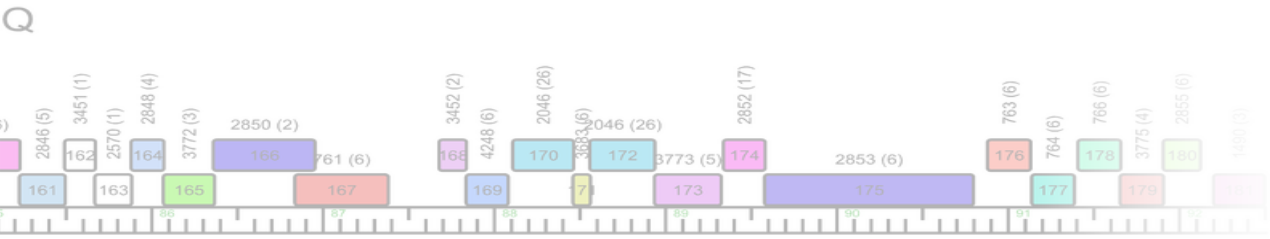
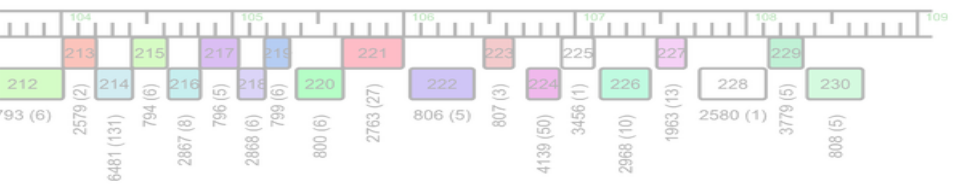








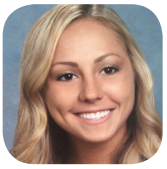




student abstracts

Poster 1

Comparative Genomics of Arthrobacteriophages



K. Long

C. Smith

Kayla D Long, Courtney L Smith, Robert M Athey, Aasmitha Chitturi, Margaret D Fitzgerald, Dalia A Hamza, Alexandria R Holden, Mackenzie A Kallemeyn, Skye J Kim, Vivian E Kwok, Tessa J Merritt, Taylor A Nesbit, Courtney N Nguyen, Eli X Ornelas-Lopez, Timothy P Philip, Caroline H Ragsdale, Aadil Sheikh, Kasey A Stokdyk, Toby N Towsley, Jeffrey D Walrod, Jennifer A Wilson, Erin N Wingerson, Tamarah L Adair

Baylor University, Waco TX

Bacteriophages, viruses that infect bacteria, have both temperate and lytic life cycles. They are found in most environments and are considered the most prolific entities in the biosphere. Arthrobacteriophages are bacteriophages that infect *Arthrobacter species*, a genus of bacteria found most abundantly in the soil. This study entailed the isolation, purification, and characterization of 22 individual Arthrobacteriophages. These bacteriophages were discovered in soil samples collected from a variety of geographic locations by Baylor University students. The main purpose of our collective investigation is to perform comparative analysis between Arthrophage genomes and to test the possibility that an Amigo-like prophage exists in our host.

Methods: Enrichment of each soil sample with *Arthrobacter sp.* produced a lysate for plaque purification. The isolated phages were characterized using transmission electron microscopy, restriction digestion using five endonucleases and gel electrophoresis. Seventeen phages were archived and ten genomic DNA samples were submitted for sequencing to the Pittsburgh Bacteriophage Institute's sequencing facility. Gene annotation was performed using DNAMAster, Glimmer, GeneMark, BLAST, HHPred, Starterator, and Phamerator to identify potential genes and assign putative functions. Further analysis of genomes used VISTA, MAUVE, PHAST, and other bioinformatic tools.

Results: All isolated phages were *Siphoviridae*. Results from the sequencing of eight phages indicated that three of the samples were identical or similar to Arthrophage Amigo, a Baylor University phage isolated and sequenced in 2013-14. This led to the design of Amigo primers to test the remaining DNA samples and the host genome. PCR indicates that Amigo sequences are present in the host *Arthrobacter sp* ATCC 21022, but this result is currently being confirmed. The class annotated four non-Amigo-like phages. Link and Courtney3 are approximately 15,500 bp in length, similar to Sandman and Tolouse. Steve and LeeroyJ are similar to Jawnski with a length of approximate 51,000 bp. These two groups of phages differ from Amigo, which is about 59,000 bp. We are currently using bioinformatics tools to investigate clustering methods and to analyze genome and protein structure features. This poster will present a comparative analysis of Arthrobacteriophages.

Conclusion: In addition to informing the field of phage genomics, further research into the genomes of Arthrobacteriophages may lead to the development of new molecular tools for research. Phages may provide an alternative to antibiotics or food preservatives. Investigation of bacteriophages may provide insights into novel bacterial strains able to reduce the accumulation of harmful inorganic compounds and agricultural pesticides in the soil.

notes

Student presenters' names appear in **bold**.

Poster 2

Isolation and Characterization of *Paenibacillus larvae* and *Brevibacillus laterosporus* Bacteriophages to Understand Their Evolutionary Relationships

Jordan A Berg, **Ian D Esplin**, Braden M Brundage, Justin T Crockett, Kyle P Esplin, Marlee R Evans, Karli E Heaton, Jonathan R Hyde, Morgan S McBride, Austin R Simister, Jared A Hilton, Jordan T Schouten, Trever L Thurgood, Bryan D Merrill, Andrew T Ward, Donald P Breakwell, Sandra H Burnett, Julianne H Grose

Brigham Young University, Provo UT

Paenibacillus larvae is a spore-forming bacterium that infects honeybees in their larval stages and is a cause of the dwindling honeybee populations worldwide through the devastating diseases American and European foulbrood. A close relative, *Brevibacillus laterosporus*, is another spore-forming bacterium that causes a secondary gut infection associated with *Paenibacillus*-inflicted European foulbrood. In an effort to better understand their host and their potential for halting the spread of this infectious disease, six novel *Paenibacillus* bacteriophages were isolated and three highly-related *B. laterosporus* bacteriophages were annotated (Xane, Jenst, and Osiris) and compared to the eight that have been previously isolated and sequenced (Abouo, Davies, Jimmer-1, Jimmer-2, Powder, Sundance). To better understand *B. laterosporus* and its bacteriophages, we clustered phages based on similarity using Gepard dotplots and Mega6 phylogenetic trees, identified and mapped DNA motifs using MIME, FIMO, and DNA Master, identified a transposable region contained in Sundance and Xane but excised from Jenst using HHPred, Phamerator, and DNA Master, and identified repeated genes and compared their predicted tertiary structures using Clustal Omega, RaptorX, and STRAP. From these data, we determined that Powder and Osiris belong in the previously described Jimmer-like cluster, Xane and Jenst form a novel phage cluster, and Sundance is a singleton. A motif repeated throughout Xane and Jenst was frequently located near genes responsible for DNA replication, nucleotide metabolism and transcription suggesting transcriptional co-regulation. In addition, paralogous transcriptional regulator genes were identified in four *Brevibacillus* phages suggesting conservation of function in the phage lifecycle. Variation at residues predicted to bind specific nucleotides indicates these paralogs likely bind different DNA sequences, exhibiting protein plasticity. Finally, a putative transposable element was identified in Sundance and Xane that carries genes homologous to those found in Brevibacillus hosts. Remnants of this transposable element were also identified in Jenst. Comparing the GC content of this region to the surrounding area indicated that Xane acquired this region more recently than Sundance. These discoveries provide a greater understanding of the unique features of these bacteriophages, their behavior, and their evolutionary relationships to one another.

Poster 3

Characterization of Palindrome Usage in Mycobacteriophage Genomes*

J. Gross

Julia Gross, Kenyon Alexander, Brandon Le, Zachary Ricca Ricca, Hwai-Ray Tung, Benjamin Siranosian, Yang Zhou

Brown University, Providence RI

Palindromes (DNA sequences with identical reverse complements) are thought to occur infrequently in bacteriophage genomes. They are often targets of Type II restriction enzymes, used by bacteria as part of restriction-modification systems. However, *M. smegmatis* does not code for any restriction enzymes and the *Mycobacterium genus* contains few restriction enzymes in general. We examined the presence of length-4 and length-6 palindromes in the genomes of all sequenced mycobacteriophages. Palindrome usage is varied across phage genomes, but we found interesting cases of both over and under-used palindromic sequences.

In accordance with the idea of under-representation of palindromes, all cluster A4 phages lack the sequence ATAT and half of cluster A2 phages lack AATT. We tested the probability of this occurring in genomes with the same length and GC content but randomized nucleotide order. The probability of a randomized A4 genome not having an ATAT sequence is less than 10^{-23}. A selective pressure must be suppressing the occurrence of these palindromes. Similarly, some genomes do not have a single example of certain length-6 palindromes; these palindromes are frequently AT-rich. Surprisingly, there is no correlation between sequence length and the number of unused length-6 palindromes.

Previous research at Brown highlighted the frequent usage of GATC and GGATCC palindromes in cluster B3 mycobacteriophages. We examined genes from B3 phages with strong homology to non-B3 cluster B phages. After alignment with BLAST, 73% of B3 GATC sites had mismatches or gaps in the comparison sequence. Most mismatch sites contained a single G to C transversion. These results argue that B3 phages commonly maintain a palindromic GATC site where related phages have NATC.

In this project, we sought to shed light on examples of both palindromic over and under-representation in hopes of elucidating what role palindrome frequencies play in phage biology and the evolutionary pressures driving outliers. Furthermore, investigating palindrome usage is an excellent way to teach the basics of computational biology, genome analysis, and data processing to students in the SEA-PHAGES program.

**Abstract selected for a talk.*

Poster 4

Analysis of Additional *Arthrobacter* Phage with Small Genomes (Cluster D) and the Singleton Kitkat*

A. Benjamin

Alyssa K Benjamin, Chaylen J Andolino, Christopher J Blasi, Katherine C DeRuff, Grace L Forster, Abigail H Garrett, Amber T Le, Spencer Li, Iris Lu, Nathan D Luftman, James H McGinnis, Jessica E Medrano, Scott M Meredith, Katie L Otero, William J Pinamont, Ross T Pirnie, Jakob R Yankauskas, Marie C Pizzorno, Emily L Stowe

Bucknell University, Lewisburg PA

We identified six new bacteriophages that infect *Arthrobacter sp.* All of these phages have small genomes of just over 15kbp and are closely related to each other and the Cluster AN *Arthrobacter* phages listed in Phamerator. Two of the phages, Yank and Decurro, were isolated from the same soil sample based on different plaque morphologies. Upon sequencing it was determined that there is a single base change at position 11,243, which changes a serine to isoleucine in gp15. The predicted amino acid sequence of gp15 has no strong similarity to any proteins in Genbank, which makes the different plaque morphologies very interesting. The genomes of the other four phages show high levels of similarity to each other and to the genome of Sandman, which we isolated last year. The major differences between these genomes are near the beginning, where some of the genomes either lack "gene 2" (Toulous) or have one of two variants of this gene (pham 892 or pham 972). The version of gene 2 found in Sandman (pham 671) is different from the other Cluster AN phages and represents an orpham. There is a single reverse gene that was not identified by DNA Master in all of these genomes. Even though all of the genomes appear to contain this reverse gene, which encodes a protein with predicted helix-turn-helix structure, small sequences deletions near the start of this orf determined whether it was called or not. This reverse gene is likely the repressor, since all of these phages appear to be temperate and upon lysogen testing are homoimmune to each other. We note that some aspect of our phage hunting technique enhances the isolation of phage in this cluster and changes in various conditions will be tested to see if they foster discovery of phage with more diverse genomes. In addition to the analysis of the Cluster AN *Arthrobacter* phages, we analyzed the genome of Kitkat, an *Arthrobacter* phage discovered last year. The genome of Kitkat is 58560bp in length and appears to be a new singleton. All of the 100 ORFs encoded by Kitkat are in the forward orientation. Kitkat is predicted to be lytic since it produces clear, small plaques and lysogens could not be isolated. This corresponds with the lack of an orf that encodes a putative repressor protein. Of the 100 proteins that Kitkat encodes, many appear to have putative enzymatic functions involved in DNA replication, recombination and repair. Some of these contain intriguing homologies to proteins involved in the CRISPR/Cas system, which have been identified in other phages and could provide a counterattack to the host defense system.

Poster 5

An optimized enrichment technique for the isolation of *Arthrobacter* bacteriophage species from soil sample isolates

D. Chudoff

C. Schoff

Dylan Chudoff, Courtney Schoff, David Dunbar, Katrina Sterling, Haley Broomell, Alexandra Correa, David Shade, Libby Graves

Cabrini College, Radnor PA

We present an enrichment protocol for the isolation of bacteriophages infecting bacteria in the *Arthrobacter* genus of bacteria. This enrichment protocol produces fast and reproducible results for the isolation and amplification of *Arthrobacter* phages from soil isolates. The enrichment technique described here, unlike many others, uses a filtered extract free of contaminating bacteria as the base for indicator bacteria growth, *Arthrobacter sp.* KY3901, specifically. By first removing soil bacteria the target phages are not hindered by competition with native soil bacteria present in initial soil samples. This enrichment method has resulted in dozens of unique phages from several different soil types and even produced different types of phages from the same enriched soil sample isolate. In the development of the procedure described here we attempted to account for selective bias inherent in any enrichment procedure by testing a range of $CaCl_2$ concentrations. What became apparent is that some phage isolates show physiological differences dependent on parameters like calcium ion concentration and temperature. The current collection of 14 phages that have had their genomes sequenced can be "clustered" with other recently sequenced *Arthrobacter* phage genomes based on the Arthrobacterphamerator database. Of the 14 phages with genomic sequencing data, 12 of them have the siphoviral morphotype and of these two of them have the prolate head morphology. Two of our sequenced phages have the myoviral morphotype with one of them being a temperate phage. Broadly speaking, the use of this enrichment technique with LB media should prove to be hardly exclusive to the isolation of *Arthrobacter* phages. While LB has been the industry standard for culturing *Escherichia coli* strains and other members of the *Enterobacteriaceae*, it supports the growth of a wide variety of bacteria in aerobic conditions. The nutrient rich composition of LB media, in one formulation or another can likely be used in this method to propagate phages in an incredibly diverse group of aerobic bacteria preventing technical challenges from hindering phage discovery.

Poster 6

An Enterobacteriophage Extravaganza: New Clusters of Enterobacteriophages Containing Fourteen Novel Isolates from Soil, Termite Guts and Snail Guts

J. Stout

R. Voogt

Jacob A Stout, **Rebecca S Voogt**, Rachel M Anderson, Stephen Buhrt, Brianna M Busscher, Kathryn M Driscoll, Kyle J Gontjes, Amanda G Hoff, Marko Ivancich, Alexandra N Kuipers, Connor E Kuipers, Thuy-Tien N Nguyen, Andrew Philipose, Morgan G Quist, Cambrynne J Rietberg, Alan W Stockdale, Jeremiah J Stout, Kristin Strydhorst, Ethan C Valentine, Sarah L VanTubergen, Tanner M Vincent, Elianna R Walters, Kelsey L Veldkamp, Serita M Nelesen, Randall J DeJong, John T Wertz

Calvin College, Grand Rapids MI

Termites and snails have complex symbiotic gut microbial communities that perform essential functions for the host. Members of the *Enterobacteriaceae* family have been implicated in several of these functions. The ecology of internal and externally-derived enterobacteriophages and their ability to stabilize or disrupt the gut bacterial community is the focus of our investigation, which hinges on the ability to isolate enterobacteriophages from various soils and geographically and temporally separated termites and snails. We isolated 22 bacteriophages from the termite gut, snail gut, and soil, using three separate snail/termite enterobacterial symbionts as hosts. Several phages appear morphologically similar to *myoviridae*, but the majority have short, non-contractile tails similar to the *podoviridae*. Ten were considered "slow-growers" (i.e. taking more than 48 hours to form plaques on isolation media). Of the 22 phages, we obtained complete genome sequence from fourteen. Eleven phage genomes are very similar in length (62,325 to 62,660 bp), associated with *podoviridae* morphologies, and are <1% detectably similar (discontiguous megablast) to sequenced phages in GenBank. Remarkably, these phages form three very distinct clusters at the nucleotide level (as little as 9% of genome detectably similar), but share genome architecture and pham identification in all but a few genes. The remaining three genomes are associated with *myoviridae* morphologies. Lily has a genome length of 92,613 bp and no nucleotide similarity to sequenced phages in GenBank. DJasperse and Sava are 178,243 and 178,429 bp, respectively, and have up to 88% average nucleotide identity (ANI) with each other and with two phages from *Cronobacter* and *Citrobacter* hosts. When combined with prior years' data, we have isolated 32 enterobacteriophages that form ten clusters, most of them novel. Hence, we have isolated a significant amount of previously untapped enterobacteriophage diversity. Two bacteriophages that were isolated from soil were highly similar (>99% ANI) to two from termite guts collected >10 miles away. An intriguing possibility is that these phages represent lysogens from the host bacterium. Further investigations using PCR on bacterial host DNA as well as termite and snail gut DNA are planned and will help resolve this question.

notes

Poster 7

Expanding our Knowledge of *Arthrobacter* and *Rhodococcus* Phage Diversity

N. Chen

K. Hanson

Nathalie Chen, **Kathryn Hanson,** Apeksha Atal, Gaurav Begur, Matthew Bofemkamp, Alyssa Brandt, Sarah Deluty, Jenny Gao, Ben Kaplan, Jonathan Kim, Hannah Nourie, Jenna Shin, Erin Sipple, Hannah Soltz, Tanya Talwar, Tuan Azmal Thahireen, Carolyn Vanek, Annabella Wong, Sharon Wu, Ling Xu, Jonathan Jarvik, A. Javier Lopez, Swarna Mohan

Carnegie Mellon University, Pittsburgh PA

Bacteriophages are the most numerous biological entities on the planet. They have important ecological roles, and there is renewed interest in their potential therapeutic applications. A continuing exploration of bacteriophage diversity in different host systems will enhance our understanding of their evolution, biological impact and potential uses.

We isolated 20 phages that infect *Arthrobacter sp.* plus one that infects *Rhodococcus globerulus* and we sequenced their genomes using IonTorrent technology. *Arthrobacter* phage Moki was also sequenced using Illumina technology at U. Pittsburgh and has been completely annotated. Genomes for the other phages have been completely assembled, but not finished, and are partially annotated.

The *Rhodococcus* phage (Tina, isolated from soil in Providence, RI) is a Siphovirus closely related to RER2 and RGL3 previously isolated from wastewater in Australia. This trio is distinct from other reported *Rhodococcus* phages. Their genomes are circularly permuted, and all three contain a cluster of three tRNA genes near the end of the right arm. The predicted lysin and holin genes are widely separated in their genomes. We showed experimentally that Tina is temperate on *R. globerulus*. This is consistent with the finding of integrase and excisionase ORFs in its genome. These ORFs are conserved in RER2 and RGL3, although these phages are reported to be virulent on *R. globerulus*.

Seventeen of our *Arthrobacter* phages are siphoviruses and 3 are myoviruses. Of these, 19 phages are closely related to previously known *Arthrobacter* phages, including 3 similar to cluster A, 6 C1, 4 C2, and 3 D. The 3 myoviruses are most similar to Steve, Brent and Shade, respectively. The last phage (Swalo, isolated in Pittsburgh, PA) is currently unique. Its left arm, comprising the structural and assembly genes, has strong similarity to ArV2, a singleton isolated in Lithuania, but its right arm is highly divergent. Both phages contain predicted integrase and repressor ORFs, suggesting they are temperate, although we have not confirmed this experimentally. The region containing these lysogeny genes is inverted between the two phages.

As observed in our first round of *Arthrobacter* phage screening (2013-2014), siphoviruses with unusually small genomes (<16 kb; cluster D) continue to be isolated frequently, suggesting that they represent a highly successful viral strategy in this niche. Interestingly, the *Arthrobacter* myovirus genomes so far are also significantly smaller (~50kb) than *Mycobacterium* myoviruses (~155 kb).

Ten of our phages belong to an intriguing group (clusters C1, C2) comprising siphoviruses with low GC content (<50%) and similarity to *Rhodococcus* phages ReqiPoco6 and Pepy6. As previously suggested for *Mycobacterium* phage Patience (Pope *et al* 2014), the discrepancy in GC content with *Arthrobacter* may indicate that this group evolved in a different host.

notes

Poster 8

Bartholomew by Land and Cobra by Sea: Mycobacteriophages from Clusters B1 and P

B. Boren

M. Wenta

Benjamin J Boren, **Meredyth D Wenta**, Aidan J Crosby, Megan J Fallert, Tristan R Grams, Emily A Gulbrandsen, Stefanie A Huttelmaier, Rehnaz Jiwani, Lucinda R Krenzke, Robert E Miner, Tanisha E Perlmutter, Perrin M Schupbach, Alexandra M Steffen, Chas F Young, Deborah M Tobiason

Carthage College, Kenosha WI

Throughout the course of the 2014-2015 Phage Hunters course, 15 novel mycobacteriophages were isolated. Two siphoviridae mycobacteriophages were chosen to be sent for sequencing based on the quality and quantity of DNA that was obtained from them. Bartholomew, a temperate, cluster P mycobacteriophage was isolated from a soil sample and had a genome with 46,484 base pairs and defined physical ends. Bartholomew's genome displayed the most homology with Fishburne, a cluster P mycobacteriophage. Cobra, a lytic, cluster B1 mycobacteriophage was isolated from a water sample and had a genome with 68,875 base pairs and circularly permuted ends. Cobra showed the most homology with PG1, a well characterized B1 mycobacteriophage. The sequenced genomes were then annotated using various bioinformatic software including DNA Master, HHpred, BLASTp, and Phamerator. Such tools allow for the identification of essential proteins related to structure and phage replication. Further bioinformatic analyses of Cobra and Bartholomew's genomes are being performed to compare their evolutionary relationships with other mycobacteriophages. All of the temperate phages isolated, including Bartholomew, are also being tested in immunity assays to further characterize their phage biology.

Poster 9

Mycobacteriophage Pipsqueaks: The Complete Genome Annotation of a New Cluster N Phage

Victoria Edmund, Heba Abdin, Brianna Harmon, J'Neice Payne, Brandi Bell, Samaria Boyd, Samantha Bridges, Maya Dilligard, Taylor Gibson, TaMia Morris, Cherie Pitts, James Solomon, Christopher A Korey

College of Charleston, Charleston SC

The HHMI SEA-PHAGES program at the College of Charleston is run with under-represented minority first-year students in a collaboration between the First Year Experience and the South Carolina Alliance for Minority Participation (SCAMP). In the fall semester, students isolated six novel bacteriophage viruses from soil samples using a variety of laboratory tools and techniques. The bacteriophage Pipsqueaks was selected from the six phage identified in the first semester to be sequenced at the University of Pittsburgh. In the spring semester, students in the First Year Experience Research Course explored basic annotation and bioinformatics. Pipsqueaks' genome sequence was annotated from the 5' to 3' end using the DNA Master Genome Annotation Software. We identified the open reading frames based on the predicted coding potential using the annotation algorithm programs Glimmer and Gene Mark. Pipsqueaks is likely a temperate phage given the presence of an integrase and a putative repressor gene. We also found that it is 99% identical to Carcharodon, which was isolated in Jacksonville, Alabama in 2013. Here we present the initial data from the annotation of this new cluster N phage.

notes

Poster 10

JetBlade: A novel mycobacteriophage isolated in Southwest Idaho

Tran C Tran, **Claire E Otero**, Evan T Fishburn, Jacob A Noeker, Steven B Schulte, Mary E Totten, Ann P Koga, Richard L Daniels

College of Idaho, Caldwell ID

Bacteriophages (viruses that infect bacteria) are the single most abundant biological entity in the biosphere, with a population estimated at 10^{31} particles. As part of the HHMI SEA-PHAGES program, the College of Idaho's Fall 2014 BIO210 class isolated and characterized 13 mycobacteriophages from Southwestern Idaho that infect *Mycobacterium smegmatis*. Electron microscopy revealed that each of these mycobacteriophages displayed morphological characteristics consistent with a *siphoviridae* morphotype. One of these newly-isolated mycobacteriophages (JetBlade) was selected for genomic analysis. Preliminary studies indicate that the Bipolar genome is 51.3kb in length and contains 86 predicted protein-coding genes, with a nucleotide sequence similar to A4 subcluster mycobacteriophages. These results are important for identifying novel genes, characterizing mycobacteriophage diversity, and for understanding the mycobacteriophages that infect not only *Mycobacterium smegmatis* but also closely related obligate human and livestock pathogens such as *Mycobacterium leprae*, *Mycobacterium tuberculosis*, and *Mycobacterium bovis*.

notes

Poster 11

Proteomic and Genomic Analysis of Novel Cluster A, B and P Mycobacteriophages*

A. Crum

T, Nguyen

Alexandra H Crum, **Trang T Nguyen,** Sharmila Y Ahmed, Stephanie M Anderson, Sydney R Burns, Jake T Elwood, Elizabeth M Gearhart, Shea L Kleinschmidt, Sarah N Kroska, Alexandra J Miller, Katharine H Roetering, Maria M Ropers, Hannah M Russert, Justina J Brusacoram, Casey L Mitchell, Carmen A Aguirre, Daniel E Westholm

College of St. Scholastica, Duluth MN

Mycobacteriophages TNguyen7, Brusacoram, and Hetaeria were isolated and characterized using proteomic and genomic tools as part of the SEA-PHAGES program at The College of St. Scholastica in Duluth, MN. All phages were isolated from soil through enrichment using *Mycobacterium smegmatis* as host. Genome sequencing placed TNguyen7 in cluster A3, Brusacoram in P and Hetaeria in B1. A total of 93, 78, and 100 protein coding genes were predicted in TNguyen7, Brusacoram, and Hetaeria, respectively. Six putative promoters were identified in TNguyen7 (-35 TTGACG and -10 TAT_CT) and Brusacoram (-35 TTGA_T and -10 TATAAT) involved with the transcription of these genes. Multiple stoperator sequences responsible for immunity repressor binding were identified in all three phages. In addition, sequences with predicted integrase function were identified in both TNguyen7 and Brusacoram. Interestingly, MEGA 6 phylogenetic tree analysis of integrase sequences indicated that the integrase in Brusacoram is more closely related to the integrases in cluster I than other cluster P integrases. Although TNguyen7 produced large turbid plaques typical of A3 phages, both Hetaeria and Brusacoram produced medium/small clear plaques. These plaque morphologies in Hetaeria and Brusacoram are inconsistent with the presence of multiple stoperator sequences in both phages, and the presence of *in silico* predicted gene functions associated with the maintenance of lysogeny in Brusacoram (e.g. integrase, immunity repressor). Thus, it appears that both Hetaeria and Brusacoram either have a preference for host cell lysis or an inability to enter or maintain the lysogenic cycle despite genome traits that suggest otherwise. In support of this, we have been unable to isolate lysogens with Hetaeria. The high degree of host cell lysis in Hetaeria and Brusacoram (as opposed to lysogeny and prophage formation) made both of these phages candidates for proteomic analysis of expressed proteins during active infection. To achieve these aims, LC-MS/MS was used identify phage proteins expressed 4 hours post infection. Proteomic analysis of Hetaeria resulted in the detection of nearly 70% of *in silico* predicted proteins, and the verification of 26% of called translational start sites. Amino acid modifications were present in over 20% of identified proteins. Gene functions were predicted for 33% of identified proteins, with functions encompassing phage structure, genome replication and assembly/release. 66% of identified proteins expressed by Hetaeria have no known function. LC-MS/MS analysis of expressed Brusacoram proteins identified approximately 50% of *in silico* predicted proteins. Identified proteins occur throughout the genome and are responsible for a variety of functions associated with phage structure and replication.

Poster 12

From Compost to Clusters: *Rhodococcus* Phages from Coastal Virginia*


S. Belay


S. Modlin

Sarah Belay, Sarah E Modlin, Adeola O Adesuyi, Andrew D Corso, Christopher J Debo, Juliet Downie, Ceyda Durmaz, Jacqueline S Espinoza, Marion L Gilliam, Milane C Gooden, Rachel L Hervey, Maran Ilanchezhian, Aboubakar Kamara, Diego A Lanao, Sri Harshavardhan Malapati, Sydney Moondra, Andrew M Mattei, Carolina J May, Imran Sadik, Katherine M Saulenas, Elizabeth A Allen, Adom L Whitaker, Olivia A Awate, Veronica C Gray, William J Buchser, Margaret S Saha

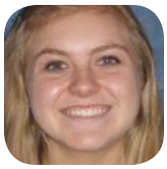
College of William & Mary, Williamsburg VA

Belonging to the order *Actinomycetales*, *Rhodococcus* bacteria display a broad geographic distribution and have been found in diverse environments including wastewater treatment plants, mills, farmlands, caves, and even deep under ice in sub-glacial lakes. There is considerable interest in *Rhodococcus* given their metabolic properties for use in detoxification and even medicinal chemistry. In order to isolate potentially novel phages that infect *Rhodococcus*, undergraduates in the College of William & Mary SEA PHAGES course took samples of soil, mud, sludge, compost, and manure from around coastal Virginia and performed enrichment with a *Rhodococcus globerulus* culture. Of over 50 different locations (including a waste treatment plant, paper mills, and livestock grazing areas) and at least three replicate cultures, we found that one specific compost pile and nearby soil with runoff from the compost was the only source of phage that would form plaques on a *Rhodococcus globerulus* lawn. These phage produced small clear and large tubed plaques and high titer lysates, and had a latent period of 1-1.5 hours. We subsequently visualized the isolates under transmission electron microscopy to find phages with approximately 60 nm diameter heads and 170 nm tails. Preliminary sequence screening of the phages using a "shotgun" cloning approach suggested high similarity among the isolates; we therefore selected those with the most distinct plaque morphology for further analysis. Four phage isolates sequenced by University of Pittsburgh revealed a 46.6 Kbp genome, with approximately 65 genes, the majority of which coded for proteins of unknown function. The four genomes shared 99% similarity with one another and 98% to the RER2 *Rhodococcus* phage. All of the known *Rhodococcus* phages appear to fall into 3-4 clusters (with six additional singletons). One of these clusters (RER2-like), in particular, contains almost 70% of the known *Rhodococcus* phages including the William and Mary isolates. Another isolate taken from the same compost soil, but in the middle of winter, and sequenced by students in a "Phage Lab Continuation" course at William and Mary, also belongs to the RER2 family and shows similarity to the other four phage isolates from William and Mary. However, it is distinctly different in that it lacks several intergenic regions, an entire gene, and 89 bp at the beginning of the integrase gene. RNA-Seq experiments are currently underway to examine phage and bacterial gene expression in response to infection.

Poster 13

Identification and Genomic Investigation of the Mycobacteriophage SamScheppers


J. Heisse


P. Pollard

James Heisse, **Patricia Pollard**, Jelani Days, Alexander Goss, Abigail Grawe, Elizabeth Hamilton, William Hill, Katherine Johnson, Dareon Jones, Grace Lancman, Cannon Ousley, Rachael Sommers, Jordan Welker, Holly LaFerriere, Muhammad Seegulam

Culver-Stockton College, Canton MO

In this study we identified and analyzed the novel bacteriophage SamScheppers. SamScheppers was first isolated and identified in 2013 by a group of students from the same institution, and the current cohort followed in 2014 by indentifying seven new phages and annotating the SamScheppers genome. All of the phages were obtained from water and soil samples in the Northeast Missouri region. Enrichment of the bacteriophage samples was performed using *Mycobacterium smegmatis* mc^2 as a bacterial host after which the samples were subjected to several rounds of purification in order to ascertain that only one phage was present. Furthermore, plaque morphology could be observed after purification and SamScheppers created round, clear plaques. Electron microscopy was performed on phage samples, leading to information about the phage characteristics such as size and morphology. SamScheppers was found to possess a *Siphoviridae* morphotype and approximately 200nm in length. Sequencing of the SamScheppers genome revealed a 58,351bp length and a GC content of 67.6%. The local phage BLAST tool available on phagesdb.org assigned SamScheppers to cluster K, subcluster K4. DNA Master was used to annotate the SamScheppers genome, which was found to contain 94 genes. While a significant number of these genes reported no known function, others were identified with functions including terminase, portal proteins, and exonuclease. One translational frameshift was found, along with a tRNA gene and three genes in reverse sequence. The translational frameshift was located between base pairs 11,957 and 12,837. HHPred, Phamerator, the Hatfull Map and BlastP were used to assign putative protein functions when appropriate.

Poster 14

Exploration of Genetic Factors Involved in Bacteriophage Temperate Life Cycle by Isolation and Annotation of the Novel Mycobacteriophage 'Chupacabra'

J. Ramirez

John Ramirez, **Sarah Mullenax,** Jamie Vulgamore, M. Clayton Speed, J. Robert Hatherill, Daiyuan Zhang

Del Mar College, Corpus Christi TX

Bacteriophages are viruses that survive by infecting and then replicating using a bacterial host's genetic machinery, leading to the destruction of the host cell. Bacteriophages' diversity of attributes, while still being specialized to their host, appeals to the need for continuing research into their survival mechanisms. Bacteriophages and their host bacteria are locked in an evolutionary struggle that has led to the development of new defense mechanisms and new methods of infection. Given the rise in antibiotic resistant bacterial species, bacteriophages may offer a new alternative for combating bacterial infections. During this project, the novel bacteriophage 'Chupacabra' was isolated and its genome was sequenced. 'Chupacabra' belongs to the cluster A and subcluster A10 of bacteriophages and infects *Mycobacterium smegmatis*. After culturing, 'Chupacabra' was revealed to have a temperate life cycle. Each plaque was approximately 3mm in diameter. Classification was assisted by TEM images taken, as well as bioinformatic analysis of phage genomic DNA. The capsid and tail of 'Chupacabra' were observed to be 60nm in diameter and 140nm long, respectively. The quality and quantity of DNA harvested from 'Chupacabra' were measured through restriction enzyme digest. After DNA sequencing, the 'Chupacabra' genome was determined to be 50,286 base pairs in length. Bioinformatic annotation of the 'Chupacabra' genome revealed that genes 4 and 46 were unique when compared to other lytic cluster A10 bacteriophages. Hypothesis: The temperate life cycle of 'Chupacabra' may be due to differences between 'Chupacabra' and other A10 bacteriophages in key DNA synthesis genes.

Poster 15

The Same and Yet Different: Two New Mycobacteriophages from Olympia, Washington

M. Horan

A. Kepler

Marissa Horan, **Aubriel P Kepler**, Keith Adams, Juniper Anzalone, Aeja Anderson, Arianne Aquino, Abigail Arnold, Megan Bennett, Alexander Bill, Jordan Billiot, Alexander Boisner, Gabrielle Bourassa, Quinn Briceno, Bianca A Brown, Elizette Brown, Nicholas (Psy) Bruni, Kaela Jo Bugg, Emily Cain, Matt Campbell, Joelle Charon, Madision Cogswell, Dawn Coleman, David Cook, Ethan Cornell, Natasha Krstvova Coumou, Jesse Craft, Cameron Crocker, Kyle Cruse, Sandra Dearinger, Daniel DeGeare, Armando DeLao, Jared Fahmy, Kayla Forayter, Isobel Francis, John Gabbard, Gabriella Greene-Dittz, Yurika Harada, Nathan Helman, Juanita Hopkins, Devin Hunter, Lukas Hunter, Kennedy Jackson, Tessa L Jacobs, Emily Donkin Jones, Andrew Kern, Andrew Kirwin, Robert Knox, Marko Krepel, Zoe Mangold-White, Ashley Marie, Patrick McGee, Jobim Morris-Gavrieli, Natalie Moss, Christian Nakabayashi, Hannah Nishman, Zachary Nixon, Ariel Page, Tyler Paradise, Edward Parnel, James Christian Perkins, Brittany Perryman, Megan Riley, Bailey Rivers, Ketl Rodakowski, Collin Ross, Crystall Searcy, Emily Smith, Ian Stehr, Lorraine Stone, Marlaina Strand, Samantha Sullivan, Sydney Sullivan, Ella Ware, Faron Watts, Jessica Wieandt, Catherine Woodward, Alberto Napuli, James J Neitzel

The Evergreen State College, Olympia WA

The student participants in our second cycle of the SEA-PHAGE project were again members of the interdisciplinary program *Introduction to Natural Sciences*, a year-long, full-time learning community with integrated instruction in biology and chemistry. During the fall quarter students collected and purified phages using enrichment cultures of *Mycobacterium smegmatis* mc^2155 as a host. This year 33 phages were isolated from local soils, purified, and entered into the PhagesDB collection. These will be archived. This set of phages had their DNA purified and analyzed by restriction enzyme digestion and gel electrophoresis. Successful DNA extractions were completed for 30 of the phages. There were 28 successful restriction enzyme experiments that revealed in some cases extremely different cutting patterns. Phages were also analyzed by transmission electron microscopy after negative staining with uranyl acetate. This resulted in clear images of 30 of the isolated phages, all of which appeared to be *siphoviridae*.

The phages sequenced were SoilDragon and Rimmer. DNA from these phages was sequenced using the Illumina process at the Pittsburg Bacteriophage Institute. The sequence of SoilDragon revealed a 50,293 bp linear double stranded DNA genome with a ten bp 3' overhang and with a GC content of 64 %. Analysis of the sequence of this phage confirmed that it was a *siphoviridae* in the A3 subcluster. We also examined an A3 phage (P28Green) in last year's work. BLASTn and dot-plot comparison (using Gepard) of

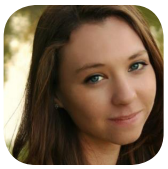
SoilDragon and P28Green indicated that although there was overall similarity in nucleotide sequence and gene content, there were two distinct areas that showed little or no nucleotide similarity between these two phages. BLASTn results showed that SoilDragon phage was most closely related to the previously annotated phage Jobu08.

Rimmer's genome is a 75660 bp dsDNA with a 9 bp 3' overhang and a GC content of 63%. BLASTn results indicated its closest relatives were Cluster E phages Willez and Sassay. Dotplot analysis indicated the presence of an inserted DNA sequence not present in Willez. Both genomes were analyzed for potential protein coding open-reading frames using Glimmer and GeneMark, and protein functions were predicted by BLASTp and HHPred, as well as examining synteny with related phages. Preliminary results suggest the presence of at least 86 protein coding genes in SoilDragon and 142 in Rimmer. The Aragorn algorithm also identified three tRNA genes in SoilDragon and two in Rimmer. Further work is being conducted to identify and confirm all protein coding regions and to identify functions for predicted protein products.

notes

Poster 16

A Comparative Bioinformatic Analysis of Four Novel *Rhodococcus* Bacteriophage*



S. Lohbauer

B. Sisson

Sara A Lohbauer, Brittany M Sisson, Amanda J Black, Victoria H Blair, Cody W Crivello, Cassandra A Ellis, Daniel J Hansen, Abigail R Huelsman, Heather S McFalls, Alec N Pica, Madeline M Quinn, Julia B Reed-Betts, Hannah B Reeves, Leah M Roach, Belen Rodriguez, Gustavo A Romero, Courtney B Sparrow, Samantha M Gatt, Kimberly A Rosales, Rachel S Walter, Santiago Yori, Scott F Michael, Sharon Isern

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Florida Gulf Coast University participated in the alternative host *Rhodococcus erythropolis* pilot project and isolated 19 temperate phage. *Rhodococcus* phage were initially elusive, but we found several sites where phage were reproducibly isolated, but only after enrichment. These phage were classified into two distinct groups: (1) stable phage producing small, lytic plaques with a typical *Siphoviridae* morphology, and (2) unstable phage producing large, turbid plaques and particles with no apparent tails. We sequenced four of the stable lytic plaque-producing isolates. Espica was isolated from horse manure in Southwest Florida, and Belenaria, Hiro, and Natosaleda were isolated from Boston soil samples. Sequencing and annotation revealed an unusually high degree of similarity (99% identity, 99% query coverage) between our phage and RER2, a previously characterized *Rhodococcus* phage isolated in Australia. We performed bioinformatics analyses comparing different aspects of our phage genomes to RER2. We compared non-synonymous and synonymous substitutions to explain how the phage evolved under selective pressure. We determined that ATG is the preferred start site codon. However, TTG start codons appeared commonly in nonstructural genes. We found evidence of use of different start site codons in nonstructural genes, but saw no variation in structural genes. To address the level of host adaptation, we looked at their t-RNA repertoire, codon frequency usage, and guanine/cytosine content. The codon frequency usage of the phage and host were similar lending support to the notion that the phage were well-adapted to the host. The same 3 tRNAs were found in each phage, and these tRNAs were for codons more frequently used in the phage genomes than in the host. By examining the guanine/cytosine content, we likewise concluded that the phage were well-adapted, but not static. The presence of genomic islands provided support for continuing horizontal gene transfer. We identified the sequence and location of the attP sites, or phage attachment site and their corresponding attB sites, or bacterial attachment site, in the host. Seven to eight sigma-70 promoters were identified in each phage. The promoter sequences were similar and found upstream of the same genes. Repeat sequences of unknown function were identified both inside and outside of open reading frames with similar frequencies. Belenaria and Espica showed virtually identical repeats in their patterns and locations, while Hiro and Natosaleda had many differences that set them apart. Investigation of potential protein functions showed an average 11.4% of proteins were predicted to have transmembrane domains and 4.1% contained signal peptides. Overall, our phage were extremely similar to each other and to RER2, despite their isolation on different continents. However, they displayed unique genomic signatures that likely played important roles in adaptation to their ecological niches.

notes

Poster 17

Evolutionary and Syntenic Comparison of Tail Proteins in Roary to Other A8 Phages

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B. Estadella

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Bacteriophages are specific to bacterial host species; however, the molecular mechanism that drives the virus/host specificity remains elusive. The analysis of tail proteins from mycobacterial phages could begin to uncover more details about the relationship between the virus and the host it infects. We tested the hypothesis that variability in amino acid sequences in major and minor tail proteins among phages could potentially reflect the binding site that allows the phage to specifically attach to the surface of the bacterial host. In this study, the synteny variation among phages was also analyzed in particular in those related structural tail proteins in comparison with the rest of the phage genome. The comparison of protein sequences was conducted within Cluster A8; which is the sub-cluster where our newly isolated phage belong, Roary. From the alignment and phylogenetic analyses, the level of amino acid differences varied depending on the protein analyzed and it ranged between 0 and 1.3% amino acid differences. These results are discussed within the context of potential bacterial host binding site. From the synteny analyses, very few changes of gene order was documented among most of the structural tail proteins found on half of the genome that had the same transcriptional orientation. However, and very interestingly, the number of gene order changes in the other half of the genome with opposite transcriptional orientation was much higher. Further research is needed to determine if the difference in tail protein sequence detected in this study mirrors the specific area of attachment of the virus on the bacterial host. This will help the field of phage biology to understand the role of tail proteins on modulating the specificity of virus/host interactions.

notes

Poster 18

Phamished and the Cluster B Phages

A. Agesen

M. Strine

Alex Agesen, **Maddi Strine,** Sarah Brantley, Elizabeth Burton, Jenna DeCurzio, Jenny Giannini, Isabella Katsanos, Aphra Murray, Julia Palmucci, Amanda Richman, Sarah Rivera, Breanna Titchen, Bobby Weisensee, Rachel Wigmore, Veronique A Delesalle, Greg P Krukonis

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The unique mycobacteriophage Phamished was isolated from the Gettysburg College campus, sequenced at the University of Pittsburgh, and assigned to cluster B1. Phamished, with a genome of 68,515 bp, 66.5% GC, 101 putative genes with an average length of 649.7 bp, and no tRNA genes, is typical of its subcluster. Phamished has no unique genes; all of its genes belong to gene families ("phams") found in other B1 phages.

A gene-by-gene analysis of amino acid composition in Phamished revealed six ORFs with disproportionate use of a particular amino acid. Five ORFs (genes 11, 15, 19, 74, and 79) had more than 20% alanine. ORF 32, a minor tail protein, contained 24.8% glycine and was the only one of these ORFs with a putative function. Given its steric flexibility, glycine may be important in tail fiber attachment. In support of that hypothesis, we found that glycine was the most prevalent amino acid for at least one minor tail protein in other mycobacteriophages (e.g., C1 Gizmo, D2 Hawkeye) and unrelated phages (e.g., phage lambda, *Salmonella* phage Chi).

The protein product of the cluster B-specific gene 52 (pham 380) fell within the PD-(D/E)XK nuclease superfamily (HHPred probability of 99.0%). This conclusion was supported by Vilnius University's Institute of Biotechnology PDEXK server, which assigned this protein to the AddAB-type helicase-nuclease complex with at least a 97.9% probability, a complex with similar functions to *E. coli*'s REC BCD.

As in other B1 phages, we added an HNH endonuclease gene, not called by Glimmer or GeneMark, at position 10. Two forms of the pham (2895) exist: a 435 bp ORF found in the majority of sequenced B1 phages and a 159 bp segment of the longer ORF found in four B1 phages. The longer ORF has a GC content of 60.5% which differed from the short version (67.9%) and the surrounding genes (68.2%). We explored whether the short version arose via a deletion in the front half of the long ORF with a loss of endonuclease function, or whether a homing event with the HNH endonuclease motif cleaved into the DNA producing the long ORF.

Start codon analysis of all B phages with available DNAMaster files showed that the frequencies of ATG, GTG, and TTG starts differ significantly between subclusters. Although ATG was the most prevalent start codon across all five subclusters, its frequency ranged from 57% to 71%. GTG was used less frequently in subcluster B1 and TTG was always the least used. Other studies have shown that modifying the start codon in a given mRNA affects protein expression levels, with ATG starts conferring the highest expression levels. Interestingly, all B tapemeasure genes start with GTG while the capsid genes in pham 9976 use both ATG and GTG codons. The variation in start codon usage within a pham suggests that factors besides control of gene expression may affect for start codon selection. The way selection operates on start codons is an area worthy of further exploration.

notes

Poster 19

Investigating Cluster O promoters at Gonzaga University

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In mycobacteriophage genomes, promoters can sometimes be predicted if they have similar sequences to mycobacterial SigA promoters, which conform to predicted consensus sequences. In the five cluster O phages, Corndog, FireCracker, YungJamal, Catdawg, and Dylan, 8 or 9 putative promoters were identified using bioinformatics (Cresawn *et. al*, 2015). However, since the promoters were identified *in silico*, biological confirmation is necessary. Upper division students at Gonzaga University amplified promoter sequences and directionally cloned them into a binary vector. Promoter activity was characterized by the cloned DNA's ability to drive expression of the red fluorescent protein mCherry following transformation into *E. coli* and *M. smegmatis*. Although promoters P_{L1}, P_{L2}, P_{L3},and P_{L6} from various phages were successfully cloned, only P_{L4}, P_{L5}, and P_{R2} resulted in red *E. coli* and *M. smegmatis* colonies. This supports their role as mycobacteria strong phage promoters in vegetatively growing cells.

notes

Poster 20

Archie14, a *Bacillus thuringiensis* bacteriophage with evidence of passage through multiple bacterial hosts

J. Dimmick

T. Meinhardt

Joshua T Dimmick, **J. Taylor Meinhardt**, Stephen L Woodall, Michael J Wolyniak

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As part of the SEA-PHAGES curriculum at Hampden-Sydney College, a men's liberal arts college in central Virginia, the Molecular and Cellular Biology class isolated and characterized bacteriophages from the local environment using *Bacillus thuringiensis* as a host. Direct plating of one environmental sample yielded Archie14, a bacteriophage that appeared to show an especially strong lytic capacity. Archie14 shows strong homology to subcluster C1 *Bacillus* bacteriophages and is 162,099 basepairs long with 298 ORFs. Of particular interest in the genome of Archie14 is the presence of several bacterial ORFs that would seemingly be dispensable for viral replication. Several *Bacillus* ORFS, including dihydrofolate reductase, adenylate kinase, and thymidylate synthase, are found clustered together in Archie14, suggesting acquisition from *Bacillus* at some point during the evolutionary history of the phage but leaving in question if the acquisition benefits the ability of the virus to be replicated or is merely coincidental. Intriguingly, we also detected a sigF-like RNA polymerase sigma factor of greater similarity to *Clostridium* bacterial species than *Bacillus*. In considering the evolution of Archie14, this suggests that the virus may have passed through *Clostridium* hosts on its way to adaptation to its current status as a *Bacillus* bacteriophage. Current work is exploring the host range of Archie14 to follow up on which bacterial hosts can support its propagation.

Poster 21

Expanded Host Range Evident in the Genomes of Glass and Bella96*

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Hope College, Holland MI

Thirty-five new mycobacteriophages were isolated from soil samples collected around the state of Michigan and parts of the United States. All were capable of infecting *Mycobacterium smegmatis* at 32°C and produced a variety of plaque morphologies based on size, shape, and clarity. Both lytic and temperate phages appear represented in this collection. All phages were experimentally characterized with respect to temperature range (32°C and 42°C). Cluster specific patterns in the temperature conditions were observed; 12 of 13 predicted Cluster K isolates failed to infect *M. smegmatis* mc^2155 at 42°C. Two mycobacteriophages, Glass and Bella96, were chosen for complete genome sequencing and comparative genomic analyses. The predominant plaque produced by Glass at 32°C was turbid and 0.5-1.0mm in diameter, while plaques produced at 42°C were clear and 1.0-1.5mm in diameter. The predominant plaque produced by Bella96 at after 5 days at 32°C was turbid and 3 mm in diameter, whereas no plaques grew at 42°C. Complete genome sequences for Glass and Bella96 revealed relationships to members of Clusters B2 and K1, respectively. The genome of Glass is 67.5 Kb, 69.0% GC, and contains 94 genes; the genome of Bella96 is 60.7 Kb, 66.1% GC, and contains 98 genes, including 1 $tRNA^{(Trp\text{-}CCA)}$ gene. Twenty-seven (29%) and thirty-two (33%) protein coding genes were assigned functions in Glass and Bella96, respectively, based on comparative analyses. An analysis of the lysis cassette (*lysA*, *lysB*, holin) reveals the mosaic nature of this region in both Clusters B2 and K1. Glass (B2) does not contain an identifiable *lysB*, consistent with other B2 phages. The arrangement of the lysis cassette in B2 phages is atypical, with *lysA*-holin genes in tandem in a forward orientation amidst a series of reverse genes. The distribution of the phams is broad for the *lysA* gene, but restricted to the B2 cluster and a single B4 phage for the putative holin gene. This pattern of pham distribution for the lysis cassette in Bella96 (K1) is similar – broad *lysA* distribution, restricted *lysB* distribution, and restricted holin distribution. The genome of Bella96 contains two novel ORFs, being found in several other draft K genomes isolated this year. Bella96 gp26 is found in the midst of well-conserved K1 minor tail proteins and replaces gp26 of other K1 phages that are part of a 40 member pham. The second ORF, Bella96 gp78, is found in a total of 8 K1 and K5 draft phages; comparative analyses suggest similarity to a membrane binding protein from *Segniliparus rugosus*, a bacterium that also contains mycolic acids in its cell wall structure. This hints at the possibility of host range expansion of some cluster K members beyond the genus *Mycobacterium*.

Poster 22

Exploring the Diversity of Mycobacteriophages Isolated from Soil at Howard University

K. Assensoh

M. Osborne

Kwadwo S Assensoh, **Morinne E Osborne**, Rasheed Nawaz, Leon A Dickson Jr., Broderick Eribo, Ayele Gugssa, Courtney J Robinson, Winston A Anderson

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Students in the 2014-2015 PHAGES course at Howard University isolated 96 mycobacteriophages. Four genomes of these phages were sequenced using Illumina sequencing at the Pittsburgh Bacteriophage Institute: Morizzled23, HUTC2, Kwadwo, and Haleema. Morizzled23 was isolated by Morinne Osborne and is cluster C1 phage. This phage contains a genome that is 53.6Kb in length, has a G+C content of 64.6%, and contains 234 ORFs and 30 tRNAs. Preliminary BLASTn analyses indicated that Morizzled23 is most similar to phage Spud (99% similarity), which was isolated in Pittsburgh, PA in 2004. Phage HUTC2, is a cluster A11 phage and is most similar to phage Fibonacci (99%), which was isolated from West Lafayette, IN. HUTC2's genome is 51.3Kb in size, contains 97 ORFs and one predicted tRNA. A cluster B1 phage, Kwadwo, was also isolated from the HU campus. Kwadwo, which was discovered by Kwadwo Assensoh, has a G+C content of 66.5% and contains 102 ORFs in its 68.3Kb genome. This phage is most similar to phage Sophia (99%), which was isolated from West Lafayette, IN. Rasheed Nawaz isolated phage Haleema, another cluster B1 phage. Similarly to Kwadwo, Haleema's genome has a 66.5% G+C content, a length of 68.4Kb and 104 ORFs. Haleema was most similar to Xavier (99%), which was isolated from soil in St. Louis, MO. Recent studies have focused on finalizing the annotations of the four genomes and using PCR to determine the cluster affiliations of the 92 remaining phages.

Poster 23

Phalcon: a Novel Mycobacteriophage from Huguenot High School

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Huguenot High School, Richmond, VA; * James Madison University, Harrisonburg, VA

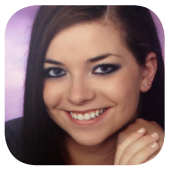
Mycobacterium smegmatis is a safe, ubiquitous soil bacterium that is genetically closely related to the organism that causes tuberculosis. Studies of bacteriophages that infect *M. smegmatis* will help to strengthen our understanding of viruses and their genomes, but may also uncover valuable information about their bacterial hosts.

This year Huguenot High School has taken part in the SEA-PHAGES program for the entire academic year. We believe this to be one of the first year-long implementations in a high school. Students collected samples from three places: the James Madison University Arboretum (Harrisonburg, VA), the holding pond behind Huguenot High School (Richmond, VA) and from homes of students (Richmond, VA). The class divided into five groups of three to four students each. Each group isolated at least one phage. From those phages, two were selected for additional study. Ultimately, we selected a single phage isolate for further study and DNA isolation. We named this phage Phalcon

Poster 24

Isolation and Characterization of two subcluster A Mycobacteriophages from Central Illinois

M. Linder



J. Spindler

Meghan L Linder, Jadeah J Spindler, Caitlin R Cadwalder, Aida X Cheung, Spencer C Eidsmoe, Jordan M Eldridge, Sarah A Gooding, Ross J Hettinger, Jordan J Miller, Megan E Mink, Tiffany H Remijas, Audrey M Smith, Ian H Taylor, Ruchen C Tian, Randi M Wilson, Richard M Alvey

Illinois Wesleyan University, Bloomington IL

Sixteen mycobacteriophages were isolated by students at Illinois Wesleyan University in Bloomington IL using a soil enrichment technique and a *Mycobacterium smegmatis* host. Each student created and archived a high titer lysate of his or her mycobacteriophage, and of these sixteen, two were selected for genomic sequencing, Eidsmoe and Morrow. Morrow was found just outside the Morrow Plots at the University of Illinois at Urbana-Champaign, and is one of 64 members of the A4 subcluster. Its two most similar phages were isolated from soil in St. Louis, Missouri and Gettysburg, PA. Eidsmoe was isolated from a small stream in Bloomington, IL and is one of just six phages that make up the A9 subcluster. Of these six phages, Eidsmoe is the only one found in the interior of the United States. The other members of this group were found in Texas, Louisiana, and Florida, states bordering the Gulf of Mexico, while two members were found in Puerto Rico in the Caribbean. Both phages were found to be highly similar to other members of their subclusters. These phages were then annotated using BLASTp, Phamerator, Starterator, and DNAMaster.

notes

Poster 25

Isolation of 11 mycobacteriophages from Florida ecosystems and genomic characterization of mycobacteriophages Lumos, Pioneer, and SnapTap

L. Molina

Laura Molina, **Chris Holland,** Nina Davis, Arthur Domingos, Nick Hueter, Michael Sonntag, Connor Swinford, Olben Saintfleur, Krystal Villalobos-Ayala, Megan Carroll, Adrienne Cottrell-Yongye, Tom D'Elia

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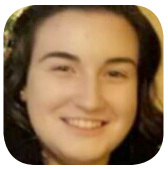
C. Holland

Mycobacteriophages capable of infecting *Mycobacterium smegmatis* mc^{2}155 were isolated through enrichment of soil samples collected in Indian River, St. Lucie and Martin Counties, Florida. This analysis represents the first look at the diversity of the mycobacteriophage population in these areas. As part of the HHMI-SEA PHAGES program, over 5,800 mycobacteriophage have been isolated and 1,233 have been classified into 23 clusters. Here we report the isolation of eleven phages representing two confirmed clusters. Electron microscopy and molecular characterization indicate that all isolates belong to *Siphoviridae*, which have double stranded DNA genomes, long flexible tails and make up 90% of all mycobacteriophage. The complete genome sequence was determined for phages Lumos, Pioneer and SnapTap. Comparative genomic analysis classified Lumos within subcluster L3, which has 2 other members. Lumos has a 75,586 bp genome with 59.3% GC content (*M. smegmatis* has 67.4% GC), and initial analysis has predicted 130 open reading frames (ORFs) and 10 tRNAs. Pioneer was identified as an A9 subcluster member, with a 53,219 bp genome and 62.6% GC content. Of the approximately 100 ORFs predicted in Pioneer, less than 20% were assigned function, which is representative of the diversity that exits in these phage genomes. Finally, SnapTap was identified as a cluster A2 phage, with high sequence identity to SweetiePie, an A2 phage with a genome of 53,184 bp. This study helps to expand the diversity of cluster L phages and provide a more thorough understanding of mycobacteriophage ecological and genomic diversity.

Poster 26

Matchsticks, Spaghettis, and Podos, Oh MY!



H. Bradley

Hannah L Bradley, Alicia D Ashby, Allie R Casto, Sunil Devkota, Laith Harb, Andy M Hoy, Qiong Kang, Andy M Hoy, Jack M Lipsky, Tzu-Jung Liu, Gregory E Mansour, Ally E Mayzel, Carly M Neville, Bryan Q Nguyen, Rachel A Policke, Alexander L Schmidt, Rowan C Shartel, Rowan C Shartel, Olivia J Smith, Augustus M Snyder, Allison P Woolf

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Interesting and varied morphologies of phages have been an exciting part of *Bacillus* phage hunting at JMU this year. We used the strain, *Bacillus pumilus*, F036B, as the host for isolation. Three different classes isolated 51 phages, of which we have obtained 25 TEM images. These are the first reported phages isolated on *B. pumilus* F036B. A mixture of morphotypes in virtually every sample led us to hypothesize that a prophage might be emerging as a result of infection of our host bacterium with environmental phages. We observed a "matchstick" myovirus frequently, in association with small podoviruses, more typical myoviruses, or siphoviruses with very long tails. Some samples had so many of the long-tailed, large head siphoviruses, that these images gave the impression of spaghetti and meatballs.

Our challenge in the discovery semester was the "case of the disappearing nucleic acid", which plagued most students in every section. Tests for RNA, dsDNA and ssDNA indicated that at least some samples contained ssDNA genomes, which was confirmed by tests at our sequencing center. Ten samples appeared stable enough for sequencing, and we obtained raw data for five of these (Angel, Boney, Chester, Hogan, and VRKOLAKAS).

Assembling contigs from raw data was a challenge our genomic sections took on, and we utilized different size datasets to experiment with how best to find authentic phage genomes. Because we expected to find a small prophage genome, we related genome size to phage head size using previous data. Based on the expected genome size for the "matchstick" head, we could not find an appropriate size contig match. We also looked for our contigs in the genome sequence of a closely related *B. pumilus* strain, but were not able to find it. It is likely that the genome of strain F036B differs in prophage content from the sequenced strains.

The five phage genomes we analyzed were all unique, meaning that no matches were found in GenBank. However, three of these had some similarity to each other. Annotation was completed for Angel (238,553bp) and Hogan (130,213bp). These have a G+C content of about 33%, similar to the *Bacillus* genomic G+C content. Like previous *Bacillus* phages we have studied, these have terminal direct repeats (17,040 and 1,762bp, respectively) that can easily be identified using the assembly view in Consed. The genome arrangement shows predicted structural genes clustered at one end, with enzymes for DNA metabolism and entry and exit from the bacterium at the other end. Hogan is predicted to be a temperate phage

due to recombinase and integrase genes. We were able to assign functions to 33/330 predicted genes in Angel and 24/236 predicted genes in Hogan.

In future studies, we would like to obtain the chromosome sequence of our host bacterium, F036B, to help figure out the prophage question, and we would like to follow up on the presence of ssDNA in our genomic preparations.

notes

Poster 27

Bioinformatic and Virological Evidence Suggests a Novel Scheme for Lysogeny in a Mycobacteriophage*

S. Jurgensen

Sophie K Jurgensen, Steven G Cresawn

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Phages that infect *Mycobacterium smegmatis* represent the largest sequenced collection for a single bacterial host species. Despite this, many relatively straightforward questions about their biology remain unanswered. In this study, 61 new phage isolates were obtained from soil samples collected in various regions of Virginia. Of these, three (Tres, Sparkdehlily, and Dione) were sequenced, annotated, and further characterized. Sparkdehlily, Tres, and Dione were determined to be members of subclusters F1, B2, and B1, respectively.

The kinetics of replication of Sparkdehlily and Tres were measured using a one step growth assay, while Tres was further characterized to determine whether it is capable of lysogeny. A spot test performed with Tres yielded a turbid zone from which viable cells could be cultured. These putative lysogens were propagated in liquid culture and shown to spontaneously release phages into the culture supernatant which could form plaques on wild type *M. smegmatis* mc^2155 but not the putative Tres lysogen. Plaques derived from the culture supernatant were morphologically indistinguishable from Tres.

The wet lab characterization of Tres indicates that it is a temperate phage, an observation that is at odds with the bioinformatic analysis of its genome. The Tres genome is not predicted to encode an integrase gene, yet there is evidence for an excise. Some temperate phages do not integrate their genome during lysogeny, and instead replicate as a plasmid. Notably, Tres does encode *ParB*, a gene implicated in plasmid and chromosome partitioning.

Lastly, we note that GeneMark coding potential plots suggest a programmed translational frameshift that occurs during translation of genes in the right arm of every B2 phage, far removed from the G/GT frameshift upstream of the tape measure gene. This predicated feature is also found within some but not all of the other B subclusters.

Poster 28

Cluster C Mycobacteriophages at Johns Hopkins: ChickenPhender and Melpomini

S. Hernandez

A. Mehta

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Two mycobacteriophages of subcluster C1 were isolated in fall 2014. Melpomini was isolated from a lawn on the JHU campus and found by enrichment. ChickenPhender was isolated from direct plating of soil from a stream near campus. Annotation yielded over 200 genes apiece and both have GC% of 64.7%. We wil present an analysis of GC found at first, second, and third codon positions in annotated genes. Conserved genes in both new genomes and other C1 subcluster members were used to create phylogenetic trees. Chicken Phender burst dynamics were investigated and found to be slow and have a very small burst. Growth of Chicken Phender in various pHs, salt concentrations, and cations were also characterized.

notes

Poster 29

Isolation, Identification, and Genomic Analyses of Mycobacteriophages Iracema64 and Lambert1

J. Hollanda Veras

R. Nork

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La Salle University, Philadelphia PA

Students from La Salle University's Integrated Science, Business and Technology program isolated two new Mycobacteriophages, Iracema64 and Lambert1. Iracema64 is a phage collected from a sand sample on the La Salle campus in Philadelphia, PA. It had a genome length of 51637 base-pairs and had a GC content of 64.0%. Characterized as a cluster A phage (subcluster A4), Iracema64 presented *Siphoviridae* morphotype and plaques of 1.4 mm of diameter. Annotation of Iracema64's genome indicated that there were 87 genes and no transfer RNAs. Phamaerator analysis indicated that Obama_12, Kampy, and Peaches were similar to Iracema64.

Lambert1 was isolated from a flower bed mulch 60 miles north of La Salle in Emmaus PA. It has a genome length of 50042 base pairs and a GC content of 64.1%. Characterized as a cluster A phage (subcluster A3), Lambert1 presented *Siphoviridae* morphotype and plaques of 1.0 mm of diameter. Annotation of Lambert1's genome indicated that there were 87 genes and two transfer RNAs. Phamerator analysis indicated that Lambert1's genome was almost identical to QuinnKiro; QuinnKiro had 24 more basepairs than Lambert1.

The most notable observation about both genomes was the large areas of no genes at the start and end of the DNA sequence.

Jefferson Hollanda Veras – Scholarship student, CAPES Foundation, Ministry of Education of Brazil, Brasilia, DF, 70.040-020

Poster 30

Bioinformatics Discoveries: Investigating Mycobacteriophage Genomic Relationships and Immunity Mechanisms

S. Sarli

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Our PHAGES program focuses on isolating and characterizing phages that infect *M.smegmatis*, thereby contributing to understanding phage genomic structure, functional diversity, and genome evolution. At Lehigh, phage research allows students to contribute new mycobacteriophage isolates to a growing collection and to participate in research developed from previous bioinformatics discoveries. Two research efforts are reported: I. Annotation of newly isolated Sneeze (G), Jane (G), and Jabiru (A5) genomes revealed novel gene features, including orphams in both G cluster phages and a new A5-specific gene found in Jabiru and Union Jack. II. Ongoing research to characterize the Butters (N) genome and to understand the biology of this phage continues. We report on experiments that explore phage immunity mechanisms using bioinformatics approaches and Butters lysogens as vehicles to investigate immunity relationships between phage clusters. Immunity tests were performed using a phage panel of clusters A – S and Butters lysogen strains (harboring wildtype or mutant prophages - the latter isolated to propagate lytic-incompetent phages in other studies) to determine which, if any, phages are homoimmune with Butters. Notably, phages Rockstar (A3), Timshel (A5), Benedict (A6), Trike (A10), and Nova (D) were unable to infect Butters lysogens, thereby suggesting a common mechanism between phages for down-regulating lytic genes. Among several mechanisms that could account for the observed homoimmunity, we favor the hypothesis that the Butters repressor binds to stoperators within these phages and blocks lytic gene expression. A corollary of this hypothesis is that the Butters genome itself contains sequences with significant homology to stoperators identified in A cluster phages. BLAST analysis reveals 53 putative A cluster stoperator hits within the Butters genome as potential repressor binding sites. Alternatively, immunity mechanisms may involve toxin production (resulting from prophage integration) that blocks superinfection by specific, but not all phage types. To determine what, if any, genes are affected by prophage integration, we used bioinformatics to predict *att*P/*att*B for Butters and *M. smegmatis* mc^2155, respectively, based on published predictions for phages encoding tyrosine integrases (e.g., Sampson *et al.*, 2009. *Microbiol*. 155, 2962), where *att*P resides between the repressor and integrase genes, and *att*B is located typically within a tRNA gene. In Butters, *att*P is predicted within the repressor and BLAST analysis shows two strong hits as putative *att*B sites overlapping *M. smegmatis* $tRNA^{Arg}$ (coordinates, 4797480-4797552) and $tRNA^{Lys}$ (5834573-5834645) genes, to be confirmed by PCR analysis. Whether or not Butters lysogens contain more than one prophage and/or produce toxins that target specific phage types preventing superinfection will be discussed.

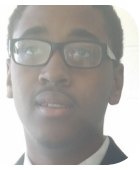
Poster 31

Finding Panchino - a novel Cluster N Mycobacteriophage isolated at Lincoln University, Pennsylvania*

E. Lockwood

Erin N Lockwood, **Nijewel X Holliday,** Nnamdi E Ihejirika, Tamara D Jones, Lisa R Mwanza, Prisca C Obidike, Jaqueline G Ruban, Nathaniel J Sangster, Anna K Hull, David F Royer

Lincoln University, Lincoln University PA



N. Holliday

Panchino, an N cluster bacteriophage, was isolated from a soil sample on the campus of Lincoln University (Pennsylvania) as part of a Phage Hunters class offered for the first time during the 2014-15 academic year. Panchino is similar to other N cluster phages in genome length, GC content and number of genes. It produces clear, 1.5mm diameter plaques. It is a member of the *Siphoviridae* with a characteristic long tail (200 nm) and a head with a 50 nm diameter. The annotation revealed 65 genes, of which 20 were assigned a putative function based on comparisons with other N cluster phages using BLASTP on PhagesDB and on NCBI. Of the 20 assigned a function, 6 code for structural proteins and 14 code for functional proteins. Two of the more interesting genes encountered during annotation were a programmed translational frameshift involving genes 15 and 16 that encode a tail assembly chaperon and a possible type 1 restriction enzyme.

Poster 32

Don't Mess with Texage

Hannah Morales, Liam Hurley

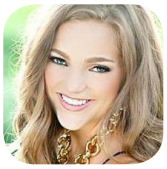
Merrimack College, North Andover MA

Texage is an A3 cluster phage isolated from the grounds surrounding Mendel pond on the campus of Merrimack College in North Andover, Massachusetts. Texage was isolated and characterized by freshmen Biology majors taking Principles of Biology I in Fall 2014 and was found to have a large and turbid plaque morphology and *Siphoviridae* phage particle morphology. Students taking Genetics in Spring 2015 carried out annotation of the Texage genome. Texage displays over 99% identity to several other A3 cluster phages isolated from a wide geographic area: Norbert, Pocahontas, Popsicle, Panamaxus, Lambert1, QuinnKiro, Veracruz, and Todacoro. Texage's genome is 50081 bp and is organized into a typical left half/forward and right half/reverse orientation of the genes. It possesses 88 putative protein encoding genes. It also carries the same two tRNA genes found in Quinnkiro, Asn (gtt) and Trp (cca). None of the other closely related A3 phage carry tRNA genes.

notes

Poster 33

Isolation and analysis of DumpstDiver, a cluster J mycobacteriophage from Southwest Ohio



E. Clenny

G. Mommsen

Elsa C Clenny, **Geneva H Mommsen**, Jana M Cable, Martha L Carter, Justin T Crookes, Rachana Gollapudi, Sarah K Hiner, Pierce N Kurek, Ciara Lawson, Svitlana A Podolyan, Leah N Stetzel, Julie M Donn, Ryann M Brzoska, Gabrielle M Lopez, David C Ream, Iddo Friedberg, Mitchell F Balish, Gary R Janssen

Miami University, Oxford OH

We have isolated a J cluster mycobacteriophage from a dumpster outside a student residence hall at Miami University (Oxford, OH) by enrichment culture using *Mycobacterium smegmatis* as host. The phage, named DumpstDiver, was isolated through six rounds of purification and consistently yielded lysogenic plaques of variable size. Electron microscopy revealed phage particles with a distinct head shape and a tail of constant length and morphology. The 112,285-base pair genome of DumpstDiver encodes approximately 230 genes (pending final annotation) and a single tRNA for glycine. There is extensive synteny with other phages from cluster J, including large blocks in which predicted amino acid sequences are 100% identical to any of various other J phages, suggesting strongly that recombination is a major evolutionary mechanism for generation of new phages, which in turn suggests that co-infection is likely. There is also a very small number of unique genes and genes that share homology only with chromosomal genes from other *Mycobacterium species*.

Poster 34

Identification and Characterization of Mycobacteriophage OwlsT2W

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Bacteriophages, the most abundant biological entities on the planet, are viruses that affect bacteria. The discovery, cataloging, and characterization of the diversity of phages is an active and fertile area of research worldwide, involving pre-college and college students through projects such as HHMI SEA-PHAGES, University of Pittsburgh's PHIRE, and NIH-SEPA's Bringing Research Into the Classroom (BRIC). Bacteriophages, or phages for short, that affect species of Mycobacteria are called mycobacteriophages, which includes the newly isolated phage OwlsT2W. OwlsT2W was isolated from a compost pile in Butte, MT and forms small plaques with well-defined edges. This phage has been classified as a temperate phage in the F1 cluster. Transmission electron microscopy revealed OwlsT2W to have *Siphoviridae* morphotype, with the capsid portion of the phage 70 nm in diameter and the tail approximately 190 nm in length. A restriction digest was performed to create a "phage phingerprint" and was compared to known phages. The genome for OwlsT2W was sequenced by the Pittsburgh Bacteriophage Institute using Illumina sequencing, and has a genome that is 56,515 bp in length and a G/C content of 61.2%. The annotation of this genome resulted in the identification of 104 separate protein-coding genes. Out of these, genes *27*, *31*, *32*, and *41* have been chosen for individual study. These genes have been determined to code for an HTH DNA-binding domain protein, lysin A, lysin B, and integrase proteins, respectively. The integration of OwlsT2W was studied by PCR using primers based from a previous study of Mycobacteriophage Tweety. OwlsT2W *att*P and the corresponding *att*B were thereby confirmed experimentally. Experiments to analyze genes *27*, *31*, and *32* by PCR and to clone for protein expression are ongoing, and a summary of OwlsT2W molecular and bioinformatics characterization will be presented.

Poster 35

Isolation, Characterization, and Comparison of Malinsilva and LugYa

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Seven bacteriophage that infect *Mycobacterium smegmatis* and *Arthrobacter sp.* were isolated and purified at Montclair State University during the 2014-2015 academic year. Of the four mycobacteriophage isolated, one was chosen to be sequenced and annotated, Malinsilva. An additional mycobacteriophage, LugYa, isolated in 2013 was also annotated. Both have a *Siphoviridae* morphotype and are type A3. The genomes of Malinsilva and LugYa were compared, in terms of similarities and differences, to determine the effect of isolation and propagation temperature. Malinsilva was isolated at 30°C while LugYa was isolated at 37°C. None of the *Arthrobacter*phage was sequenced due to problems obtaining medium or high titer lysates.

notes

Poster 36

The discovery and annotation of mycobacteriophages BigPhil and Sotrice96*

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Morehouse College, Atlanta GA

Mycobacteriophages BigPhil and Sotrice96 were isolated from soil samples in or near Atlanta, Georgia, using *Mycobacterium smegmatis* as the host. BigPhil is a Cluster F1 virus 53,618bp long and Sotrice96 is a Cluster E virus 76,299bp long. The purpose of our research was to finalize draft annotations of both phages by manually confirming potential genes and identifying gene functions. Utilizing the annotation program, DNA Master, and guided by heuristic GeneMark output for both phages, we determined the most likely open reading frames to identify each gene in these two genomes. We also performed BLASTp searches, in GenBank, and used the Starterator application in the Phamerator program, to ascertain whether the start site designated for each gene aligned with other start sites of homologous genes, and the probability of these matches. Lastly, we utilized Phamerator, GenBank BLASTp searches, and HHPred searches to assign gene functions to these two phages. Both BigPhil and Sotrice96 have programmed translational frame shifts in the tail protein chaperone genes just upstream of the tapemeasure gene in each genome. In BigPhil, this was a +1 frameshift and in Sotrice96 this was a more common -1 frameshift. We confirmed 100 genes and no tRNA sequences in BigPhil. Sotrice96 has approximately 145 genes and two tRNA sequences. The BigPhil genome is very similar to the F1 mycobacterial phage Sparticus and Sotrice96 is very similar to the E mycobacterial phage Henry.

Poster 37

Mycobacteriophages "Dreamcatcher" and "Legolas", two *Siphoviridae* collected in Lincoln, Nebraska

P. Fawns

T. Osborne

Pamela Fawns, **Taylor K Osborne**, Anna Daly, Gunnar Garner, Mitchell Heavican, Leigh Heese, Taylor James, Morgan Mickey, Joseph O'Connor, Gwennan Plouzek, Justine Blumenstock, Anna Donley, Matthew Harrison, Alexander Kuszak, Connor Springman, Riley Taylor, Taylor Zitek, Jerald S Bricker, Angela L McKinney

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The SEA-PHAGES program grants undergraduates the opportunity to isolate a novel mycobacterial phage from the environment and sequence its DNA. Phages DreamCatcher and Legalos were isolated on Nebraska Wesleyan University in Lincoln, Nebraska. The placement of phage "DreamCatcher" and "Legolas" as a member of the *Siphoviridae* is supported by plaque morphology and electron microscopy. The genome size of DreamCatcher is 97 putative genes comprised of 52,821 base pairs. Whereas, the genome size Legolas is 104 putative genes comprised of 68,555 base pairs. DreamCatcher has been analyzed as cluster A1, and Legolas as cluster B1.

notes

Poster 38

Isolation and Annotation of "Bactobuster", a Novel A2 Mycobacteriophage

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During the inaugural SEA-PHAGES Phages Hunters course at North Carolina Agricultural and Technical State University, we used the enrichment method to isolate and characterize four bacteriophages that infect *Mycobacterium smegmatis*. Three of the mycobacteriophages were isolated from soil samples in the Greensboro, NC area while the other, "Schmeg Griffin", was isolated from Chesapeake, VA. While three of the four phages appeared to be characterized by a lytic life cycle, mycobacteriophage "Bactobuster" appears to exhibit a temperate life cycle based on several factors, including plaque morphology, size and number. Given its distinctive life cycle among the isolated phages and its unique banding pattern during restriction mapping, Bactobuster was chosen for further characterization. The genome was thus submitted for whole genome sequencing at the University of Pittsburgh and annotated using DNA Master, as well as associated bioinformatics software programs. The Bactobuster genome consists of 52,129 bp, with a GC content of 63.1 %. Autoannotation identified 92 open reading frames (ORFs), with approximately one-third of the ORFs being in the forward direction. Based on sequence similarity, Bactobuster appears to be a member of cluster A, sub-cluster A2. Among the 67 family members in sub-cluster A2, Bactobuster appears to be most closely related to the mycobacteriophages Pukovnik, Power, Trixie, and Adzzy. Bactobuster and Pukovnik, a mycobacteriophage isolated from Fort Bragg, NC, exhibited the greatest similarity, sharing 94 % sequence identity at the nucleotide level. Interestingly, Bactobuster gp2, which was highly similar to Pukovnik gp2, showed high sequence similarity to the *Bacillus anthraxis* lethal toxin gene, according to HHPred. On the other hand, several ORFs with strong coding potential did not align well with any known proteins in either the PhagesDB or the NCBI databases, suggesting that they may correspond to novel genes.

Poster 39

A Predicted Uncommon -2 Programmed Translational Frameshift in a *Paenibacillus larvae* Phage Transposase Gene

C. McKenney

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Paenibacillus larvae is a firmicute bacterium that infects honeybee larvae and causes American Foulbrood Disease in hives. The 2013-2014 North Carolina State University Phage Hunters class isolated several *P. larvae* bacteriophages in North Carolina, and among these was a *siphoviridae* phage named Tripp. During the spring 2015 semester, NC State Phage Hunters students annotated the Tripp genome. While analyzing the genome, we found two consecutive genes that were called to encode partial transposases. If the two genes were joined, they would encode a more typical full-length transposase, which is an enzyme that facilitates movement of genes within and across genomes. We have found evidence to suggest there is an uncommon -2 programmed translational frameshift linking these partial genes. A programmed translational frameshift is an infrequent event that occurs when the ribosome stalls during translation, causing a shift along the RNA and a change in reading frame. Frameshifts of +1 and -1 have been found to occur in forming full-length transposases, but we are unaware of any -2 programmed frameshift in a transposase gene. Features of a frameshift can include a slippery sequence of repeated bases and a downstream RNA pseudoknot; a pseudoknot is a complex RNA structure that forms when mRNA folds on itself and creates a knot-like roadblock for ribosomes during translation. We identified a slippery sequence and probable pseudoknot at the junction of the partial transposase genes in the Tripp genome. When the translating ribosome reaches the pseudoknot structure, it stalls and slips back two nucleotides along the slippery sequence while the knot unfolds, causing the frameshift to occur. The change of reading frame caused by a -2 frameshift would result in a readthrough of the original stop codon, linking the segments of the transposase. Only 1-5% of translating ribosomes undergo a frameshift at most programmed frameshift sites, which for Tripp would result in low level transposase expression. This would allow for some genetic variation without greatly altering the genome or causing detrimental effects. In the future, we hope to experimentally confirm the presence of the RNA pseudoknot and identify the product of the frameshift, which will reinforce our discovery of an uncommon -2 frameshift in bacteriophage Tripp. Additionally, characterizing the features of the Tripp -2 frameshift may improve our understanding of the frameshift mechanism and allow us to determine why the -2 frameshift occurs in Tripp, rather than a ±1 frameshift.

Poster 40

Characterization of the Temperature-Sensitive K2 Mycobacteriophage Findley

P. Burrell

A. Elliott

Paige Burrell, **Abigail Elliott**, Ashley Bazemore, Emily Beard, Ryan Campbell, Aisha Castro, Patrick Findley, Kumarie Francis, Michelle Fraser, Justin Gabriele, Jack Henley, Corens Moreau, Tesla Murillo, Piotr Piasecki, Hwa Soo Ryoo, Maria Shields, Drew Walker, Matthew Yun, Lanese Henry, Peter J Park, Jacqueline M Washington

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In the last three years, Nyack College Phage Hunters have isolated a total of 45 phages by using *Mycobacterium smegmatis* mc^{2}155 as a host at 37ºC. This year, Biology majors as well as non-majors isolated and characterized 20 phages. All the newly isolated phages were screened for similarity to previously isolated phages by homoimmunity experiments. Five phages heteroimmune to Subcluster A1, A3 and F1 lysogens were sequenced and classified as new members of A2, B1 or K2 subclusters. Fameo and Journey13 are A2 phages with Journey13 at 48,502 bp being the being the smallest A2 phage isolated to date and is most closely related to D29. BatteryCK and ProfessorX are circularly permuted B1 phages. Finally, Findley, a K2 phage, is most similar to Milly, Mufasa and TM4; TM4 is widely used in mycobacterial genetics.

Bioinformatic characterization of Findley reveals that it contains no tRNAs, 94 genes, four of which are in the reverse direction, and in this respect, it is identical to its nearest relative Milly which is 98% similar at the amino acid level. Cluster K phages typically contain Start Associated Sequences (SASs), or short repeated sequences just upstream of predicted translation start sites and due to their location, are thought to be involved in translation initiation. Findley contains 16 SASs, 1 more than Milly (15) and five more than TM4 (11). No SASs were found in the intergenic regions missing in TM4.

In situ characterization show that similar to other K phages, Findley forms stable lysogens, unlike TM4 which is missing an approximately 5kb segment in the region where genes including the tyrosine integrase and immunity repressor are typically found. Interestingly, experiments revealed that Findley is very sensitive to temperature, with no plaques forming at 42ºC but plaques forming efficiently at 37ºC. Similar findings have been reported in Anaya, a K1 phage which fails to form plaques at temperatures above 33ºC. Ongoing experiments will provide insight on the nature of Findley's temperature sensitivity and its host range, and SDS-PAGE will be used to analyze its structural genes

Poster 41

Four years of mycobacteriophage isolation at Ohio State*

G. Brockman

E. Lemanski

Gina Brockman, Elise Lemanski, Sarah Ball, Caroline Breitenberger, Charles Daniels, Laurence Webb

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Since joining the SEA-PHAGES project in 2011 as a member of cohort IV, students at The Ohio State University have isolated more than 300 phages (with over 100 posted on phagesdb), resulting in the addition of 13 sequenced genomes to the ever expanding understanding of mycobacteriophage diversity. Sequencing revealed the discovery of an A1 cluster phage, 4 belonging to B1 subcluster, 4 C cluster phage, an E cluster phage, a J cluster phage and 2 F cluster phage, including the first F3 phage. Genome sizes range from 51,409 bp to 155,916 bp and the average GC content is 64.3%. The number of open reading frames predicted by auto-annotation with DNAMaster ranged from 90 to 244. Six of the genomes encode tRNAs, with the majority being found in the phages belonging to C cluster.

The SEA-PHAGES lab was expanded from approximately 50 students in autumn semester (2012 and 2013) to >400 students over two semesters of the 2014-15 year. Lack of incubator space led to the change of the temperature used for incubation from 37°C to room temperature (as suggested by others who have large numbers of students isolating mycobacteriophages). While students were able to successfully isolate phage from their soil samples, we were curious if the change in incubation temperature may impact which phage we are able to isolate. Using the archived lysates of previously isolated phages, we are using plaque and other assays to test the ability of phages isolated at two different temperatures to form plaques on *Mycobacterium smegmatis* at various temperatures. This information may be useful for other investigators considering different incubation temperatures in their phage isolation protocols.

Poster 42

Isolating and Characterizing Different Mycobacteriophages at Old Dominion University

C. Henderson

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Old Dominion University, Norfolk VA

Six newly discovered bacteriophages from the Tidewater region of Virginia were isolated and characterized by the ODU Research Community Alliance (ORCA) members. The bacteriophages were enriched from different soil samples using *Mycobacterium smegmatis* mc^2155 as the host organism. Numerous rounds of purification and isolation using spot plates and stick streaks were used to purify the phages. Subsequently, the isolated phages were visualized using transmission electron microscopy which revealed that the 6 phages all had siphoviral structural morphology. One of the six phages, named Bricole, was sequenced revealing a genome length of 81.1 kbp. The Bricole genome sequence placed the phage in the Cluster M phage group with mycobacteriophages Bongo, PegLeg, and Rey. Bricole is a temperate phage and the genome contains interesting features such as a large number of tRNA elements and a unique *lysA-gp36-gp37-lysB* lysis cassette.

notes

Poster 43

Genomic Analysis of Cluster T Mycobacteriophage*

Buzz R Hardin, **Daniel N Games,** Alex Abbott, Meredith Bolin, Rachel Dilatush, AlleaBelle Gongola, Taylor Johnson, Nathan Malone, Hannah Pagan, Nathan Terry, Tyler White, Sam Wilson, Joseph Koon, Colby Smith, Ben Blankenship, Ruth Plymale, Nathan Reyna

Ouachita Baptist University, Arkadelphia AR

Cluster T consists of only phages Mendokysei (UC-Santa Cruz), Bernal13 (University of Florida), and RonRayGun (Ouachita Baptist University). Students at Ouachita Baptist University conducted a bioinformatic analysis of phages in Cluster T in collaboration with both UC-Santa Cruz and U of Florida. At OBU, we focused on characterizing the location of promoters, terminators, integrase, attachment sites, transfer RNA, and repetitive elements found in all three phages. Using either DNAMaster or the web base software PePPER, 6 SigA-like promoters were identified in all members of Cluster T. Seven hair-pin terminators were identified using ARNold and Softberry. Repetitive elements were discovered through the use of Gepard dot plots and MEME logo analysis. Interestingly, only Bernal 13 and RonRayGun contained a potential repetitive motif in Pham 8914 (GP48) However, it appears this pham is deleted from the Mendokysei genome. Nether ARAGORN or tRNAscan-SE found tRNAs in the genomes of phages in Cluster T. However, while all three phage contain Pham 694 (GP25) only in Mendokysei does Pham 694 contain a tRNA like secondary structure. The genomic identification of regulatory sequences in Cluster T has allowed us to begin wet-lab type projects that will lead towards the verification of bioinformatically identified elements in the future.

Poster 44

Isolation, Characterization and Analysis of EricMillard, a Novel Cluster J Mycobacteriophage and WaterDiva, a Novel Cluster B1 Mycobacteriophage

Hee Gun Eom, **Nicole Griffin**, Jonuelle Acosta, Pedro Ajsivinac, Mark Aronson, Lauren Bailey, Ryan Benczik, Michelle Bonahoon, Yiqun Chen, Henry Clarke, Hannah Cook, Mokun Fatukasi, Anthony Fontana, Emma Fort, Vasupradha Girish, Jaycey Hardenstein, Matt Holderbaum, Xueqi Huang, Nathaniel Hunnewell, Hyun Joo Kim, Arren Liu, Tamara Lofton, Kate Lowrey, Weichuan Luo, Nick Palcheff, Rushi Patel, Ben Pfister, Haley Roos, Matt Saaco, Lindsey Saari, Cameron Smith, Stephanie Tucker, Jeier Yang, Lingyu Yang, Naina Zachariah, Xinchen Zhang, Yi Li, Kari Clase

Purdue University, West Lafayette IN

Two phages from diverse clusters were selected for annotation based upon large differences in genome size and diverse putative proteins towards the long term goal of further exploring changes in protein expression in response to changes in the lifecycle of the host bacteria, in the presence and absence of a putative canonical integrase.

EricMillard (http://phagesdb.org/phages/EricMillard/) and WaterDiva (http://phagesdb.org/phages/Waterdiva/) are novel mycobacteriophage isolated from environmental samples collected in 2012. Both phages induce lysis in the mc^2 strain of *M. smegmatis*, have a *Siphoviridae* morphotype, and were sequenced using Illiumina sequencing. The genome of WaterDiva contains 68,886 base pairs of circularly permuted DNA, 103 putative proteins, GC content 66.5% and is classified as a Cluster B, Subcluster B1 with 101 total members. The genome of EricMillard contains 113536 base pairs of DNA with defined physical ends, 251 putative proteins, GC content 60.9% and is classified as a Cluster J with 18 total members.

Annotation of both phages was executed using bioinformatics programs including DNA Master, Glimmer, GeneMark, Phamerator and BLAST. ORFs were assigned a predicted function based on homology to previously characterized proteins, location in the genome, or the presence of conserved protein motifs, using programs such as BLAST, Phagesdb, HHPred and Phamerator. EricMillard contains a putative frameshift between gene 33, a tail assembly chaperone, and gene 34, the tape measure gene. Comparison of EricMillard to RedNo2 in Phamerator aided in the identification of the +1 frameshift on "slippery sequence" CCCCAAAA resulting in a change of a serine to a valine. Scanning of the EricMillard genome with tRNA machine learning algorithms ARAGORN and tRNAscan-SE, revealed a tRNA. EricMillard also encodes a putative protein with homology to the FtsK superfamily, proteins that participate in chromosome segregation, and a methyltransferase within a non-conserved region of the phage genome. Methyltransferases can methylate phage DNA and thus provide protection from host-encoded endonuclease restriction endonucleases.

Many of the putative gene products from EricMillard contained an interesting conserved region identified with HHPRED known as Outer Membrane Proteins (OMPs), specifically Beta-barrels or prepilin-type processing-associated H-X9-DG, a "targeting signal for outer membrane insertion." This suggests that many proteins in EricMillard may be targeted to specific locations within the bacterial host cell.

Finally, several large intergenic gaps and putative overlapping genes were also identified in both WaterDiva and Eric Millard that did not share homology with similar regions of other bacteriophage in the same cluster. We are further exploring the gaps and putative reverse genes more closely by mass spectrometry and examining the expression pattern of proteins associated with the conserved OMP targeting domains.

notes

Poster 45

Genomic Analysis of Mycobacteriophage Smeadley*

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Smeadley was found in 2011 in Conyers, GA by Krystle McMinn student of Georgia State University. We received its genome through adopt a phage program, annotated it and looked at some of its genes more closely. Smeadley belongs to the subclaster A8 together with four other phages: Astro, Saintus, Expelliarmus and Roary. Two of them are submitted to the Gene Bank: Astro and Saintus. Saintus is also from Georgia, Astro is from South Carolina and Expelliarmus is from Michigan. Smeadley has a genome with a length of 52392 bps and has 98 predicted genes. This genome is very packed, with few long gaps and multiple instances of a 3 base pair overlap. Another feature of Smeadley genome is that the first half of the genome is forward and the second is reversed similarly to all A cluster phages. BLAST of the full genome of Smeadley returns 99% identity with Astro. BLAST of individual genes revealed that 48 of Smeadley's putative genes have genes from Astro as their best match, 31 have Saintus and 19 have Fredward that is not a part of Phagesdb. Smeadley has 1 tRNA gene, corresponding to tryptophan. The first part of the genome contains many genes with well predicted functions such as structural proteins: minor tail subunit, major tail subunit, tai assembly chaperone, tapemeasure, portal and terminase and functional proteins: lysin A, lysin B, integrase, protease. We identified programmed frameshift in front of the tapemeasure. The end of the genome has many short genes that don't have functional domains. Smeadley does not have any unique genes or genes matching other clusters than A.

Poster 46

Discovering novel *Arthrobacter* phages

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T. Buerkert

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Students in the 2014-2015 Phage Safari lab isolated *Arthrobacter* phages from soil samples, extracted DNA from the phages, and characterized them by restriction endonuclease digest and electron microscopy. The genomes of three phages - Brent, Wilde, and Tank - were sequenced. Phages Wilde and Tank have the *siphoviridae* morphologies; both have long non-contractile tails. Phage Brent has a relatively short tail and resembles a *myoviridae*. All three were lytic phages since they produce clear plaques; plaques produced by Wilde and Tank were much smaller than those by Brent. Sequence analyses revealed that Brent (49,879 bp long and isolated from Broomall, PA) is similar to phage Jawnski, isolated near the University of Pittsburgh in 2012 and vB_ArtM-ArV1 from Lithuania. Phage Wilde (68,203 bp) isolated from Montclair, PA and Tank (67,592 bp) from the Saint Joseph's University campus were highly similar and represent a novel cluster of all *Arthrobacter* phages discovered thus far. We have found many common features shared between Wilde and Tank. For example, they both have a frameshift mutation in the same gene features. Despite being unique phages with very little known about *Arthrobacter* phages, we have been able to identify some gene features that encode structural proteins such as the tape measure and tail sheath proteins, common in a wide variety of phages. Since phages are viruses that infect bacteria, learning about them will shed light on viral strategies and new bactericidal therapeutics as well as further the understanding of evolution and biodiversity.

Poster 47

Isolation and genomic characterization of Mycobacteriophages Wilbur and Romney2012*

I. Owen

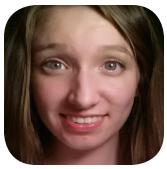
Izzy Owen, Kaylee Anderson, Tyler Breth, Hannah Brichacek, Victoria Caruso, Dilveer Chaggar, Delaney Cortazzo, Karlee Criado, Tanner Druck, Denali Espy, April Falkosky, Jusdan Griffith, Cierra Harrington, Briana Kostan, Kaitlyn Kutchman, James LaGrosse, Anna Lasslo, Dustin Martin, Samantha McKee, Elizabeth Powell, Corey Pruzinsky, Zachary Sheffler, Emma Snyder, Jackson Towers, Alicia Walsh

Seton Hill University, Greensburg PA

Mycobacteriophages Wilbur and Romney2012 (Cluster A4) were isolated from separate soil samples collected from geographically distinct locations on the campus of Seton Hill University in Greensburg, PA via enrichment culture on *Mycobacterium smegmatis* mc^{2}155 and characterized by students at Seton Hill University participating in the SEA-PHAGES research program. Both phages form clear plaques of 2.5 mm in diameter after 24 h incubation at 37°C. Both genomes are highly similar (51.4 kb, 63.9% GC, defined linear ends, 99% nucleotide similarity) despite being isolated from unique soil samples collected on different days. Phage genomes were annotated using DNA Master, HHPred, Phamerator, and the BLAST database suite. Wilbur and Romney2012 each contain 84 predicted protein-coding genes. Several noncoding features in the Wilbur genome were identified and annotated. Various phage characteristics were explored, including the permissive temperature range for plaque formation, ion co-factor requirements, and lysogen formation. Despite having genetic features common to temperate phages, Wilbur forms clear plaques under laboratory conditions, and no lysogens were able to be isolated using this phage. Future directions include examination of Wilbur and Romney2012 under various conditions to assess lysogeny and broader bioinformatic searches for noncoding features and mapping of membrane spanning domains.

Poster 48

Genomic analysis of four mycobacteriophages from Southern Connecticut: Two A3's, EpicPhail & Veracruz, and two new N cluster phages, Xeno and Phrann*



A. Sciola

K. Wielechowski

Amanda N Sciola, **Kamil Wielechowski**, Kathleen K Aberger, J. Carlos Barranco, Katherine E Brown, Christopher A Curtin, Jonathan D Gonzalez, Samantha M Groskritz, Jeff J Haney, Kara L Jones, Annie E Kaczmarczyk, Amy K Karlberg, Nina C Kestenbaum, Scott W Lucero, Francisco J Salinas, Deanna Scotto, Nicholas P Edgington

Southern Connecticut State University, New Haven CT

SCSU Honors College Freshman isolated 16 new mycobacteriophages that have the ability to infect *M. smeg*. mc^{2}155 at 42°C. We selected this temperature for several reasons. One reason is that we hoped that the increased temperature would perhaps select for novel cluster members. A second reason is that many other microorganisms cannot grow at 42°C, and thus this temperature would help to reduce contamination problems that could arise. Finally, the higher temperature facilitated a growth rate that allowed plating results to be seen in approximately 24 hours. The sequence was determined for three genomes which revealed their membership in the A11 subcluster (Snape--which we will discuss next year), and the N cluster (Xeno and Phrann). One might predict that phages isolated at 42°C may have a higher genomic GC% than phages isolated at 25°C or 37°C, and indeed the N cluster phages have a relatively high GC% compared to the remaining clusters. Also, Xeno has the highest GC% of all of the sequenced N cluster phages. Notwithstanding the previous two points, in all likelihood there is not a significant correlation between isolation temperature and genomic GC content. Currently, the sample size is too small to make a definitive conclusion. In addition to the phages isolated in the fall of 2014, two A3 phages (EpicPhail & Veracruz) isolated in 2011 were annotated and analyzed using comparative genomics.

SCSU isolated half of the new N cluster phages found in 2014, with Phrann being the largest N cluster phage genome sequenced to date. Phrann has a rare orpham (p) ppGpp synthetase/hydrolase (gp30) that is similar to another orpham, Squirty_gp29 (in the F3 subcluster), as well as a large number of other *Mycobacterium spp*. (p) ppGpp synthetase/hydrolases. We present an analysis of this important gene family. Xeno has many genes that, while present in other sub/clusters, are only present in this one particular N cluster phage. These are discussed in greater detail. The fifty-eight sequenced A3 mycobacteriophages seem to fall into four major groups based on whole genome multiple sequence alignments and phylogenetic tree construction. Interestingly the group to which Veracruz belongs is a very tightly clustered group of eight members, whereas two groups have five members each, and a final large diverse group captures most of the remaining members. Comparative genomics of this subcluster are presented.

Poster 49

Mariner: A novel marine phage which may affect marine biofilms

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Marine phage are ubiquitous and increasingly recognized as an important component of the aquatic environment. For instance, phage have been shown to control dynamics of some phytoplankton blooms. The role of phage in the composition and function of marine biofilms is largely unknown. Epizootic Shell Disease (ESD) in American Lobsters (*Homaus americanus*) is an emerging disease in the Gulf of Maine which can have a negative economic impact on its fishing industry. Although significant work has been done on the prokaryotic composition of biofilms in ESD lesions, we have found no published research on the role of phage in these biofilms. In this study, we cloned DNA fragments from a phage which infects a bacterium isolated from ESD lesions for partial genome sequencing.

Poster 50

Functional clustering of Mycobacteriophage proteins using phylogenetic profiles

D. Fox

Dylan Fox, Samantha Parsons, Margaret Walsh, Charles Hauser, Michael Kart

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Annotating protein functions from genomic sequences is a central aim of the SEA-PHAGES project. One assumption relevant to annotation efforts is that proteins that function together in a pathway or structural complex are likely to evolve in a correlated fashion, and that during evolution, all such functionally linked proteins tend to be either preserved or eliminated in a new species (phage). Phylogenetic profiles describe this property of correlated evolution by characterizing each protein by a string that encodes the presence or absence of a protein in a given genome. The aim of this project was to cluster Mycobacteriophage phylogenetic profiles to aid in annotation. Phylogenetic profiling has previously been applied to a variety of systems and shown to identify previously unknown enzymes in metabolic pathways, transcription factors, and explanations for roles of certain mutations in human disease.

notes

Poster 51

An Evolutionary History of A4 Cluster Phage, Skipitt, and the Study of its Infection Potential

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The annotation of the A4 cluster mycobacteriophage, Skipitt, provided a basis for further study of the phage's evolutionary history and infection potential. Through beginning with the first documented mycobacteriophage, D29, and following its rate of mutation to determine its most recent ancestor, both codon bias and the infection potential of Skipitt were discovered. This also allowed for insight as to how the A4 subcluster is characterized compared to other subclusters. Genome annotation and DNA sequence analysis tools, including DNA Master, NCBI Blast and conserved domain searches, phages.db Blast, Phamerator, and HHPred, were used to determine proper starts and reading frames of Skipitt's genome and assign gene functions to the reading frames.The same tools were used along with literature research to answer our experimental questions. It was determined that the tape measure protein gene has been used to distinguish phages within their cluster as well as allow for a tracing back of the phage's history. A 2,649 year period of evolution occurred between Skipitt and its most closely related ancestor, Medusa, since their divergence. Also, Skipitt showed codon bias similar to other A4 cluster phages and although Skipitt showed almost identical codon usage to phage, D29, Skipitt is more closely related to *M. tuberculosis*. A4 cluster phages are classified in such a way due to various factors including the tape measure protein, but there is no one definitive rule when placing a phage in this subcluster. Skipitt's relation to other phages within this subcluster was telling of both its history and the evolution of the A4 cluster overall.

Poster 52

Isolation and Characterization of Bacteriophage Appletree2, A Cluster L, Subcluster L1, *Siphoviridae*

C. Hines

K. Penrose

Christina Hines, **Kimberly Penrose,** Mary Darby, Anna Hardiman, Briana Nickelson, Jocelyn Perez, San Tat, Julie Ellsworth, Tina Slowan-Pomeroy, Laura A Briggs

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The goal of our research was to capture and isolate novel bacteriophage from Northern Nevada in conjunction with the Howard Hughes Medical Institute Science Education Alliance PHAGES program. Bacteriophages are relevant for their potential as an alternative treatment for pathogenic bacterial infections due to antibiotic resistant bacteria. The source location of this bacteriophage was 39°35'35"N & 119°48'39"W, collected on August 8, 2014 at 11:00 am in moist soil 3 cm below the surface underneath an apple tree. After isolation and purification the phage was given the name Appletree2. *Mycobacterium smegmatis* mc^2155 was used as the host for this project. After soil collection the bacteriophage were purified and plated with *M. smegmatis*. Bacteriophage was isolated until uniform phage were found resulting in clear plaques 0.8 mm in diameter. A High Titer Lysate (HTL) was prepared from pure culture and DNA was isolated for analysis. The bacteriophage was imaged by transmission electron microscopy at the University of Nevada, Reno showing a head diameter of 75 nm with a tail length of 275 nm. Appletree2 DNA was then sent to North Carolina State Genomic Sciences Laboratory for sequencing using Illumina Sequencing. The sequence was annotated using DNA Master and Phamerator. Appletree2 genome length is 73,808 base pairs, contains 58.9% GC content, features 132 genes, 124 ORFS, and eight tRNAs. It is a *Siphoviridae* morphotype classified as cluster L, subcluster L1. Utilizing Phamerator Map, Appletree2 was compared to closely related L1 subcluster bacteriophage UPIE, LeBron, and JoeDirt.

Poster 53

Genomic Characterization of 3 Novel Phage Infecting *Acinetobacter*


A. Nichols

Alexandria L Nichols, **Ben J McCafferty**, Omar K Bilbeisi, Shequori K Clyde, Katherine T Elmore, J. Alex Lushington, Laith E Matalka, Alicia C Moczynski, Mohamad M Moughnyeh, David P Saleeby, Jason A Shryock, Samantha A Turner, James C Whitfield, Denise L Monti


B. McCafferty

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A rise in antimicrobial resistance is a number of bacterial species has led to a resurgence of research to design new therapeutic agents and develop novel strategies for combatting antimicrobial resistant pathogens. The Centers for Disease Control and Prevention has identified 6 ESKAPE pathogens of particular concern: *Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas*, and *Enterobacter species*. Barton, JeffCo, and Effie are novel phage infecting the bacteria host *Acinetobacter* that were isolated from filtered sewage influent samples. Following purification of phage genomic DNA, we sequenced and analyzed each phage's full-length genome. Freely available bioinformatics software (DNAMaster, Glimmer, GeneMark, Aragorn) and NCBI Blast were used to assist in the identification of putative genes. Phamerator was also used to identify phams and assist in function determination. Our findings show that Barton and JeffCo share a significant number of genes and contain a similar tRNA gene. In contrast, Effie has little gene homology with either Barton or JeffCo. A number of novel genes were also identified in the newly sequenced *Acinetobacter* phage. This work represents the first set of *Acinetobacter* phage analyzed by the UAB Phage Genomics team and expands the pool of available phage infecting *Acinetobacter* for comparative genomics studies.

notes

Poster 54

Genomic and proteomic analysis of Mycobacteriophage Kersh, an F1 cluster phage


D. Hollingsworth

Derek Hollingsworth, Siddhant Ambulkar, Jaidev Bapat, Nathan Board, Teva Bracha, Eric Byeon, Joseph Chang, Chandra Couzens, Dillion Dorsey, Areian Eghbali, Danielle Escueta, Alison Fanton, Lynley Fernandez, Sean Guy, Taylor Harmon, Brooke Hawkins, Alyssa Hu, Emelie Knight, Gregory Lum, Rishi Modi, Rachan Narala, Joseph Park, Marissa Parkhurst, Kimia Tahami, Amanda Tjitro, Elena Westeinde, Madeleine Yu, Lianne Cohen, Eammon Riley, Kit Pogliano, Joseph Pogliano, Madeline Butler

University of California, San Diego, San Diego CA

Students in the Phage Genomics Research Initiative course at UCSD isolated and characterized 27 *Mycobacterium smegmatis* bacteriophage from collection sites in San Diego, CA in the Fall quarter of 2014. All of the isolated phage were members of the family *Siphoviridae*. One of the phages, Kersh, appeared to be a temperate phage based on plaque morphology and was selected to be further characterized. The genome of Kersh was sequenced and assembled by the Pittsburg Bacteriophage Institute using Illumina sequencing technology; the Kersh genome is 60190 base pairs in length and has defined ends. Using NCBI BLASTn, it was found that Kersh is a member of the F1 cluster. Students annotated the Kersh genome using the computer program DNA Master, and together with maps of predicted coding potential, found 107 putative protein coding genes, all of which but nine were transcribed in the same direction. Comparisons of the predicted proteins with NCBI BLASTp and Phamerator provided tentative functional assignments for many of the gene products, including several structural proteins and proteins involved in cell lysis. An integrase as well as a putative repressor and antirepressor were also tentatively identified. Kersh appeared to contain several bacteria-derived genes including a gene for a lipase that is a member of a pham with only one other member. Tandem mass spectrometry of phage lysates is currently being used to increase the accuracy of the annotation by identifying the production of gene products and by confirming the predicted start site for some of the proteins.

Poster 55

Comparing Codon Usage and tRNAs Present in a C1 Phage, Sprinklers, and a A6 Phage, ToneTone

B. Morgan

W. Stanton

Brianna Morgan, Whitney Stanton

University of Colorado at Boulder, Boulder CO

We compared the tRNAs and codon usage in two mycobacteriophages; ToneTone, a *siphoviridae* A6 cluster phage and Sprinklers, a C1 *myoviridae* phage. ToneTone contains three tRNAs while Sprinklers contains thirty tRNAs and one tmRNA. ToneTone's tRNA's are clustered at the beginning of the genome, while Sprinklers tRNA's are distributed in several clusters localized mostly in the second half of the genome. *Mycobacterium smegmatis*, the host organism used to isolate these phages, contains 47 tRNAs. In Sprinklers' genome we found the translational associated proteins peptidyl tRNA hydrolase type 2 and peptide chain release factor 1, which were not found in ToneTone's genome. These tRNAs and translational factors may contribute to the fitness of these phages.

notes

Poster 56

The annotation of 3 new Mycobacteriophages: ArcherNM, Petra64142 and Wooldri

E. Gunn

Eliot Gunn, Inbar Aberman, Daniel Aldridge, Neha Bapatla, Connor Carlton, Sophie Charles, Alicia Kianes, Katerina Lambrinos, Megan Laughrey, Yianni Protopapadakis, Riddhi Ramanlal, Carey Roberts, Justin Yeung, Guanqiao Feng, William B Davis*, Brad Barbazuk

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The HHMI SEA-Phages class at UF this year concentrated on annotating the genomes of three Mycobacteriophages isolated by students of Washington State University, Pullman. WA. and provided by Dr. William Davis: ArcherNM, Petra64142 and Wooldri. ArcherNM is an A2 phage with high sequence similarity (96%) to Changeling–another A2 phage also isolated and annotated at Washington State University. ArcherNM is 52,561 bp in length with a GC content of 64.2%. Genome annotation suggests that there are 94 protein coding genes and no tRNAs.

Petra64142 exhibits highest nucleotide sequence similarity (99%) to OrionPax, and has been assigned to phage cluster E. Petra64142's genome is 75,271bp in length and exhibits 63% GC content; these values are typical of cluster E phages. Also typical of cluster E phages are a large number of protein coding genes. Petra64142 has 143 annotated protein genes and 2 tRNAs. Finally, Mycobacteriophage Wooldri is a member of the phage cluster A, subcluster A3. Wooldri is 50,797 bp in length, exhibits 64% CG content and contains 98 protein coding genes and 3 tRNAs. Details of the genome characterization of these 3 Mycobacteriophages are discussed, including search results for promotors, stoperators, translational frameshifts and a comparative analysis of gene content between A2 and A3 cluster phages.

Poster 57

Isolation and Characterization of A, B, C and K Cluster Mycobacetriophages

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Mycobacteriophages are viruses that infect mycobacteria and are composed of genetic material encapsulated in a head made of proteins. These phages can be found in places populated with their host bacteria such as soil. Goal of this project was to isolate and characterize a bacteriophage from soil sample. In the fall semester of 2014, sixty students were involved in a project based phage hunting lab course offered as General Biology lab I course (Biol 1101) at University of Houston-Downtwon (UHD). Ten phages named Midas, Duchessdung, Lesgirval, Coog, Bigswole, Phinci, Xiaopingtu, UHDChampion, Greedylawyer and Edugator were isolated using enrichment method, and electron micrographed. DNA of only six phages names; Midas, Duchessdung, Coog, Bigswole, Greedylawyer and Edugator from four three different clusters A, B, C and K were sequenced. The annotation studies are currently under progress.

notes

Poster 58

Characterization of Potter and Polka14, two mycobacteriophages isolated from soil samples in Lawrence, Kansas

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Mycobacterium smegmatis is a common soil bacterium that is a non-pathogenic close relative of *Mycobacterium tuberculosis*, the causative agent in Tuberculosis. One laboratory section of the introductory biology course at the University of Kansas took part in a Howard Hughes Medical Institute-sponsored project to isolate and characterized bacteriophages that can infect *M. smeg*. Fifteen unique phages were isolated from soil samples collected throughout Lawrence, Kansas, purified to high concentration, and examined by electron microscopy and DNA fingerprinting. Genomic DNA from two selected bacteriophages was subjected to whole genome sequencing at the University of Pittsburgh. Phage Potter is a lytic phage that was found near Potter lake on the KU campus. Its genome sequence indicates that it belongs to subcluster B1 mycobacteriophages. Polka14 is a lytic phage producing very small plaques that clusters with F1 mycobacteriophages. During the annotation of Potter, we discovered that within B1 phages, gp10 comes in two forms, either an 87 amino acid protein or a 45 amino acid protein that results from a mutated start codon in the shorter protein phages. We are exploring the evolution of gp 10 in B1 phages, and are attempting to express the longer and shorter products in bacteria so that we can isolate and begin biochemical characterization of the proteins. To gain a better appreciation of the diversity in the samples collected by the class, we are using the Phage Enzyme Tool (PET) to estimate the cluster and subcluster of each sample and are comparing these results with a method using polymerase chain reaction of tape measure genes to predict cluster assignment. Together these results will contribute to a better understanding of the diversity of mycobactriophages, and may provide insights into the structure and function of viral proteins that can potentially be useful in understanding the viral life cycle and its interaction with host bacteria.

Poster 59

Annotation of *Rhodococcus erythropolis* Phage Singletons Trina and Chewy VIII*

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Four novel *Rhodococcus erythropolis* phages were isolated from northeast Louisiana area soil samples. To date, *Rhodoccocus* phages Chewy VIII and Trina have been sequenced (Illumina Sequencing, University of Pittsburgh) and their genomes annotated with the DNA Master bioinformatics platform. Chewy VIII is a *siphoviridae* phage with 69,165 bp including a 10 bp 3' overhang, 100 open reading frames (ORFs) and no tRNA gene coding regions. Trina is 139,262 bp including a 2860 bp terminal repeat and has 249 ORFs and 25 tRNA genes. While functional gene calls can be identified for both genomes, the majority of open reading frames have no sequence homology to any known gene product currently found in GenBank and are consequently labeled as 'orphams'. Phamerator comparison of a number of recently sequenced *Rhodococcus* phages indicates that both ULM isolates are significantly different from other existing isolates and are currently designated as 'Singletons'. Chewy has some BLASTn hits to Gordonia phages but only ~20% query coverage. While Trina is about the same size as *Rhodococcus* phages Grayson and Peregrin, it is only ~10% similar at the nucleotide level. A BLASTn comparison of Trina and Chewy VIII against the Mycobacteriophage database revealed that Trina displays some limited similarity to L1 cluster mycobacteriophages while Chewy VIII shows a higher degree of similarity to M cluster phages.

Poster 60

Annotation of PeterPeter, a Cluster A Phage

Z. Beal

P. Nelson

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University of Maine, Fort Kent, Fort Kent ME; *University of Maine, Machias, Machias ME

Students at the University of Maine-Fort Kent isolated and purified a novel organism, Mycobacteriophage PeterPeter, from a soil sample taken outside a University residence hall next to a dumpster. Students then analyzed PeterPeter's 51,366 bp genome. PeterPeter's genome has a GC content 63.9% and begins with a reverse gene. This is uncommon, with only 30% of A4 phages having this as a start. PeterPeter is a member of the largest cluster and the second largest subcluster of the A phages (subcluster A4).

notes

Poster 61

Going to the Gap for more than genes

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Bacteriophage (phage) are the most numerous and diverse biological entities on Earth. This diversity provides a reservoir of information about not only gene and genome structure and function but about mechanisms of evolution. This year we explored the genomes of two mycobacteriophage, Phaja and Pippin. Phaja is a Cluster E phage with a genome size of 75685 bp. Pippin is a Cluster A1 phage with genome 52034 bp in length. To explore these two genomes we focused on the gaps. One gap visible on Phamerator is created by the tRNA genes. Phaja carries two, both encoding Gly tRNAs. They do not reflect alternative codon use when comparing Phaja to *Mycobacterium smegmatis*. Rather they are codons used at a higher frequency by Phaja suggesting a need for a larger tRNA pool. The mismatch of codon frequency of use with the host, suggests that *M. smegmatis* may not be Phaja's natural host. Another gap we explored was one created by an approximately 500 bp insertion immediately upstream of the tapemeasure protein gene in Phaja. The insertion contains two overlapping reading frames. BLAST analysis shows that both reading frames have the potential to encode an endonuclease. The endonuclease has been annotated in other phage. However, the structure suggests the possibility of a reading frame shift. It is intriguing that that the structure occurs immediately downstream of the tail chaperone ORFs with their reading frame shift. Phaja also contains an endonuclease/recombinase that it shares with a small subset of the Cluster E phage, downstream of gp94. Most of the E cluster phage have a methylase at this position. The endonuclease is a candidate for horizontal gene transfer. Gaps are usually mined for promoter sequences and in Pippin we explored the promoter region of the putative repressor protein. Pippin forms lysogens at a very low frequency and the plaque morphology is essentially clear. The peptide is 100% conserved with the putative repressor of BxB1 as is the sequence in the gap. The area has a weak promoter based on sequence and the same sequence is found in other cluster A1 phage suggesting there is another reason for the clear plaques.

Poster 62

Isolation and Characterization of Mycobacteriophage Zenon

Zach Beal*, **Kaleigh Carroll, Monique Mills,** Jeremy Bishop*, Isaac Caballero, Bryce Carter*, Colby Drexel-Harmon*, Corey Henderson*, Kate LaPlante, Jessica Laytart, Jessica Walker, Gerard Zegers, Shallee Page, Peter Nelson

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Mycobacteriophage Zenon is a newly isolated phage from a soil sample collected at a farm in Maine, using *Mycobacterium smegmatis* mc^2155 as a host. Zenon is one of five Cluster R phages. Cluster R phages display a high degree of similarity to each other (≥98% nucleotide sequence identity). A complete annotation of Zenon and its relationship to Cluster R are presented.

notes

Poster 63

Characterization of the *Bacillus thuringiensis* subsp. *Kurstaki* Bacteriophage Channel Fever

J. Esler

K. Littlefield

James N Esler, **Kirsten E Littlefield**, Shannon R Becker, Samuel J Clark, Kendra B Ganser, Samantha L Gorton, Jenifer M Grove, Juliette M Guilloux, Polly R Healy, Mariah A Jensen-Wachspress, Skyler C Larsen, Lynn O Lewis, Matthew J Mahoney, Kimberly N McFarland, Lesya M Melnychenko, Parker A Campbell, Alexandra M Piercy, William Rodriguez, Heather L West, Benjamin A Whipkey

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Since 2011, the UMW Phage Hunters classes have been isolating phages that infect the host *Bacillus thuringiensis* subsp. *Kurstaki* (BtK). We chose this particular host as it is used as a simulant for *Bacillus anthracis* by the Naval Surface Warfare Center in Dahlgren, VA (with whom we have a partnership) and BtK has been widely used as a bioinsecticide for the control of Gypsy moths, making discovery of novel phages very likely along the East Coast. Further, there are relatively few *Bacillus* phage genomes published in GenBank, making *Bacillus* phages a fertile area for student study. This year, UMW students chose whether to incubate their BtK and phages at room temperature (approximately 23oC) or at 30oC in hopes of isolating some *podoviridae*. However, all phages isolated were *myoviridae*, as demonstrated by electron microscopy. UMW students also tested their phages against ten *Bacillus* species to determine host range. One phage, which infected almost all the hosts we examined, was chosen for sequencing. Bacteriophage Channel Fever was isolated from direct plating of soil collected from White Stone, VA. Channel Fever produced mostly lytic plaques, approximately 1.7 – 3 mm in diameter, after incubation for 48 h at room temperature. The capsids of Channel Fever, as examined by transmission electron microscopy, had heads of approximately 96.5 nm in height and 84.2 nm in width, and contractile tails approximately 222 nm long. On autoannotation, Channel Fever was found to contain approximately 297 features in a genome of 164,974 bp, with a 2808 direct repeat containing 4 genes repeated at each end. There were 7 singleton genes on autoannotation. The closest match to this phage in GenBank is BigBertha, isolated at UMW and annotated by Texas A&M, followed by UMBC's Troll. According to Phamerator, Channel Fever is also a good match to UMBC's Anthos, and UMW's phages Adelynn and Phabio. As found with other *Bacillus* phages, there is a large gap in the genome, but Channel Fever does not code for any tRNA within that gap. While analyzing the Channel Fever genome, students also designed primers to test their specific phage by PCR. We chose two genes that are found in Troll and have functions – a capsid gene and the tape measure gene. Several of the phages matched one or the other, but not both, while the rest either matched both genes or neither gene. We are hoping to develop a method for testing our phages prior to sequencing so we can determine the diversity of *Bacillus* phages.

Poster 64

TsarBomba, a multi-host sub-cluster forms deep inside Saratov, Russia

F. Hamedooni Asli

E. Kawecki-Wright

Faranak Hamedooni Asli, **Elizabeth L Kawecki-Wright**, Chan H Ahn, Arash Bahari, Gabrielle M Boyd, Sean D Cordova, Nicholas M DiLosa, Joshua A Dutterer, Julie A Gessler, David N Ma, Hassan M McLain, Anu Michael, Julie E Norton, Kahye Park, Clarence Pascual, Mayuriben Patel, Brittnie S Prakash, Jesse V Schoen, Katie G Seong, Eric Sohn, Mimi Way, Grant L Wunderlin, Ravali Yanamaddi, Hassaan K Yousufi, Sefa Kılıç, Steven M Caruso, Ivan Erill

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Bacillus cereus group phage TsarBomba was isolated from soil samples collected from Saratova, Russia on *Bacillus thuringiensis* subsp. *Kurstaki*. The phage was characterized by plaque morphology, electron microscopy, and cluster analysis using primer extension prior to genomic sequencing by Illumina next-generation sequencing. TsarBomba was determined to have a genome of 162,486 bp in length, with 40.1% GC content, and 6,364 bp direct terminal repeats. Detailed examination of the genome revealed TsarBomba to contain 247 protein coding genes and 20 tRNA genes. Comparison of TsarBomba with other phages belonging to the proposed C cluster by genomic synteny and phylogenetic methods suggests the inclusion of a fourth sub-cluster containing *B. cereus* group phages BCP78, TsarBomba, Hobo, and IceQueen. Host range for TsarBomba was assessed experimentally with a cohort of *B. cereus* group hosts. TsarBomba was found to target all the tested *B. thuringiensis* strains, several *B. cereus* strains and a *B. anthracis* strain, but not more distantly related species, such as *B. pumilus*, *B. megaterium*, or *B. subtilis*. The ability of bacteriophages to infect different hosts depends largely on the ability of tail fibers to interact with specific membrane elements, enabling the assessment of host range through comparative phylogenies of housekeeping and membrane proteins in bacterial hosts. Codon usage bias has also been postulated as a putative determinant of host range, especially for bacteriophages harboring tRNA genes in their genomes. Analysis of the translational component of codon usage bias using the scnRCA index inferred from multiple hosts reveals that codon usage patterns have not changed significantly across the *Bacillus* genus and, therefore, should not have an impact on *B. cereus* group bacteriophage host range. Furthermore, comparison of codon usage bias among several *B. cereus* group phages harboring and not harboring tRNA genes suggests that codon usage patterns are not optimized among the former to exploit their genomic tRNA component.

Poster 65

Isolation and Characterization of *Streptomyces* bacteriophage Chymera, OlympicHelado, Verse, and Amela

I. Delwel

Isabel O Delwel, Emory Barnes, Sierra Barnett, Alec Bond, Cheyenne Colley, Gabriel Doria, Alex Eller, Ryan Hemenway, Connor Kennedy, Ashley Millican, Christine Munyoki, Jessi Narvaez, Stetson Norried, Jazmine Rosado, Christie Sirisakd, Brandt Smith, Justin Swain, Orooj Syed, Swapan Bhuiyan, Sonya R Layton, Robert C Benjamin, Lee E Hughes

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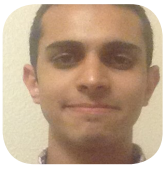
Streptomyces are Gram-positive, filamentous bacteria. Members of this genus are used to produce antifungals, antivirals, antitumoral, anti-hypertensives, antibiotics and immunosuppressives. *Streptomyces griseus* and *Streptomyces venezuelae* were used as the host bacteria in this research for the isolation of bacteriophage. *Streptomyces griseus* is most often found in soil and was first used to produce the antibiotic streptomycin. *Streptomyces venezuelae* can also be found in the soil, and was used to manufacture the antibiotic chloramphenicol.

The diverse group of *Streptomyces* bacteriophage isolated in this study were Chymera, OlympicHelado, Verse, and Amela. Chymera, OlympicHelado, Verse, and Amela came from enriched soil samples from Texas and New York. Amela and Verse both have a 3' sticky overhang of 11 base pairs CGGTACGTGAT, 65.6% GC content, and are only 31 base pairs different in genome length. Verse has a 49,483 genome base pair length, and Amela has a 49,452 genome base pair length. These two phage are in the same cluster. OlympicHelado and Chymera both produce extremely small, lytic, and circular plaques. OlympicHelado is the only phage that was isolated from a soil enrichment that was not collected in Texas, and has the shortest 3' sticky overhang length of 9 base pairs CGCCCGCCT. OlympicHelado has a 56,189 genome base pair length, and a 59.5% GC content. OlympicHelado produces plaques that are approximately .5 mm in diameter. OlympicHelado established a new *Streptomyces* phage cluster. Chymera was isolated from a prophage on the host strain and was also isolated as Dayspring in a separate class section. Chymera has a 34,742 genome base pair length, and the highest GC content of the phages in this study at 71.4%. It has a 3' sticky overhang length of 10 base pairs CGCGGGGGGG. OlympicHelado has a 56,189 genome base pair length, and a 59.5% GC content. OlympicHelado produces plaques that are approximately .5 mm in diameter. Chymera is currently a singleton. Chymera, OlympicHelado, Verse, and Amela all appear to have normal synteny in the structural protein genes. Using these phage to infect either *Streptomyces griseus* or *Streptomyces venezuelae* helps further the research into not only bacteriophage, but also the *Streptomyces bacterium.*

notes

Poster 66

Isolation and Annotation of a group of similar *Streptomyces* phages: Excalibur, Danzina, Hydra, Izzy and Lannister



M. Mehany

Mark Mehany, Tunazzina Ahmed, Danial Ali, Guadalupe Alvarez, Gretchen Clark, Elizabeth Cox, Isabel Delwel, Cynthia Garcia, Frederic Johnson, Connor Kennedy, Aaron Lara, Jessi Narvaez, Naomi Niyah, Brian Okundaye, Sekinat Quadri, Jazmine Rosado, Owen Saenz, Brandt Smith, Orooj Syed, Dat Tran, Katelyn Williamson, Jennifer Zinanti, Swapan Bhuiyan, Sonya R Layton, Robert C Benjamin, Lee E Hughes

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Streptomyces are genus of filamentous bacteria. They are complex, gram-positive aerobic bacteria that exhibit a complex form. Certain *Streptomyces* are used in antifungals, antivirals, immunosuppressants, and especially antibiotics. In this research, we have used *Streptomyces venezuelae* and *Streptomyces griseus* as hosts bacteria for our isolated phages. *Streptomyces griseus* is commonly found in soil and resembles a fungi in shape. It is a well-known producer of antibiotic. *Streptomyces venezuelae* can be found in the soil and above ground as aerial hyphae. The first antibiotic to be manufactured on a mass scale was derived from *Streptomyces venezuelae*. The large group of similar phages we studied during the annotations portions of the semester include: Excalibur, Hydra, Lannister, Danzina, and Izzy. There are many similarities between these genomes along with a few differences.

Lannister, Izzy, Excalibur, Hydra and Danzina were isolated from enriched soil samples. These phages tend to have typical synteny of structural bacteriophage genes. All these phages contain a 3' sticky overhang that is 11 base pairs long. Specifically, Hydra, Excalibur, and Izzy have the same 3' sticky overhang consisting of CGGGCAGTGAT while Danzina and Lannister have a CGGCCAGTGAT 3' sticky overhang. All the phages are roughly 50,000 base pairs long, and the genomes contain around 65% of G and C's. Hydra and Excalibur have the most similar genomes based on the Phamerator maps, while Izzy, Lannister and Danzina exhibit more variation.

The phage Lannister was collected in Texas. Its genome is around 50,165 base pairs long, consists of 65.7% G and C's and potentially codes for at least 73 genes. The phage Izzy was collected in Texas. The GC content of this genome is around 65.9% and is 50,113 base pairs long. The phage Excalibur was collected in Texas. The genome is 49,949 base pairs long and contains 66.2% GC content. The phage Hydra was collected in Texas. It is 50,727 base pairs long and has GC content of 66.2%. The phage Danzina was collected in Texas. It is 50,773 base pairs long with a 65.7% content of GC. All of the phages contain a gene in the middle of the DNA that is responsible for tape measure and a gene towards the end of the DNA that has a function of DNA Polymerase II. Many of the reads and BLASTS of these phages relate to Zemlya, Sujidade, and Lika. It is also worthy to note that Danzina has an immunity repressor gene that could possibly indicate that this is a temperate phage. Because the phages Lannister, Izzy, Excalibur, Hydra and Danzina are in a closely related group, they are similar and exhibit comparable functions, shapes and characteristics. In this research, the phages Excalibur, Danzina, Hydra, Izzy and Lannister were isolated from *Streptomyces venezuelae* or *Streptomyces griseus* bacteria, sequenced, annotated, and finally compared to further expand the research of bacteriophages in *Streptomyces*.

notes

Poster 67

Gee Whiz: Emerging diversity of the Cluster G mycobacteriophages*

J. Schiebel

M. Ulbrich

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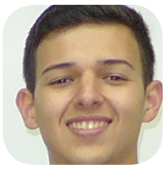
Bacteriophages are the most numerous biological entities on the planet, with an estimated 10^{31} globally, and phages influence gene flow in microbial populations and processes such as the global carbon cycle. More than 627 phages of *Mycobacterium smegmatis* mc^2155 have been sequenced and characterized as part of the SEA-PHAGES program; genome analysis puts these phages into 28 different clusters and subclusters. Furthermore, rarefaction analysis of phage "phams" suggests that the mycobacteriophage population is not a closed set, but continually acquires new genes. Recent isolates Cambiare, FlagStaff, and MOOREtheMARYer are new members of the Cluster G mycobacteriophages, a cluster within which the current members are all closely related at the DNA sequence level. The three new Cluster G phages share similar G+C content, genome length, and pham content with other members of Cluster G, but the average nucleotide identity (ANI) shows they span a greater degree of diversity than previous seen. We propose that the three newly isolated phages represent new members of Subcluster G2 (Cambiare and FlagStaff) and G3 (MOOREtheMARYer) with all extant Cluster members forming Subcluster G1. The Subcluster G2 and G3 members lack the integration cassette that characterizes their integration-dependent immunity systems, and do not contain the mycobacteriophage mobile elements discovered in many other G phages. Finally, Cambiare, FlagStaff, and MOOREtheMARYer encode an additional tail fiber-like protein not present in Subcluster G phages, host range analyses have not yet shown any differences in host preferences.

notes

Poster 68

Morphologic, Genomic, and Proteomic Characterization of Mycobacteriophage InterFolia Isolated from Tropical Soil of Puerto Rico



L. Reyes-Pena

I. Torres-Rivera

Luis A Reyes-Pena, **Ivanice Torres-Rivera,** Fernanda Bonilla-Gierbolini, Paola A Figueroa-Solano, Molishka A Flores-Narvaez, Alexander J Jaramillo-Canas, Dariel D Mojica-Gomez, Robert Nieves-Ayala, Daniel Nurse-Corso, Sorelly Ojeda-Centeno, Yolymar Oquendo-Alvarado, Ricardo Ortiz-Rodriguez, Paola M Padilla-Alicea, Laura Perez-Nuñez, Francisco Perez-Rivera, Luis A Perez-Rodriguez, Laura V Rivas-Vega, Nashaly M Saldana-Santiago, Kevin R Sanchez-Garces, Windelys M Sanchez-Torres, Andrea Sanchez-Velazquez, Kenia Santa-Rosario, Isamarie Santiago-Figueroa, Keily Santiago-Morera, Haydeeliz Santos-Sanchez, Zulmari Silva-Pedraza, Leroy A Smith-Torres, Gabriel Solis-Diaz, Steven Soria-Ramos, Adelbert Soto-Gonzalez, Juan A Vargas-Alvarez, Juan J Apiz-Saab, Eduardo Correa-Vazquez, Mariceli Fernandez-Martinez, Natalia Maldonado-Vazquez, Jose Pabon-Lopez, Joseph Perez-Otero, Laura I Ramos-Flores, Myrielis Rivera-Burgos, Christopher A Quintanal-Segarra, Michael R Rubin, Edwin Vazquez

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Student engagement in original scientific research is an effective method to provide meaningful educational experiences in introductory biology. Over the past six years, approximately 200 undergraduate freshman biology majors have participated in the SEA-PHAGES Program at UPRC, a project to isolate and characterize mycobacteriophage genomes, previously shown to present variable and mosaic gene organization. Understanding mycobacteriophages is important for evolutionary studies, novel gene identification, and development of therapies against antibiotic resistant pathogenic mycobacteria. We hypothesize finding unique bacteriophages with useful properties.

Tropical soil samples were collected throughout Puerto Rico and high titer phage lysates were prepared following enrichment with *Mycobacterium smegmatis* mc^2155 and plaque purification. Phage morphologies were characterized using electron microscopy. Virion coat proteins were resolved using SDS-PAGE and will be followed by protein identification using mass spectroscopy. Mycobacteriophage genomic DNAs were purified, digested, and visualized using gel electrophoresis. Selected genomic sequences were determined and analyzed using bioinformatics tools.

This year, 25 novel lytic and lysogenic bacteriophages were isolated followed by purification and characterization. The genomic sequence was determined and annotated for the virulent *Myoviridae* mycobacteriophage InterFolia. InterFolia belongs to cluster C1, is 156,221 base pairs in length, with 64.7% GC content, circularly permuted ends, and encodes approximately 239 putative protein coding genes including proteins similar to capsid and tail structural proteins, terminase, HNH endonuclease, band 7 protein, LysM domain, helicase, primase, recombinase RecA, nucleotidyltransferase, phosphoesterase, FAD-dependent thymidylate synthase, lysins A and B, and many novel proteins with unknown functions. The InterFolia genome also encodes 32 tRNAs and one tmRNA.

During the past six years, more than 150 bacteriophages have been isolated and partially characterized at UPRC and additional bacteriophages for this and other bacterial hosts continue to be found, increasing our understanding of the biology of this important group of viruses. Eight UPRC mycobacteriophage genomes have been sequenced and annotated, and four have been submitted to GenBank so far: Lilac (JN382248.1), Cuco (JN408459.1), Kratio (KM923971.1), and Murucutumbu (KM677211.1). The unique organization of these mycobacteriophages presents an interesting challenge in understanding evolution and genome conservation maintained through distant geographic dispersal. SEA-PHAGES, HHMI, and UPR-Cayey supported this research.

notes

Poster 69

Characterization of Leston, a novel Mycobacteriophage of the subcluster K5

Ibrahim Salama, Luisa Mata, Jaqueline Olivas, Ashley Shamaley, Leslyann Mercier, Jazmin Okwunwanne, Adilene Chapina-Guizar, Infinity Tucker, Alicia Ramos, Kurt Boots, Enrique Rangel, Lakishia Muniz, Dakota Saavedra, Kenyon Behrens, Katherine Granillo, Jaime Morales, Angel Caro, Yosaira E Flores, Eduardo Urias, Sergio L Munoz, Manuel Llano, German Rosas-Acosta

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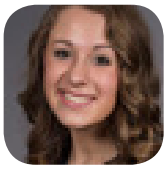
Studies on the composition and action of bacteriophages can provide insight into the physiology of bacteria and, in the case of pathogenic organisms, suggest mechanisms for their control or elimination. In collaboration with the Science Education Alliance, National Genomic Research Initiative, and Graham Hatfull's laboratory at the University of Pittsburgh, we are investigating the diversity and genomic strategies of action of bacteriophages that are capable of infecting a laboratory strain of *Mycobacterium smegmatis*.

Investigators collected environmental samples from the El Paso area and assayed them for phage activity. Positive samples were processed to enrich and isolate bacteriophages. Eleven independent phage isolates were purified and characterized by electron microscopy and by DNA restriction analysis. Structural features and DNA fingerprints were compared to mycobacteriophages reported on phagesdb.org. On the basis of its unique characteristics, the bacteriophage Leston was selected for genomic study. Purified DNA was sequenced at Virginia Commonwealth University using 454 technology and assembled to a finished genome of 61,898 bp. Leston was isolated from soil obtained from the Franklin Mountain range, in west El Paso, TX. Its capsid morphology identifies it as a member of the *Siphoviridae*, and its genome is linear with defined ends and a 3' overhang. Initial BLAST comparisons to known phage genomes reveal that Leston is a class K5 *Mycobacterium* phage. Preliminary analysis using Glimmer and GeneMark identified 98 protein coding genes and one tRNA-Trp gene. We have annotated each of the predicted genes, including phage structural proteins and DNA replication enzymes, and we are investigating additional potential coding regions. Interestingly, scattered along this phage genome we found multiple gap regions between genes, with the longest being 359 bp. The complete annotation of Leston's genome and its comparison with other mycobacteriophages will provide important insights on the biology and evolution of the most abundant members of Earth's biome.

notes

Poster 70

From Australia to Wisconsin: Isolation and characterization of several members of a related group of *Rhodococcus* phage*



M. Kosch

K. Schuette

Madeline G Kosch, **Kaylah J Schuette**, Taylor G Adams, Patrick O Anigbogu, Codi R Braden, Diego C Carneiro, Ariadine A Casas, Megan L Caturia, Leah S Chally, Brent B Cunningham, Nicole M Debace, Alyssa J Gunderson, Nicole K Hastings, David A Jacobsen, Krystal J Kleven, Bradley J Koch, Eric W Liesse, Thalles S Lopes, Anna N Lowery, Brittany A Lubich, Kristi L Mahoney, Michelle L Mattison, Connor D Satterlund, Robyn A Syverson, Bee Thao, Soua Thao, Kali J Westwood, Mary Xiong, Jorge A Bonilla, Karen K Klyczek

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Students in the UWRF Phage Hunters courses set out to isolate phage from *Rhodococcus erythropolis* (formerly *Rhodococcus globerulus*). We used the standard *M. smegmatis* soil enrichment protocol, substituting LB broth with 4.5 mM $CaCl_2$ and culturing at 30°C. We also tried a soil extract method, and tested enrichment culture times from 2 to 5 days. Only four soil samples out of more than 80 samples tested yielded phage. The positive samples included garden compost, riverbank and field soil. Several phages were isolated from these samples, and we obtained DNA sequences for six phages (Krishelle, AppleCloud, RexFury, Alatin, Naiad, StCroix). All are very similar to each other at the genome level, and are closely related to *Rhodococcus* phage RER2, isolated in Queensland, Australia, as well as to many of the *Rhodococcus* phages isolated at other SEA-PHAGES schools. They have genome sizes between 46,300-46,700 bp, with 68-70 ORFs. This nucleotide similarity was surprising, since the plaque morphologies exhibited by these phages varied. Alatin produced much smaller plaques than the others. StCroix produced turbid plaques, though it differs by only 2 nucleotides from Naiad, which yielded larger, clear plaques. One of these nucleotide differences is located in a possible repressor protein gene. Preliminary results of temperature range testing suggest that some of the other phages yield turbid plaques when cultured at 37°C.

Each of these phage genomes appears to encode an integrase and excisionase, and we have recovered putative lysogens from Naiad, Krishelle and Applecloud. Preliminary immunity testing suggests that these phages may not all be homoimmune despite their genetic similarity. Host range testing revealed that these phages do not multiply in *M. smegmatis*, even though they share significant sequence identity with cluster A Mycobacteriophages. However, they are able to lyse *Corynebacterium xerosis* at a titer similar to that seen in *R. erythropolis*.

Additional research projects include analysis of phage structural proteins by SDS-PAGE and characterization of the repeat sequences observed in these genomes. We also are trying to optimize the phage isolation procedures, based on information provided by other SEA-PHAGES schools participating in the *Rhodococcus* phage pilot, including a comparison of different culture media and $CaCl^2$ concentrations. PCR primers are being designed that will identify phages in the RER2-like group. Results of these experiments are still pending, but we anticipate that they will yield information that will enhance our ability to isolate *Rhodococcus* phage, and contribute to our understanding of the biology of this new group of phage.

notes

Poster 71

OutPHAGEous Discoveries*

Kaivalya Dandamundi, Shelby Edling, Alina Afzal, Nasser Alali, Robert Allison, Rahaf AlQahtani, Demetrius Carter, Erin Cochran, Jordan Davis, Ryan Duong, Samantha Foltz, Sailasya Gundlapudi, Livia Horton, Damien Islek, Deeksha Jain, Monica Jeyasankar, Michael Livingston, Amanda Luong, Tom Matthew, Andrew Miller, Rachel Miller, Herleen Mokha, Natalia Olszewski, Bharath Peddibhotla, Aarthi Prakash, Lucas Rizkalla, Christopher Rowe, Neha Sehgal, Yash Singh, Morgan Van Driest, Emma Weber, Erik Wolfsohn, Kristen Wade, Allison Johnson

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Over the course of the year, our class discovered 20 bacteriophages infecting *Bacillus thuringiensis* subspecies *Kurstaki*. These phages were purified and tested for their ability to infect and lyse (host range) 16 different strains of *Bacillus* bacteria. We observed a broad spectrum of host range, with some phages infecting only a few hosts and other phages infecting many host bacteria. We sequenced the genomic DNA of six of these novel phages. The genes in each of the genomes were annotated to compare and contrast genome features. We used dot plot and average nucleotide identity analysis to form two groups of myovirus phages in our collection. We completed comparative genomics projects examining both small and large scale genome characteristics. We explored genome features related to host range looking for the long tail fiber and receptor binding proteins. We examined the unique location of endolysin and holin in these genomes compared to a canonical lytic cassette, confirmed each predicted holing has three transmembrane domains, and examined potential promoter elements to explore regulation of expression of these proteins. A DNA Polymerase containing a Bastille-like HNH endonuclease in some phages, and an unrelated HNH endonuclease in other phages highlights a unique recombination event in these phages. Other topics to better understand genomic diversity include analyzing promotor sequences and sigma factor proteins to understand regulation of phage gene expression, and comparison of tape measure proteins. Finally, we've characterized one podovirus low sequence similarity to any published phages. Combined, our results show these phages represent a dynamic and and diverse collection of *Bacillus* phages.

Poster 72

Isolation and Analysis of Cluster O Phages Mori and Vorrps and Cluster C Phage Clarice

M. Perumattam

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Through the Phage Hunters program at Washington University in St. Louis, approximately 120 mycobacteriophages have been isolated, sequenced, and annotated over the last seven years. This year, five new mycobacteriophages have been analyzed: Clarice, Enkatz, Mori, Phlavio, and Vorrps. Four of the phages were isolated from soil samples collected in the St. Louis area. Clarice was isolated from a soil sample collected in front of a restaurant in O'Fallon, Illinois, which is an area that has not often been used as a sampling site for Washington University's phage hunting initiative. Clarice has an isometric head and a short, stubby tail, which is characteristic of a Cluster C phage. Vincent, another phage that was isolated but not annotated this year, is also a member of the Cluster C group. Only three other Cluster C phages have been isolated by Washington University. They also produce small, clear plaques, suggesting a lytic phage. Phages Mori and Vorrps have very distinct prolate heads, indicating that they are members of the unique Cluster O group. These phages are the first Cluster O phages that have been isolated at Washington University. Both phages were collected on the Washington University campus, but from different locations. They have a 99% sequence similarity, and have the same number and pattern of unique seventeen base pair repeat sequences. The Clarice genome is 154,450 base pairs in length. Overall, 232 genes, all transcribed in the forward direction, were annotated through bioinformatics analysis programs such as DNA Master, BLAST, Phamerator, and GBrowse. The function of 32 of the genes was determined through comparative analysis with other phage proteins. Phamerator was used to generate a map of predicted gene calls, and provided comparisons to two other highly similar phages as well. Detailed analysis of particularly interesting observations will be presented. Some examples include tmRNA comparisons, investigating features of the tapemeasure proteins, the LysM protein, the histidine triad domain, and phosphoesterases. Further analysis will be necessary to understand the evolutionary history of these novel phages.

Poster 73

The Genome Discovery and Exploration Program at Western Kentucky University isolated, purified, and characterized mycobacteriophages Badger and TheloniousMonk

M. Ronkainen

Millicent M Ronkainen, Whitney N Heard, Austin C Beaver, Nolan S Calhoun, Melissa Y Camargo, Lincoln T Chasteen, Maritza V Chavez, Maycon A DeCesare, Rohan R Deshpande, Jenna N Ellis, Linyue Fan, Olivia M Gearner, Sarah E Herricks, Dustie L Hoffman, Esther C Huggins, Kimberly Ibisate, Emily L Keeter, Malia L Latimer, Maria D Long, Kayla M McKnight, Craig A Meers, Emily M Noel, Eric D Parker, Leslie A Pike, Nathaniel A Powers, Kayleigh M Profumo, Elizabeth A Pulsifer, Eura E Shin, Eayvon B Smith, Stefan M Stryker, James M Styers, Jeremiah L Wayne, Amanda J White, Landon E Wolford, Rodney A King, Naomi Rowland, Claire A Rinehart

Western Kentucky University, Bowling Green KY

Badger, a *siphoviridae* phage isolated from a soil sample taken in Bowling Green, Kentucky, is a member of the A4 subcluster and exhibits homology to Mycobacteriophages Achebe and Wile. Badger has an average capsid diameter of 55.2 nm and an average tail length of 182.5 nm, and produces bullseye plaques measuring approximately 1-3 mm in diameter. Badger's genome is 51,274 bp long and includes a 10 bp 3' overhang with the sequence (CGGCCGGTTA). It is predicted to have 86 genes.

TheloniousMonk, a *siphoviridae* phage isolated from a water sample taken from the Barren River in Bowling Green, Kentucky, is a member of the A1 subcluster and is similar to mycobacteriophages Museum and PattyP. TheloniousMonk has an average capsid diameter of 46.7 nm and an average tail length of 132.3 nm, and produces plaques of varied morphologies. The most common morphology consisted of a turbid plaques with indistinct boundaries encompassing smaller halo plaques. TheloniousMonk's genome is 52,055 bp long and has a 10 bp 3' overhang of (CGGATGGTAA). It is predicted to have 88 genes. Further genomic analysis and characterization of Badger and TheloniousMonk reveals additional evidence supporting mosaicism among mycobacteriophages.

Poster 74

From little Specks to a big genome: the continuing story of bacteriophages from the bayou (sort of)

A. Ahmad

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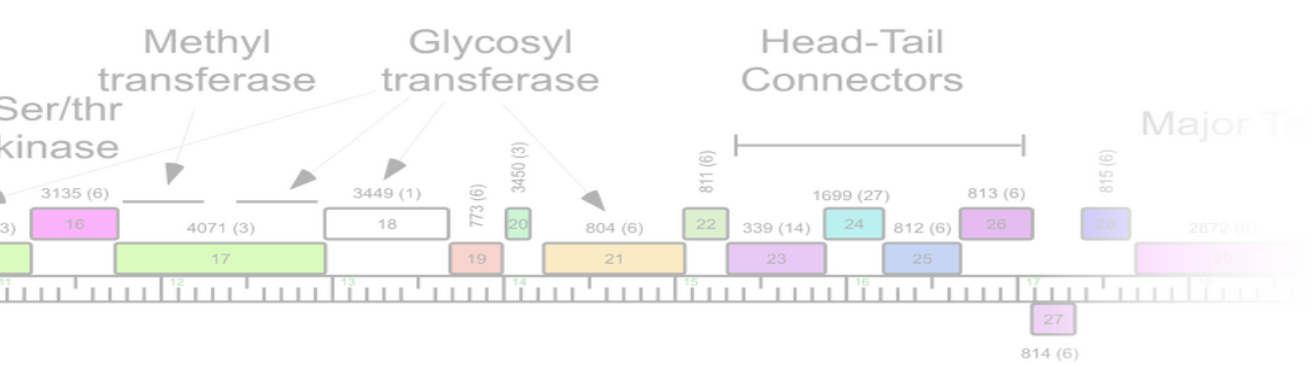
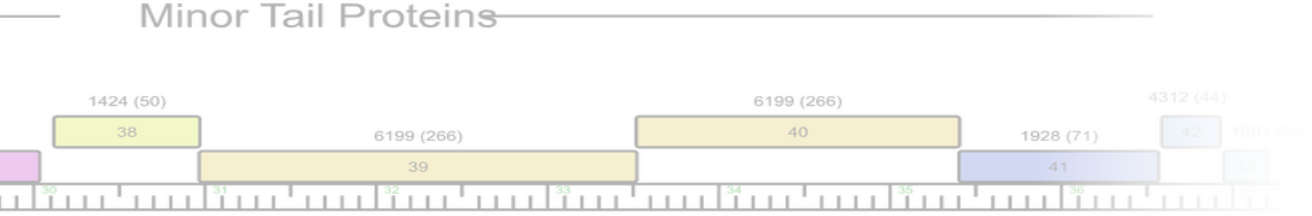
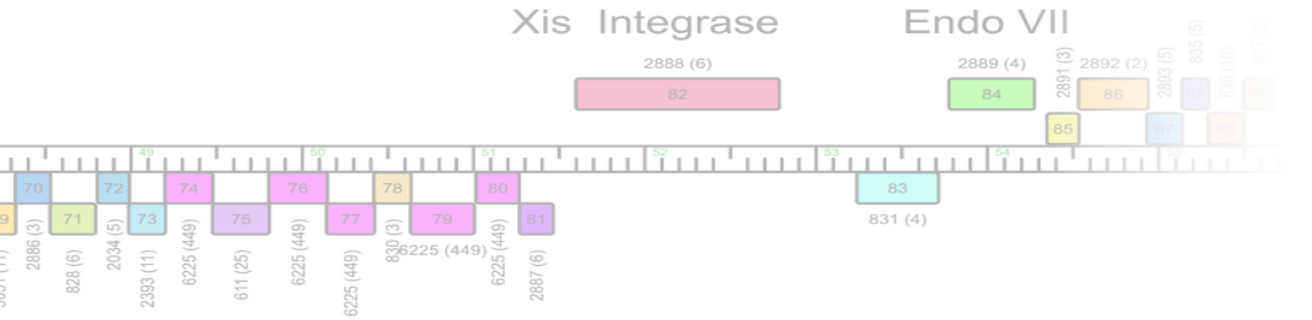
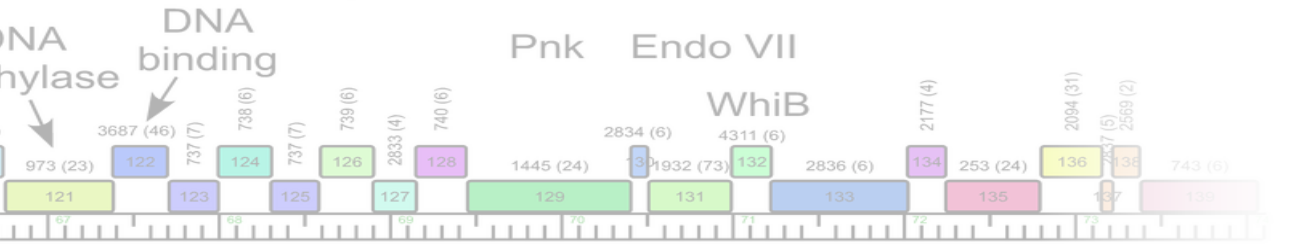
During the past year, students in the SEA-PHAGES program in the Department of Biology, Xavier University of Louisiana have isolated a collection of 12 mycobacteriophages capable of growth on *Mycobacterium smegmatis* mc^2155 (ATCC 700084) as a host.

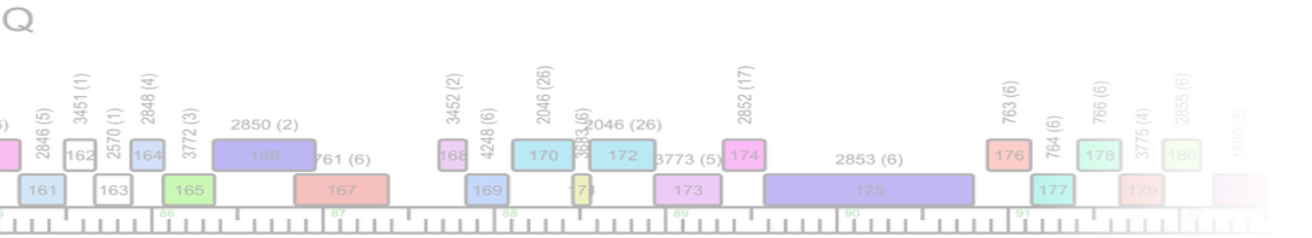
Phages were isolated from a variety of locales in and around the Greater New Orleans, Louisiana area using standard microbiological techniques. When allowed to form plaques in a soft-agar overlay culture with *M. smegmatis* mc^2155 as host, phages displayed a variety of plaque sizes and morphologies. Titers of high titer lysates varied from roughly 10^8 to 10^{14} plaque-forming units per milliliter. One phage, Specks, was selected for sequencing based in part on the purity and quantity of its DNA. The Specks genome is somewhat more than 156,000 bp in length, with circularly permuted ends. BLASTn analysis reveals considerable nucleotide homology with the genomes of other known mycobacteriophages, including Catera, Teardrop (a Xavier phage from last year), TinyTim, Lysocool, Astraea and Zygotaiga. These homologies support assignment of Specks to the C cluster and specifically the C1 subcluster. BLASTn data along with genome size and structure also support its assignment to the family *Myoviridae*. Similarly, BLASTp analysis reveals a number of amino acid sequence homologies and putative functions that support this assignment and in general comport well with the maps of well-characterized phages. DNA Master autoannotation employing Glimmer and GeneMark calls about 240 protein-encoding genes. Analysis with Aragorn via the World Wide Web, external to the DNAMaster environment, calls 34 tRNA-encoding genes. Most of these tRNA calls are located in a single cluster not far from the center of the genome, from about 94,700 bp to about 102,200 bp as measured from the left-hand end. Included are calls for genes encoding two noncanonical tRNAs, one for selenocysteine and one for pyrrolysine. External Aragorn also calls a tmRNA gene. All these calls are uncertain, however, as they are not corroborated by corresponding calls in tRNAscan-SE. tRNAscan-SE calls neither the tmRNA nor the selenocysteinyl-tRNA; and calls the pyrrolysyl tRNA as a suppressor tRNA.

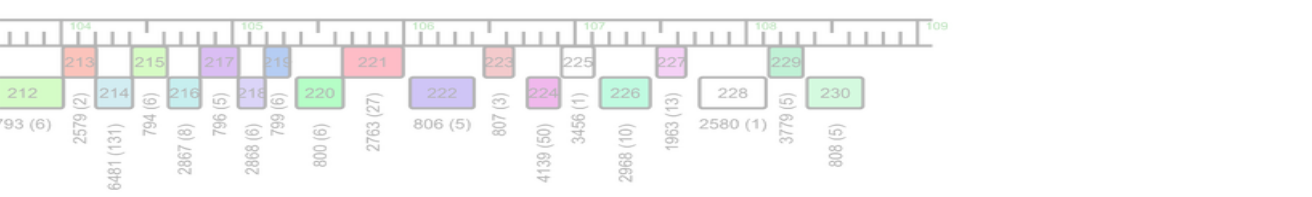












Poster F1

Investigating the Relationships of Bacteriophages with a Class Reveals Obvious Boarders Between Bacterial Orders

J. Grose

Julianne H Grose, Ruchira Sharma

Brigham Young University, Provo UT

With an estimated greater than 10^{31} bacteriophages in the earths biosphere, and only a small fraction studied, our understanding their diversity and evolutionary relationships is just beginning to emerge. The ability of bacteriophages to rapidly evolve through genetic mutation or exchange with their hosts and with one another adds complexity to discerning their evolutionary origins. In an attempt to understand the natural boundaries for such genetic exchange, 337 bacteriophages that infect the Enterobacteriaceae Order of bacteria (18 genera and 31 species) were recently analyzed. This analysis revealed 56 diverse clusters of related phages (32 lytic and 54 temperate). Although little correlation between the bacteriophage host and its cluster assignment was seen, most bacteriophages naturally divided into subclusters with a 78% correlation with their host genera. The fact that so few clusters correlated with the bacterial host lead us to investigate whether clusters correlated with bacterial orders rather than genera. Bacteriophages that infect the Pseudomonadales, another order within the Gammaproteobacteria class, were then compared with those that infect the Enterobacteriaceae. The correlation with cluster when comparing these different orders was remarkable, at greater than 90% for many clusters. These results suggest that although a bacterial Genus may not describe strong boundaries for genetic exchange, a bacterial order may.

notes

Poster F2

Phamerator Database Manager: A web-based application for building customized Phamerator databases

R. DeJong

Randall J DeJong, James G Lamine, John T Wertz, Serita M Nelesen

Calvin College, Grand Rapids MI

The number of phages sequenced and analyzed by SEA-PHAGES students and faculty continues to grow each year. In addition, unique sampling environments and an expanding list of bacterial hosts continue to diversify the types of phages sequenced. This has resulted in a need for individual faculty to create, manage, and provide to their students unique Phamerator databases tailored to their sequence data and research questions. Constructing databases in Phamerator currently requires comfort with the Unix command line, the completion of a number of sequential steps, learning which error messages to respond to and which to ignore, and investment of faculty time. Furthermore, at the time that this project was begun, Phamerator used CLUSTAL and BLASTP to compute pairwise alignments and assemble related proteins into phamilies, a process that took many hours to construct a database of very modest size (10^{-30} phages). Troubleshooting sometimes required communication between SEA-PHAGES staff and the faculty member. As a senior project, a computer science major designed, coded, and tested an application for creating such databases. The application is web-based and allows the user to upload genbank-format files, which are then checked for proper elements and format. The user can deselect uploaded phages or add additional phages before creating a database. The application incorporates the new 'kClust' algorithm which allows modestly-sized databases to be computed and constructed in minutes (kCLust is also now incorporated into the existing Phamerator). After database construction, users of the app can remove or add phages and reconstruct the database. Instructions for pointing Phamerator to the correct server and database are displayed for the user to easily copy and paste into Phamerator preferences.

Poster F3

The SEA-PHAGES Program at Del Mar College: Indisputable Student Outcomes and Assessment Tool Development


D. Overath

R. Deborah Overath, Daiyuan Zhang, J. Robert Hatherill

Del Mar College, Corpus Christi TX

While self-reporting surveys of attitudinal changes and motivation of STEM students has its place in understanding the benefits of SEA-PHAGES and other course-based undergraduate research programs, we have focused on the tangible outcomes that our students experience by participation in this program. At Del Mar College, we implement the program with a section of our introductory biology for majors linked with our introductory biotech course each semester and taught in tandem. In the spring, the lab sections of the courses meet in joint session for much of the semester for annotation sessions. Students are required to present their annotation results at a regional meeting or at our annual Student Research Day. This program affords our students experiences that are not possibility in a traditional bioscience course. For example, annotation of novel phages leads to GenBank submissions. SEA-PHAGES student researchers have also published articles and abstracts, won poster/presentation awards, received offers of employment (before they graduated), been recruited into other schools, and successfully competed for and procured scholarships. In addition, these students have greater participation in campus activities (e.g., Student Research Day) and integration into the culture and profession of the scientific discipline from presenting at scientific meetings. Another benefit of the SEA-PHAGES program is that it incorporates a competency-based testing of laboratory techniques by its nature. For example, students that do not use proper aseptic technique will experience contamination and will have to repeat the experiment. The project team has observed that these students are able to grasp laboratory techniques faster and work more independently. We have also observed SEA-PHAGES students have superior skills, knowledge, and technical competencies compared to traditionally taught students. In addition, both the faculty and student assistants have noted an elevated level of student excitement and engagement compared to the traditionally taught science labs. In the long-term, the project team anticipates that our implementation of the SEA-PHAGES program will engage students and faculty in a common experiment, building a student/faculty community that has shared laboratory resources and expertise. The SEA-PHAGES research-mentoring program, therefore, is providing student outcomes that simply cannot result from traditionally taught freshman science classes.

Poster F4

Using the SEA-PHAGE Program in an Interdisciplinary Context


J. Neitzel

James J Neitzel, Alberto Napuli

The Evergreen State College, Olympia WA

The Evergreen State College was giving a mandate to experiment in education, with an emphasis on collaborative interdisciplinary learning. Much of our curricular content is delivered by team-taught multi-quarter programs in which all students and faculty stay together as a learning community for the duration of the program. For two years the SEA-PHAGES program was used with the interdisciplinary program *Introduction to Natural Sciences*. In the 2013-14 academic year this program combined work in introductory biology, introductory chemistry, and algebra based physics, taught by three faculty to 75 students. In the 2014-2015, the program was offered again with introductory chemistry and biology, but with the third faculty member being an additional biologist with a stronger background in ecology and evolutionary biology. This work will describe in more detail how the SEA-PHAGES program is integrated into the work the students are doing in this broadly focused introduction to science.

Specific examples will be provided of ways in which course content related to chemistry, physics, quantitative skills, and reading of scientific literature was integrated and linked to ideas and methods students were using with their SEA-PHAGES work.

Our student mix in the SEA-PHAGES program also differs in that few of the students enrolled in this program are freshman direct from high school. A large number are students who are returning to school after pauses for work, family, or military service. Another significant portion of our students are individuals who are turning to a science based major relatively late in their college career. We hope to provide these groups of students with the educational benefits of this course research experience and will provide reactions from these groups of students to the SEA-PHAGES experience.

In the future we are planning to add a computer scientist as a member of the faculty team to take advantage of the opportunities the bioinformatics work would provide for teaching concepts in computer science.

We have also experimented with methods to stream-line the delivery of sterile supplies to larger numbers of students and to manage the storage and self-management of students' Petri dishes. We will describe some inexpensive solutions we have found for some of these laboratory issues that can arise as program size increase.

Poster F5

Cluster O promoter investigations at Gonzaga and Ouachita Baptist Universities

Nathan S Reyna, Marianne Poxleitner*

Ouachita Baptist University, Arkadelphia AR; *Gonzaga University, Spokane WA

Putative promoters from Cluster O mycobacteriophage were identified through bioinformatic analysis by a multi-university consortium at the Advanced *In Silico* Workshop (Summer 2014). However, since the promotors were only identified *in silico*, there is still a need for biological confirmation. Advanced phage research students at Gonzaga University and Ouachita Baptist University started projects characterizing these Cluster O promoter sequences. Each university used a different plasmid vector and promoter analysis system. Gonzaga students measured promoter activity by driving expression of the red fluorescent protein mCherry in a binary vector. A major advantage of this approach is the ability to validate promotor expression directly in *M. smeg*. Ouachita Baptist students used Golden Gate Assembly and the pClone Red vector. Golden Gate Assembly allows for digestion and ligation to occur as a single step in the same tube. The result is a rapid and cost effective way to validate promoter expression; however analysis is limited to *E.coli*. Overall, (five) cluster O promoters were confirmed in *E.coli* and (three) confirmed in *M. smeg*. This is just one of several multi-school projects that is currently being developed as a result of the phage lab. Other projects include the T-Phage genomics project and the terminators analysis projects (to begin this summer). This project exemplifies the need for scaffolding of research into multiple classes and into independent research projects. Additional it is hope that it will serve as a vehicle to recruit other faculty at smaller institution to participate in similar project with us in the future.

notes

Poster F6

Exploring the Impact of Bacterial Growth on Mycobacteriophage Protein Expression by Mass Spectrometry

K. Clase

Kari Clase, Yi Li

Purdue University, West Lafayette IN

The fundamental knowledge of phage has led to many applications in biotechnology. Phages are engineered to be nano-carriers for drugs and anti-bacterial agents based upon their target specificity and simple virion structure. The limited information of phage-host interactions and expression of phage proteins, however, constrains maximizing their full potential for biotechnology.

The life cycle of a phage begins with attachment to the host cell. After injecting genomic DNA into a bacterium, a phage may further enter either lytic or lysogenic cycle. In lytic cycle, the phage replicates DNA and produces virions by hijacking host machinery, eventually resulting in lysis and the release of virions. The lysogenic cycle is distinct from lytic cycle, as phage DNA is integrated into the bacterial chromosome and replicated during bacterial cell division. Under certain conditions of a lysogenic cycle, the phage DNA can be released from the host chromosome and subsequently enter a lytic cell cycle.

Previously, we reported a novel mass spectrometry (MS) method that facilitated the identification of peptides produced in phage-infected *Mycobacterium smegmatis* (*M. smegmatis*) culture. We conducted further analysis by searching mass spectra in six-reading frames of the phage genomes as reported by Pope *et al*, 2014, and detected many unexpected out-of-frame peptides that were not predicted by the genome annotation (manuscript in preparation). We hypothesize that the peptides we observed were produced in response to specific phases in the growth cycle of the host and highlight the importance of examining the phage-host system in more detail. In order to test this hypothesis, we are currently investigating the impact of bacterial cell cultures from different growth phases (exponential, stationary and death) on phage life cycle and protein expression. We selected phage with and without canonical integrase proteins to infect *M. smegmatis* and harvest at different phases of growth for subsequent protein extraction and analysis by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

Our long-term goal is to identify those proteins that are mediating the lytic and lysogenic cycle and characterize peptide markers that are expressed during key transition points in the life cycle of the phage. Our findings will provide a better understanding of the interactions of the phage-host system over time and contribute towards novel applications in biotechnology.

Poster TA1

Mycobacteriophage Discovery: Potential Applications for Phage Therapy of Mycobacterial Diseases

Fernanda L Alonzo, Gabrielle B Morgan, Chris R Gissendanner, Ann M Findley

University of Louisiana at Monroe, Monroe LA

Nontuberculosis mycobacterial (NTM) infections cause a wide range of debilitating diseases and are increasing in prevalence in the U.S and worldwide. Antibiotic therapies for NTM disease are complex and poorly efficacious. One potential option for both prevention and treatment of NTM disease is bacteriophage therapy. In the age of extensive antibiotic resistance for many bacterial pathogens, phage therapy is gaining renewed attention as an alternative for the treatment of infectious bacterial diseases. Despite the potential of phage therapy, many factors complicate phage therapy development and use. One of the more important considerations is the availability of a diverse pool of candidate phages that can be tested for virulence and host-range, two factors that influence suitability and potential effectiveness of phage for therapeutic use. Due to extensive sampling using the non-pathogenic *Mycobacterium smegmatis*, it is known that a large and genetically diverse pool of bacteriophage exists in the environment that are capable of infecting *mycobacterium* species. The genomes of nearly 700 of these phage have been sequenced and can be clustered based on genomic sequence similarity. The bacteriophage discovery group at ULM is now exploring the use mycobacteriophage as potential phage therapy for NTM. Specifically, we are investigating the potential of the A3 subcluster of mycobacteriophage. A3 is one of three subclusters, out of 39 distinct clusters/subclusters, that have the ability to infect a broad host-range of pathogenic mycobacterial species. We have identified genetic characteristics of the A3 phage that further advance this group of phage as therapeutic candidates and we are initiating studies to test the 48 known A3 phage against the major NTM pathogens.

Poster F7

Giving it a GO: enhancing bacteriophage genome annotation and student learning with the Gene Ontology

S. Caruso

I. Erill

Steven Caruso, Ivan Erill

University of Maryland, **Baltimore County,** Baltimore MD

Bacteriophage genomes are notoriously devoid of functional annotations. This limits the interpretability of newly sequenced bacteriophage genomes, as well as the ability to leverage bacteriophage genomes for the automated inference of biological knowledge. The *in silico* phage hunters courses offered by many SEA-PHAGES participating schools provide a unique opportunity to address the lack of gene product annotations by leveraging the enthusiasm of undergraduate researchers characterizing their own bacteriophage genome. To this end, the SEA-PHAGES consortium has established guidelines for functional annotation, but these are self-enforced and often rely on long chains of orthology inference. Furthermore, current guidelines do not provide well-defined standards for some aspects of the functional annotation process, such as naming conventions or the systematic identification of references. The Gene Ontology (GO) is a global community effort to annotate gene products across all domains of life. As such, it provides robust definition of terms describing gene products and their relationships, as well as unique identifiers for gene products, their associated terms and the type of evidence supporting those associations. In addition, the GO provides rigorous protocols for annotation, extensive documentation and a wide array of tools for knowledge inference on annotated terms.

Here we report on a pilot experience to implement GO annotations in an *in silico* phage hunters course. To enable annotations based on orthology assessments by undergraduate curators, we developed a GO Reference based on the SEA-PHAGES guidelines that can be cited as the source for annotation from an ortholog to a bacteriophage gene. To streamline the annotation process, we made use of the GONUTS wiki interface and organized an in-class CACAO annotation competition. Collectively, student teams made over 30 GO annotations, including experiment-backed annotations on several phages and bacteria. This pilot experience has shown that GO annotations provide a suitable means to perform high-quality functional annotation on bacteriophage genomes and that exposing students to the idea of ontologies in biology and related concepts is in itself a valuable outcome of the experience. By leveraging a publicly available and open tool for the annotation, students become plainly aware of the impact of their work in the scientific community. Upholding the standards for GO annotations means that students become intimately familiar with the SEA-PHAGES procedures for functional annotation and must read several research articles to annotate gene products, enhancing their reading skills and their critical understanding of functional orthology across domains of life. We will discuss the perceived benefits of this pilot experience, as well as practical components associated with it, such as the amount of time that should be allotted to it and any other practical considerations the audience may have.

Poster F8

Exploring Bacteriophage Diversity in *Streptomyces*

L. Hughes

Lee E Hughes

University of North Texas, Denton TX

When the SEA-PHAGES Program launched in 2008, the scientific focus was on the isolation of a collection of bacteriophage that were capable of infecting a single host organism, *Mycobacterium smegmatis* mc^{2}155. This effort has grown to involve several thousand students each year and has resulted in almost 6,000 phage isolates. Recently, the program has expanded to additional host organisms, including *Arthrobacter* and *Rhodococcus*. In each case, a single species has been utilized as host.

At the University of North Texas (UNT), we have begun to pursue an alternate strategy within the genus *Streptomyces*. Like the other SEA-PHAGES hosts, streptomycetes are high G+C, gram-positive bacteria in the order Actinomycetales. This genus is extremely diverse, with almost 600 named species, and includes a number of industrially important organisms such as antibiotic-producing strains. In our studies, we have begun to explore the variety of phage types across species in this diverse genus. Rather than focusing on a single host species, we are seeking to develop collections of phage from several different *Streptomyces* species. To date, we have successfully introduced two *Streptomyces* species for use in our undergraduate phage hunting sections, *S. griseus* and *S. venezuelae*. Other host species on which phage have been isolated in the research laboratory are also currently being evaluated for suitability for the classroom, including *S. finlayi, S. toxytricini*, and *S. albus*. To date, approximately 120 phage have been isolated using members of the genus *Streptomyces* as host. The 52 genome sequences available at this time show a great deal of diversity, grouping into 9 clusters and 7 singletons.

There are several reasons why additional institutions should consider adding *Streptomyces* phage hunting to their courses. First, the expected diversity of phage types in the genus promises to be extensive, providing exciting opportunities for phage discovery by participating undergraduates. Second, the procedures for growth of *Streptomyces* cultures are generally no more difficult than the procedures for *Mycobacterium*, although there is a slight learning curve for methods specific to the harvesting of spores for cultures and for obtaining dispersed liquid cultures. In general, the procedures utilized for phage isolation in *Streptomyces* require simple media (Nutrient agar with a media supplement) and do not require the use of top agar, further simplifying the isolation process. As well, an adapted version of the SEA-PHAGES Resource Guide is available for use with *Streptomyces*.

For institutions looking for expanded opportunities for phage discovery, *Streptomyces* species can serve as an exciting option with a growing collection of proven host species from which to choose.

Poster F9

Active Assessment in the SEA-PHAGES Course: A Report from the Faculty Assessment Development and Validation Workshop

David Hanauer, Kristen Butela[1], Kari Clase[2], Bill Davis[3], Sally Dixon[4], David Dunbar[5], Ann Findley[6], Emily Fisher[7], Urszula Golebiewska[8], Julianne Grose[9], Latanya Hammonds-Odie[10], Rob Hatherill[11], Shuba Ireland[12], Allison Johnson[13], Michael Rubin[14], Margaret Saha[15]

University of Pittsburgh, Pittsburgh PA; [1]Seton Hill University, Greensburg PA; [2]Purdue University, West Lafayette IN; [3]Washington State University, Pullman WA; [4]University of Maine, Honors College, Orono ME; [5]Cabrini College, Radnor PA; [6]University of Louisiana at Monroe, Monroe LA; [7]Johns Hopkins University, Baltimore MD; [8]Queensborough Community College, Bayside NY; [9]Brigham Young University, Provo UT; [10]Georgia Gwinnett College, Lawrenceville GA; [11]Del Mar College, Corpus Christi TX; [12]Xavier University of Louisiana, New Orleans LA; [13]Virginia Commonwealth University, Richmond VA; [14]University of Puerto Rico at Cayey, Cayey PR; [15]College of William & Mary, Williamsburg VA

This poster reports on the current assessment development work being conducted by the SEA-PHAGES faculty who participated in the assessment workshop directed by Dr. David Hanauer in March, 2015 at HHMI HQ. At this workshop faculty who had already started the process of in-class assessment tool development were organized into working groups to continue their development and validation of their tools. Active assessment divides relevant inquiry course knowledge into four areas: physical (procedural) laboratory knowledge, cognitive (science content and scientific thinking) knowledge, representational knowledge and presentational knowledge (Hanauer, Hatfull & Jacobs-Sera, 2009). Using this scheme, faculty worked collectively in one of these four areas and assessment tools for potential in-class use across the SEA-PHAGES program are currently being developed and validated. The aim of this faculty development project is to provide faculty with assessment tools that they could choose to use for course and institutional grading and formative assessment purposes.

Poster F10

Host range of *Bacillus* phages

Allison Johnson[1], Lynn Lewis[2], Steven Caruso[3], Ivan Erill[3], Louise Temple[4]

[1]**Virginia Commonwealth University**, Richmond VA; [2]**University of Mary Washington**, Fredericksburg VA; [3]**University of Maryland, Baltimore Count**y, Baltimore MD; [4]**James Madison University**, Harrisonburg VA

The *Bacillus* group of bacteria is important to human health and agriculture due to the different pathogenic profiles of members of this genus. For example, *B.anthracis* is a human pathogen and important in bioterrorism research, while *B. thuringiensis* is an insect pathogen and of economic importance to agriculture. Students from several SEA-PHAGES programs have been isolating and characterizing *Bacillus* phages for several years, primarily using *B. thuringiensis* subspecies *Kurstaki*, but also other *Bacillus* species, as the host bacteria. The host range of a phage is determined by its ability to bind carbohydrates and proteins on the surface of bacteria, leading to a productive infection and bacterial lysis. Last year, we joined forces to do parallel host range testing in each of our phage hunting courses. Combined, our Fall 2014 SEA-PHAGES students tested 127 phages for ability to infect and lyse a common set of *Bacillus* isolates. We profiled phage infection in each of our classes, found both 'narrow' and 'broad' host range phages, and can characterize some of the bacteria species as relatively 'permissive' and 'susceptible'. This knowledge can be used to select host bacteria for future courses, as well as inform our genomics analysis. Together, ~30 phages in this collection were sequenced, including 17 from this year. We are examining the comparative genomics of the tail and baseplate protein region, looking for long tail fiber and receptor binding protein homologs that might be useful for future research defining the phage-bacteria infection interaction, as well as predicting host range of future characterized phages.

notes

sea-phages **phun phind**

Do your best to see how many of the SEA-PHAGES words you can find in this puzzle game.

B T C Z Q E C T V D E B T E R M I N A S E Q P H E
D P O D O V I R I D A E F Z H B J W G J Z F R B A
V F D G H K N A B N E G H L V L E C E T O P O O D
T S O E A V R A L S U L L I C A B I N E A P T J I
S E N G I D Q G Y O W I Y T O Q V X O F I R E F R
U M S M E G M A T I S M T E D B F D M H V O I C I
C O F B V I R U C L W M I C L U S T E R D T N J V
C R Z Q R E D H B W V E C Z T E I Q S J B E H D O
O D E D S E I C E P S R E T C A B O R H T R A N Y
C N V G J Q M D J C E L M Y C O B A C T E R I U M
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D L D N A M A S T E R B Q C L E A U T E U Q A L P
O A C E T C E D W R E R V I L T S R Z A H F R E X
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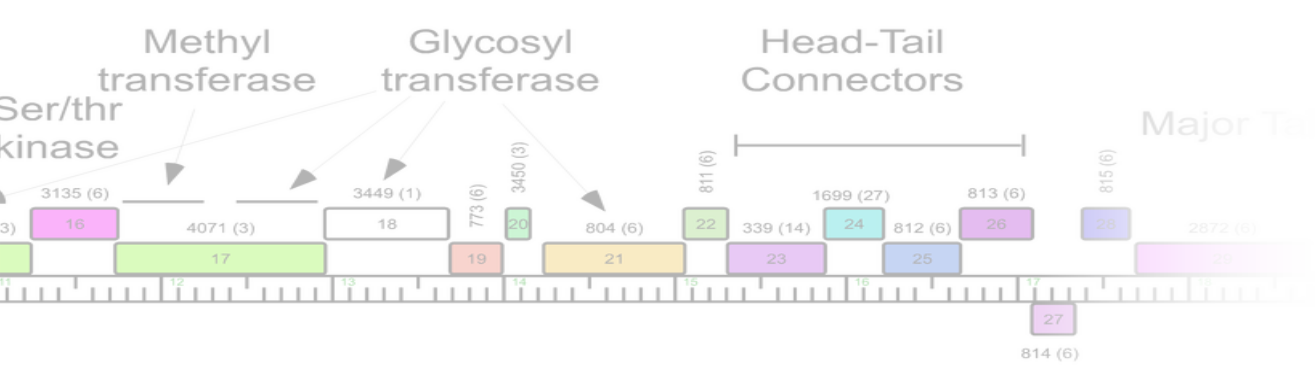
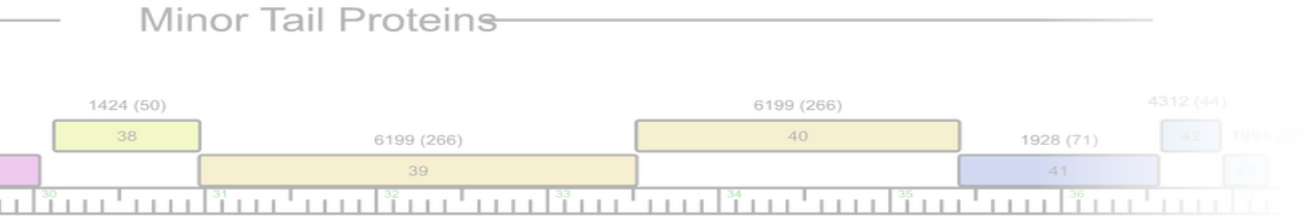
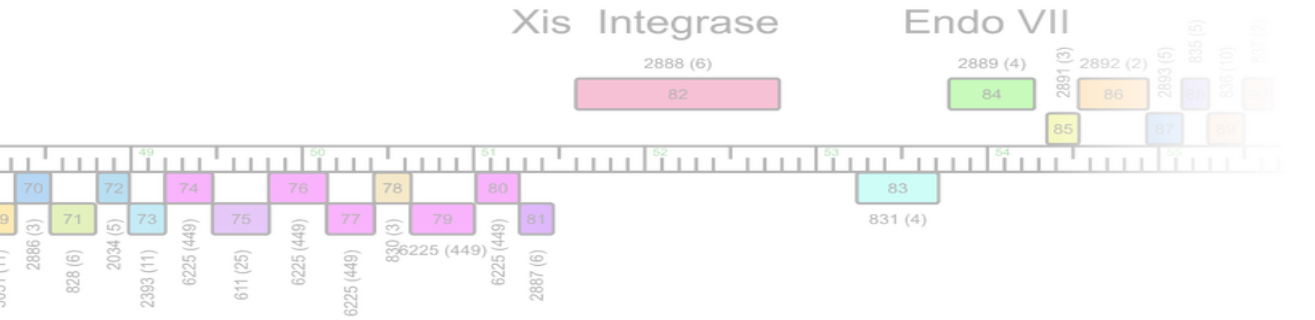
E N T E R O B A C T E R I A C E A E B Z I V R I P
B E E Y P O C S O R C I M N O R T C E L E E E D U
C A M I N O A C I D R D E W C R O N O B A C T E R
E L A P W H E E J L S I M V M P L A Q A A N O S I
C H I Z X T U G R P O L Y M E R A S E S I E M D F
N L D T E M P E R A T E Z E D M P H U E A U O S I
E G A M G L S T X H K O N D I C O D I P M Q R O E
U R A N Y L A C E T A T E W A H G E N A E E P D D
Q L E N R I C H M E T N O I T A L O S I T S G O X
S E Q U C L U S C R D N A U R E S E A R C H L C T

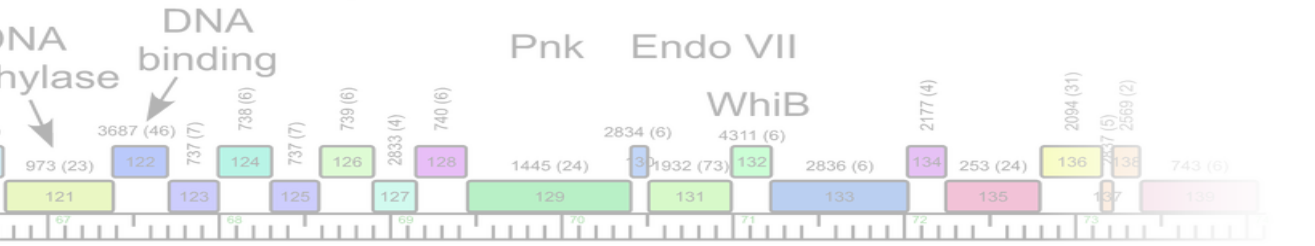
AMINO ACID
ARTHROBACTER SPECIES
BASE PAIR
BLASTP
CELLS
CLUSTER
CODON
CRONOBACTER
DIAMETER
DNA MASTER
DOTPLOT
ELECTRON MICROSCOPY
ENRICHMENT
ENTEROBACTERIACEAE
ENYZYME
GENBANK
GENES
GENOMES
GLIMMER
ISOLATION
LYTIC
M. SMEGMATIS
MEDIA
MYCOBACTERIUM
MYOVIRIDAE
NUCLEOTIDE
PAENIBACILLUS LARVAE
PALINDROMES
PLAQUE
PODOVIRIDAE
POLYMERASE
PROMOTER
PROTEIN
PURIFIED
RESEARCH
RHODOCOCCUS
SEA-PHAGES
SEQUENCE
SOIL
TERMINASE
TEMPERATE
URANYL ACETATE
VIRUS

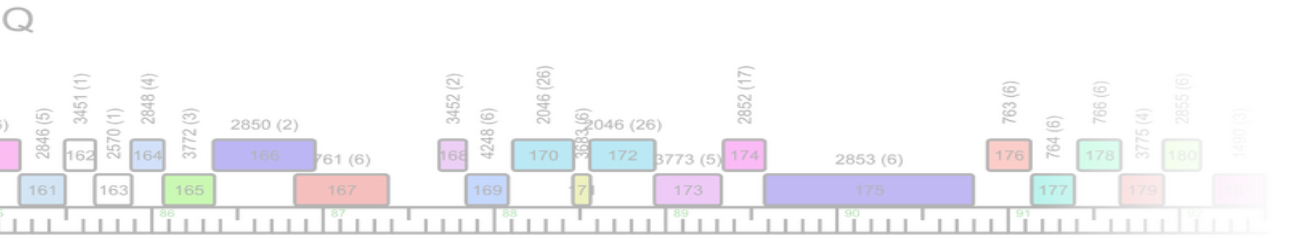
Solution in the back of the program book.

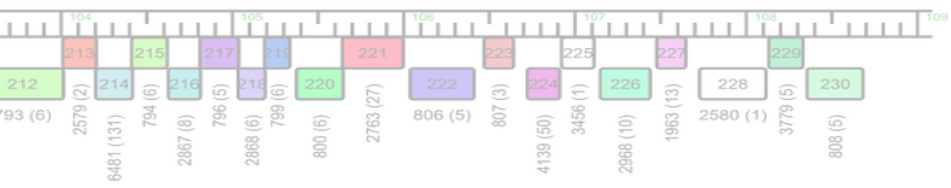












speakers' bios

Eric Betzig, PhD
Group Leader
Janelia Research Campus

After obtaining a BS in Physics from Caltech, Eric Betzig moved to Cornell, where his thesis involved the development of near-field optics -- the first method to break the diffraction barrier in light microscopy. Betzig became a PI at AT&T Bell Labs in Murray Hill, NJ, where he further refined the technology and explored many applications, including high density data storage, semiconductor spectroscopy, and superresolution fluorescence imaging of cells. In 1993, Betzig was the first to image single fluorescent molecules under ambient conditions, and determine their positions to better than 1/40 of the wavelength of light. Tiring of academia, he served as Vice President of R&D at his father's machine tool company, developing a high speed motion control technology based on an electrohydraulic hybrid drive with adaptive control algorithms. The commercial failure of the technology left him unemployed and his search for new directions culminated in the invention and demonstration of the superresolution technique PALM by himself and fellow Bell Labs expatriate, Harald Hess. Since 2005, Betzig have been a Group Leader at Janelia, developing new optical imaging technologies for biology. Betzig shared the 2014 Nobel Prize in Chemistry.

about dr. betzig

Graham F. Hatfull, PhD
Professor of Biological Sciences
Eberly Family Professor of Biotechnology
Howard Hughes Medical Institute Professor
University of Pittsburgh

Dr. Hatfull is Professor of Biological Sciences at the University of Pittsburgh. He received a B.Sc. (Hons) degree in Biological Sciences from Westfield College, University of London in 1978, and a Ph.D. in Molecular Biology from Edinburgh University in 1981. He did postdoctoral work at Yale University in the Department of Molecular Biophysics and Biochemistry with Dr. Nigel Grindley, and at the Medical Research Council at Cambridge University, with Drs. Fred Sanger and Bart Barrell. He has been at the University of Pittsburgh since 1988 and served as Chair of the Department of Biological Sciences from 2003 to 2011.

Dr. Hatfull's research focuses on the molecular genetics of the mycobacteria and their bacteriophages. These studies take advantage of the intimacy of phage-host interactions to gain insights into the genetics and physiology of *Mycobacterium tuberculosis*, the causative agent of human TB. Through integrated research-education programs such as the PHIRE and SEA-PHAGES programs, the large collection of completely sequenced mycobacteriophage genomes provides insights into viral diversity and evolution, and represents a rich toolbox of new approaches to understanding *M. tuberculosis*. Development of vector systems, selectable markers, recombineering approaches, expression tools, and insights into mycobacterial biofilms reflect some of the useful applications of this genomic resource.

Highlights of Dr. Hatfull's research accomplishments include publication of more than 150 peer-reviewed research articles, 36 book chapters or reviews, and four books. He has received funding from the National Institutes of Health since 1989, mentored 20 Ph.D. students, over 100 undergraduate student researchers, and 16 postdoctoral associates. Dr. Hatfull has received the University of Pittsburgh Chancellor's Distinguished Research Award at both the junior and senior level, the University of Pittsburgh Chancellor's Distinguished Teaching Award, the Carski Award (ASM), and holds the Eberly Family Professorship in Biotechnology. He is a fellow of the American Academy of Microbiology, a fellow of the American Association for the Advancement of Science, and a teaching fellow of the National Academy of Science. He has been a Howard Hughes Medical Institute Professor since 2002.

about dr. hatfull

Fill in the blank squares with the numbers 1 through 9. Every row, column and 3x3 region of a 9x9 grid must contain each number only once.

Sudoku 1 (*Level of difficulty = easy)*

8					1		4	2
5								
	9		8	2	3			
		1		3		7		
					8			5
				5	9			
1		6			4	8		
					5		6	7
3	8			7		2		

Sudoku 2 (*medium)*

	6				1		8	3
		3		2				4
	4				5			
	8		7					
							5	8
		7	5			2		
6		2		8				
							3	
1	7			6				

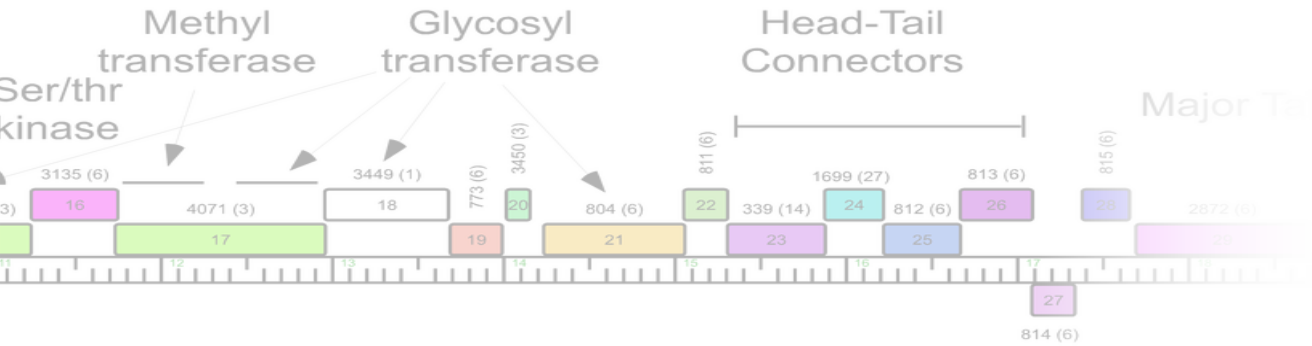
Sudoku 3 (*hard)*

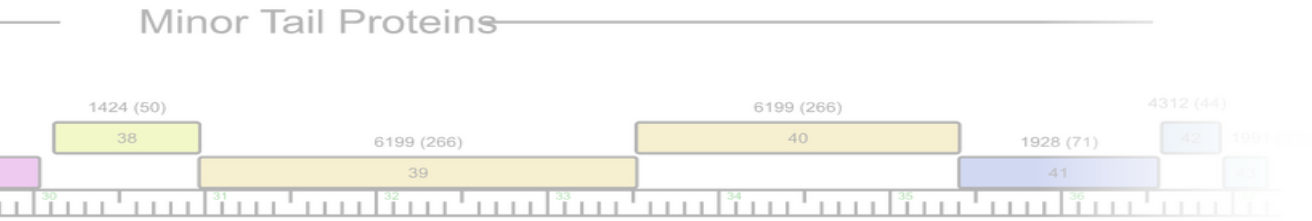
3								
6			1	7	9			
	5							4
1				6		5		
				5		2	9	3
		2			3			7
		6		8				
	9							
			5				6	

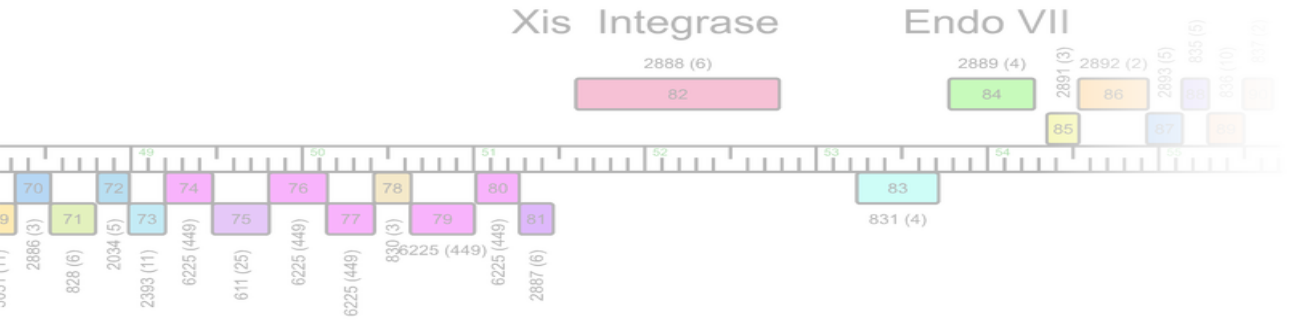
Sudoku 4 (*hard)*

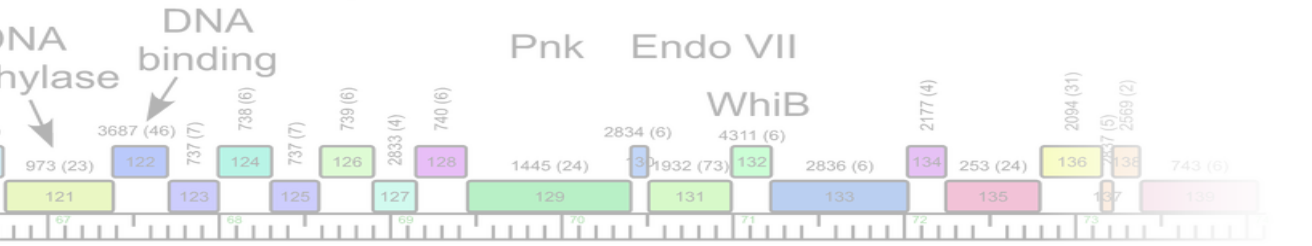
8				2				
	6		7					
	7				4			
			6		3		4	
1								
				1	8	5		3
		1	3			6		
			5			9		4
2		7					1	

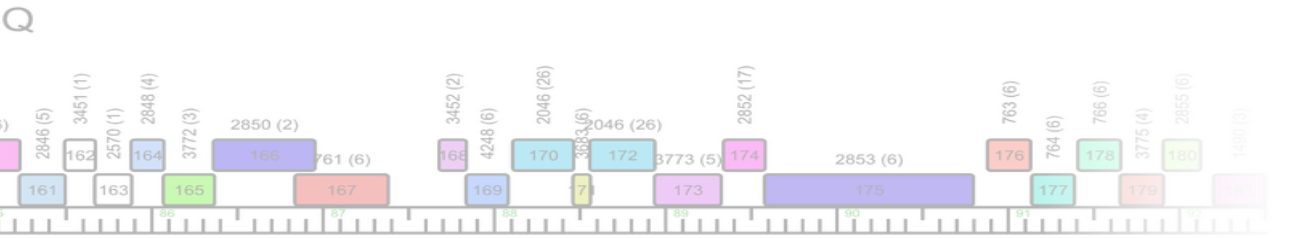
Solutions in the back of the program book.

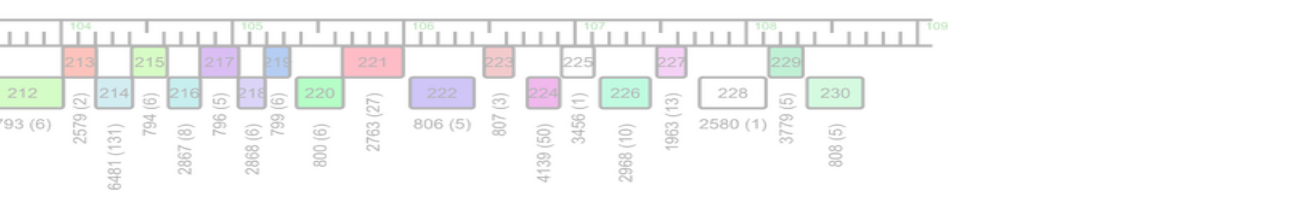












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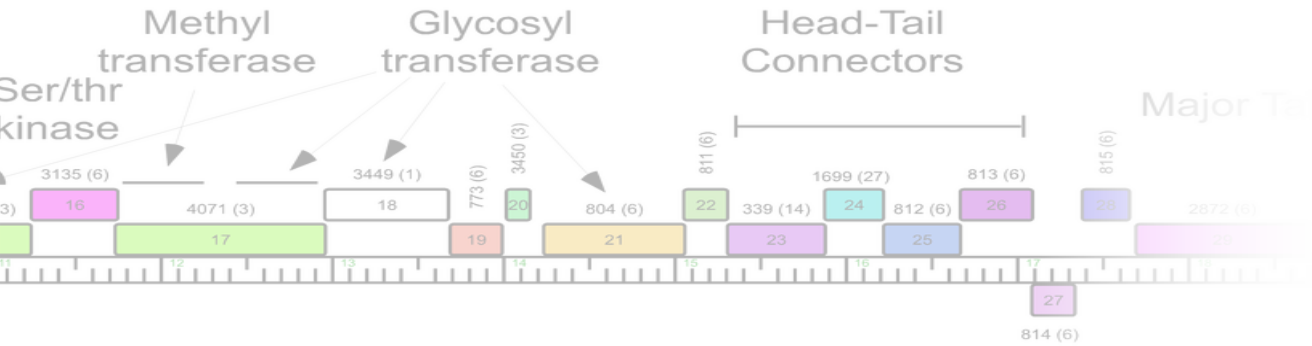
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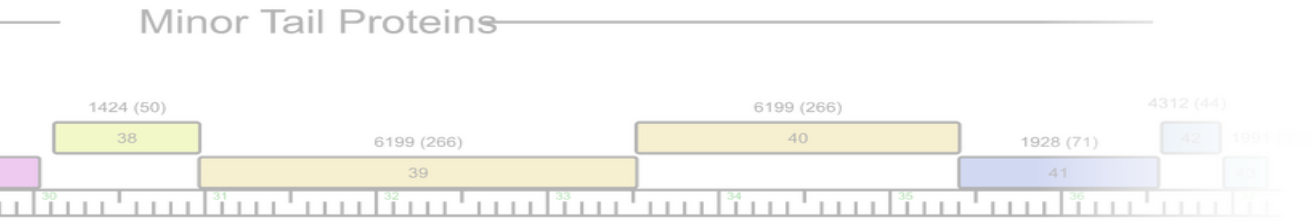
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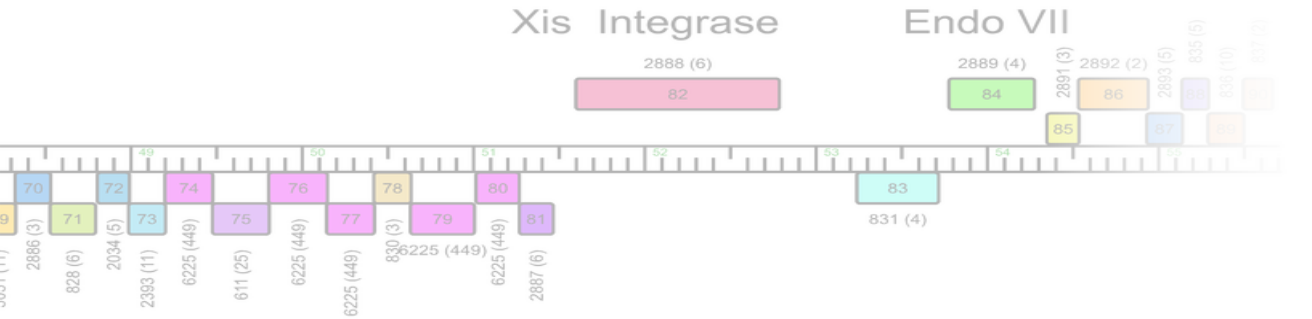
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Integrase
Endo VII
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835 (5)
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84
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2034 (5)
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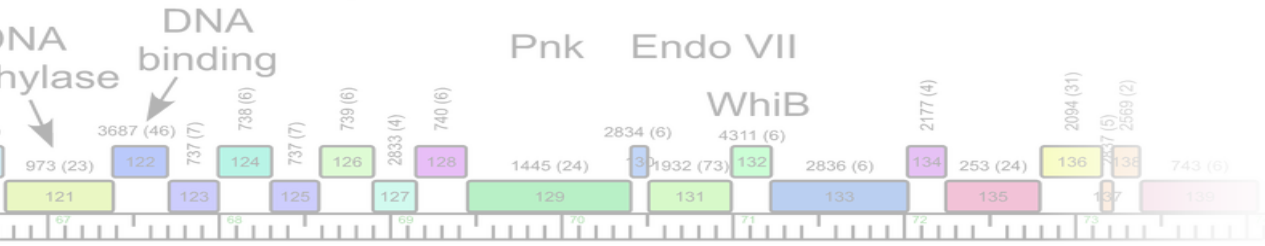
DNA binding
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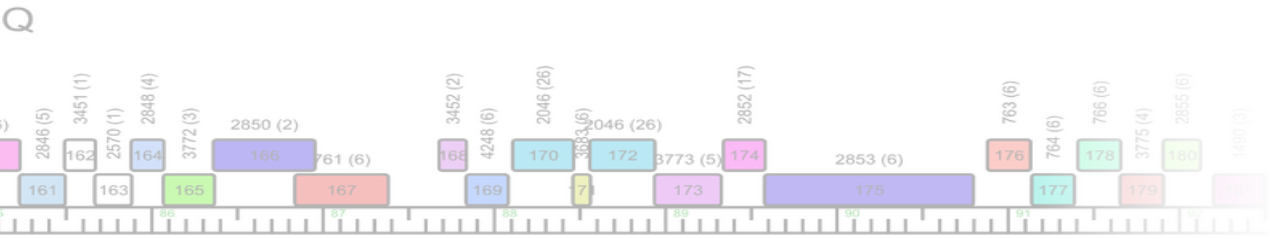
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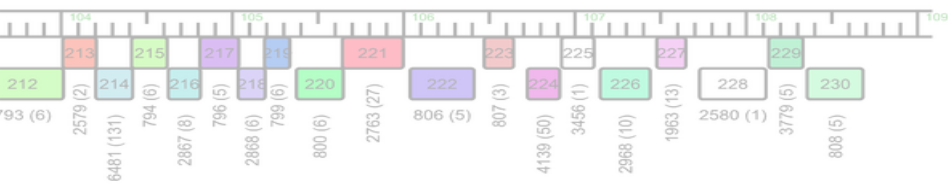
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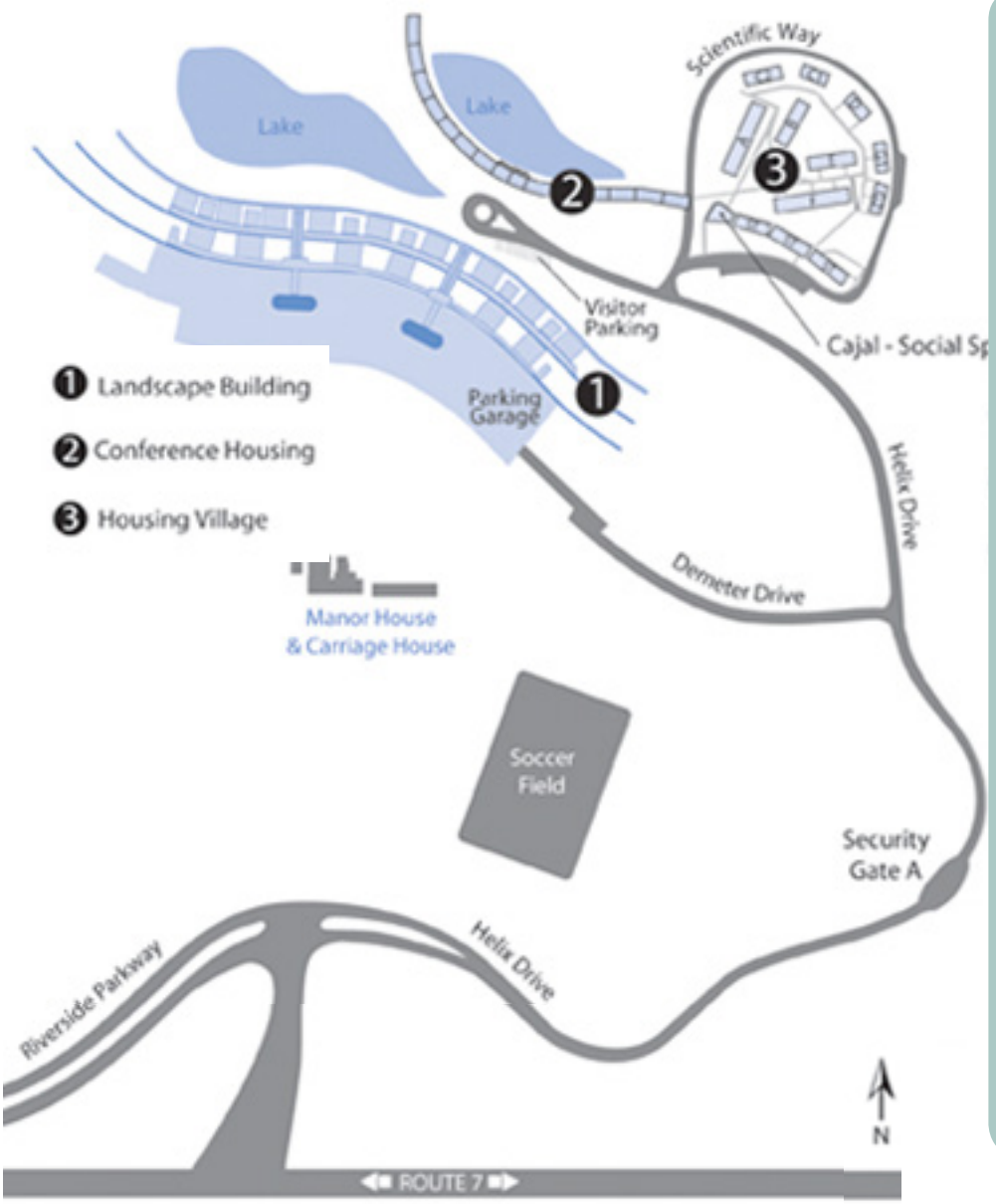
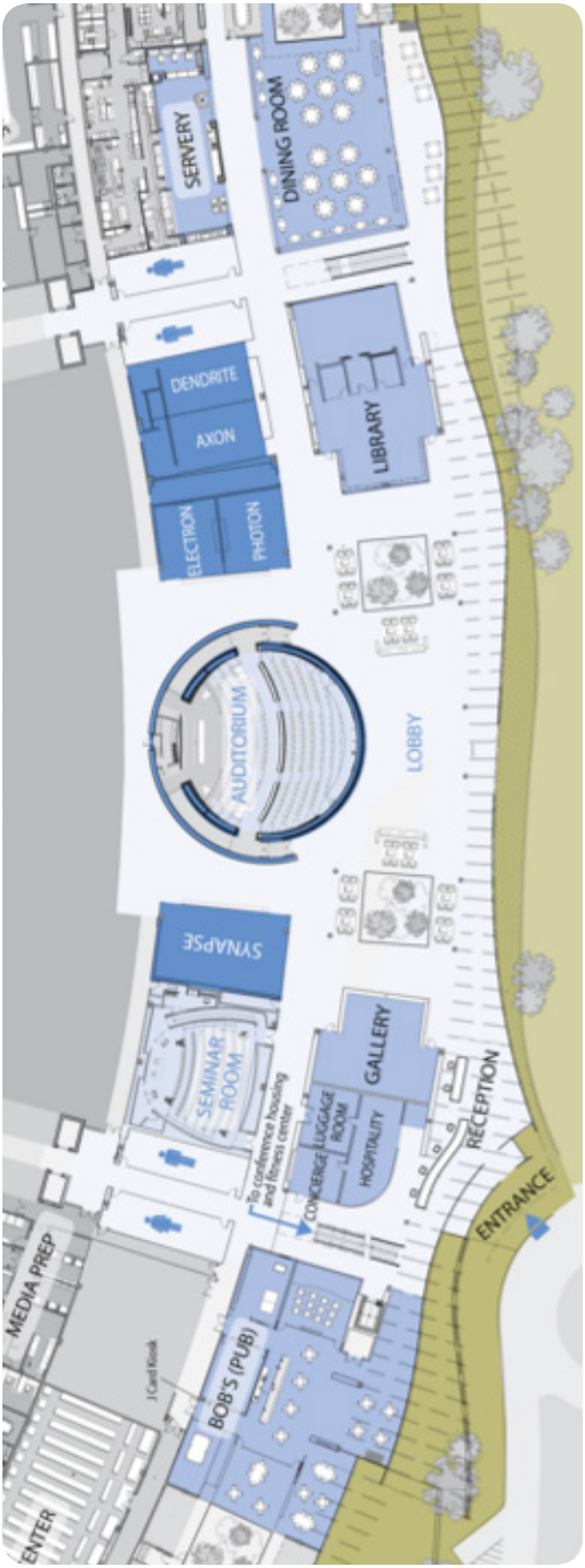
808 (5)
6481 (131)

JRC Landscape Map



The Howard Hughes Medical Institute founded Janelia in 2006 to complement the long-standing HHMI investigator program. The Janelia campus includes 700 acres of woods and meadow on the banks of the Potomac River, in Ashburn, Virginia, 35 miles from Washington D.C. Dulles International Airport is just 15 minutes from the Janelia campus, offering easy access for visiting scientists and conference participants. Today, Janelia includes 50 labs, nine project teams, and additional scientific support staff. In addition to lab heads, our scientists include over 380 researchers, more than 100 postdocs, and roughly 20 graduate students.



JRC First Floor Map

Janelia Research Campus
19700 Helix Drive
Ashburn, VA 20147
571.209.4000
www.janelia.org

Receptionist Desk
Dial "0" from any JRC telephone.

Security
Dial "4040" from any JRC telephone.

Fitness Center
Open 24 hours a day.

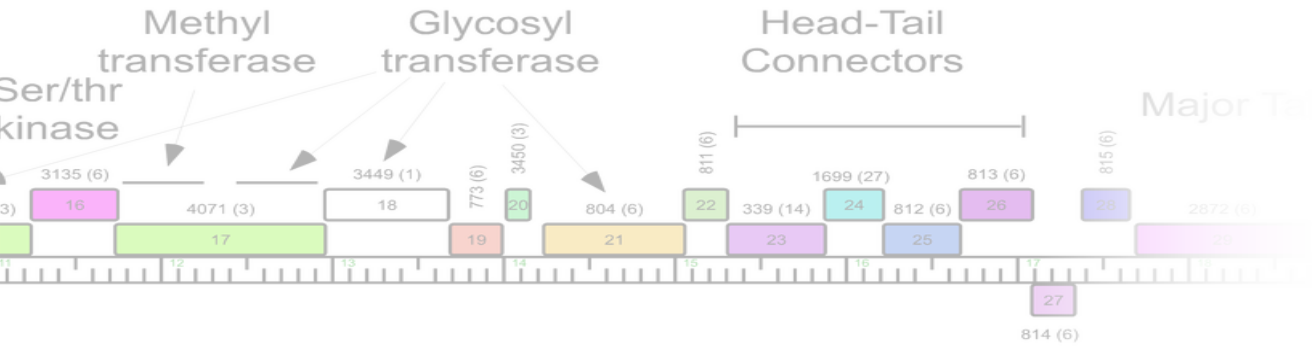
Food and Beverage
Coffee machine is available in the Guest House Lobby at 6:30 am.

Bob's Pub
Monday-Friday
7:00 am - 10:00 pm

Saturday-Sunday
9:00 am - 10:00 pm

Overflow Hotel

DoubleTree by Hilton Hotel Sterling - Dulles Airport
21611 Atlantic Boulevard
Sterling, VA 20166
703.230.0077


Methyl transferase
Glycosyl transferase
Head-Tail Connectors
Ser/thr kinase
Major Tail
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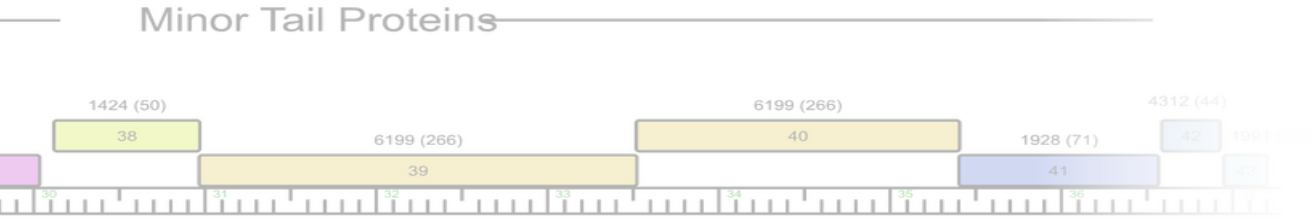
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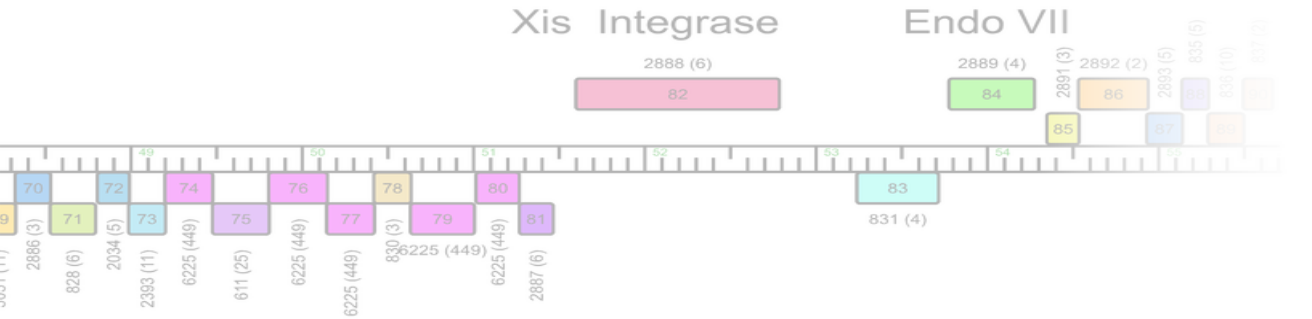
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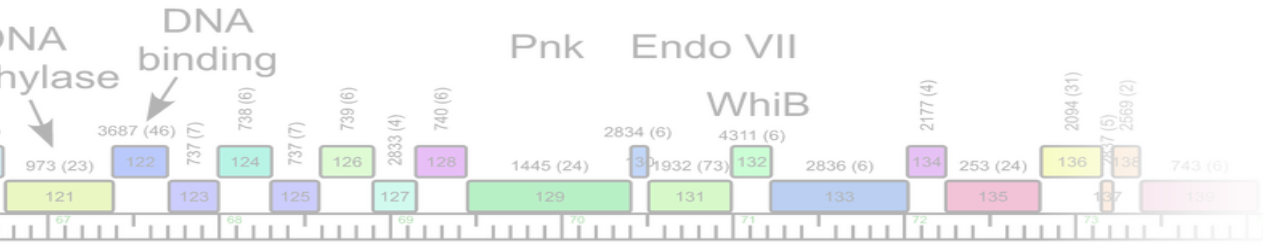
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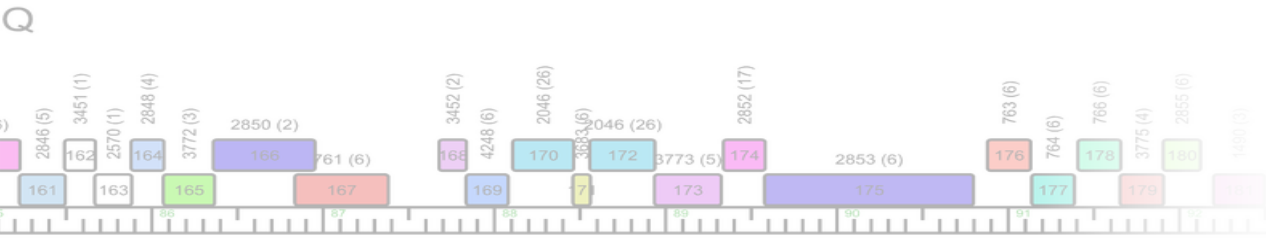
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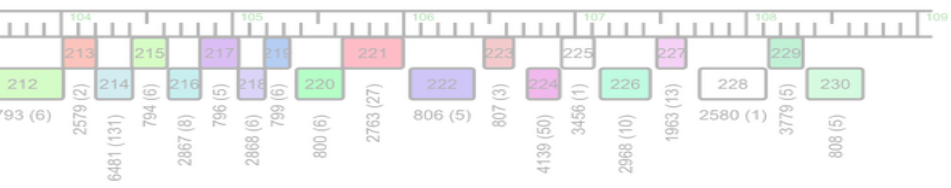
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1963 (13)
2580 (1)
3779 (5)
808 (5)

index

Abstracts, Faculty and Teaching Assistant

(alphabetical by title)

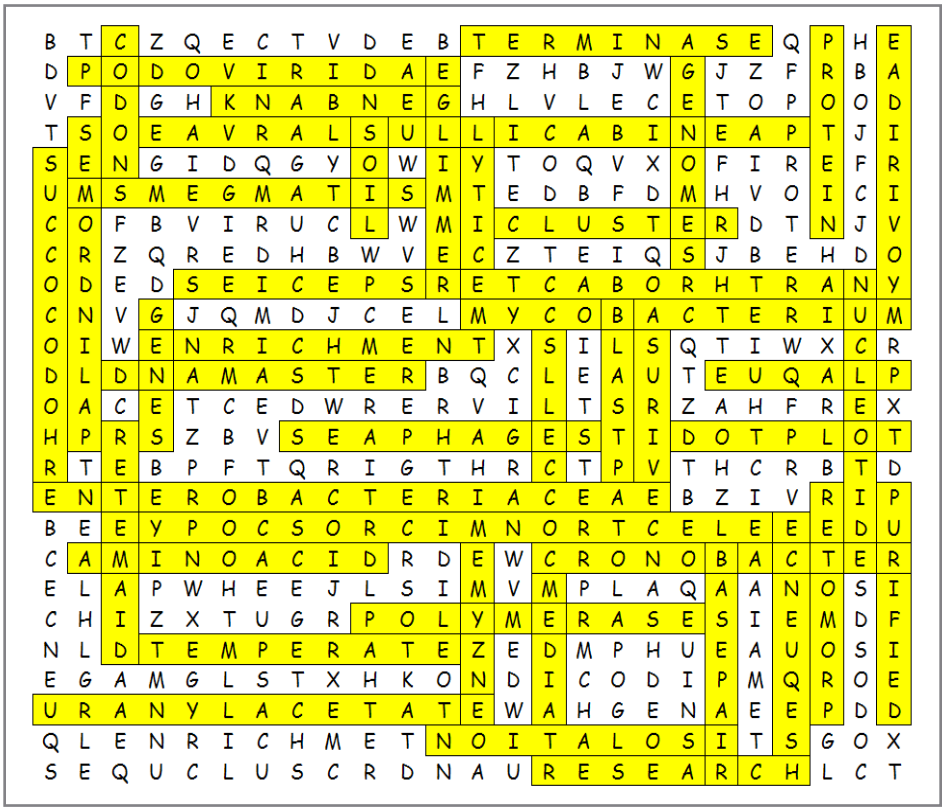
Abstracts, Student *(alphabetical by title)*

**Abstract selected for a talk.*

Presenters, Faculty and Teaching Assistant

Presenters, Students

Solution to the Phun Phind on p. 60.



Solutions to the Sudoku Phun on p. 64.

Sudoku 1 (easy)

8	3	7	5	6	1	9	4	2
5	1	2	9	4	7	6	3	8
6	9	4	8	2	3	5	7	1
4	5	1	6	3	2	7	8	9
9	6	3	7	1	8	4	2	5
7	2	8	4	5	9	3	1	6
1	7	6	2	9	4	8	5	3
2	4	9	3	8	5	1	6	7
3	8	5	1	7	6	2	9	4

Sudoku 2 (medium)

2	6	9	4	7	1	5	8	3
7	5	3	8	2	6	9	1	4
8	4	1	9	3	5	6	2	7
5	8	6	7	9	2	3	4	1
9	2	4	6	1	3	7	5	8
3	1	7	5	4	8	2	6	9
6	3	2	1	8	9	4	7	5
4	9	8	2	5	7	1	3	6
1	7	5	3	6	4	8	9	2

Sudoku 3 (hard)

3	8	1	2	4	5	9	7	6
6	2	4	1	7	9	8	3	5
9	5	7	6	3	8	1	2	4
1	3	9	7	6	2	5	4	8
7	6	8	4	5	1	2	9	3
5	4	2	8	9	3	6	1	7
2	7	6	9	8	4	3	5	1
4	9	5	3	1	6	7	8	2
8	1	3	5	2	7	4	6	9

Sudoku 4 (hard)

8	1	5	9	2	6	4	3	7
4	6	2	7	3	5	1	8	9
3	7	9	1	8	4	2	5	6
5	2	8	6	9	3	7	4	1
1	3	6	4	5	7	8	9	2
7	9	4	2	1	8	5	6	3
9	5	1	3	4	2	6	7	8
6	8	3	5	7	1	9	2	4
2	4	7	8	6	9	3	1	5

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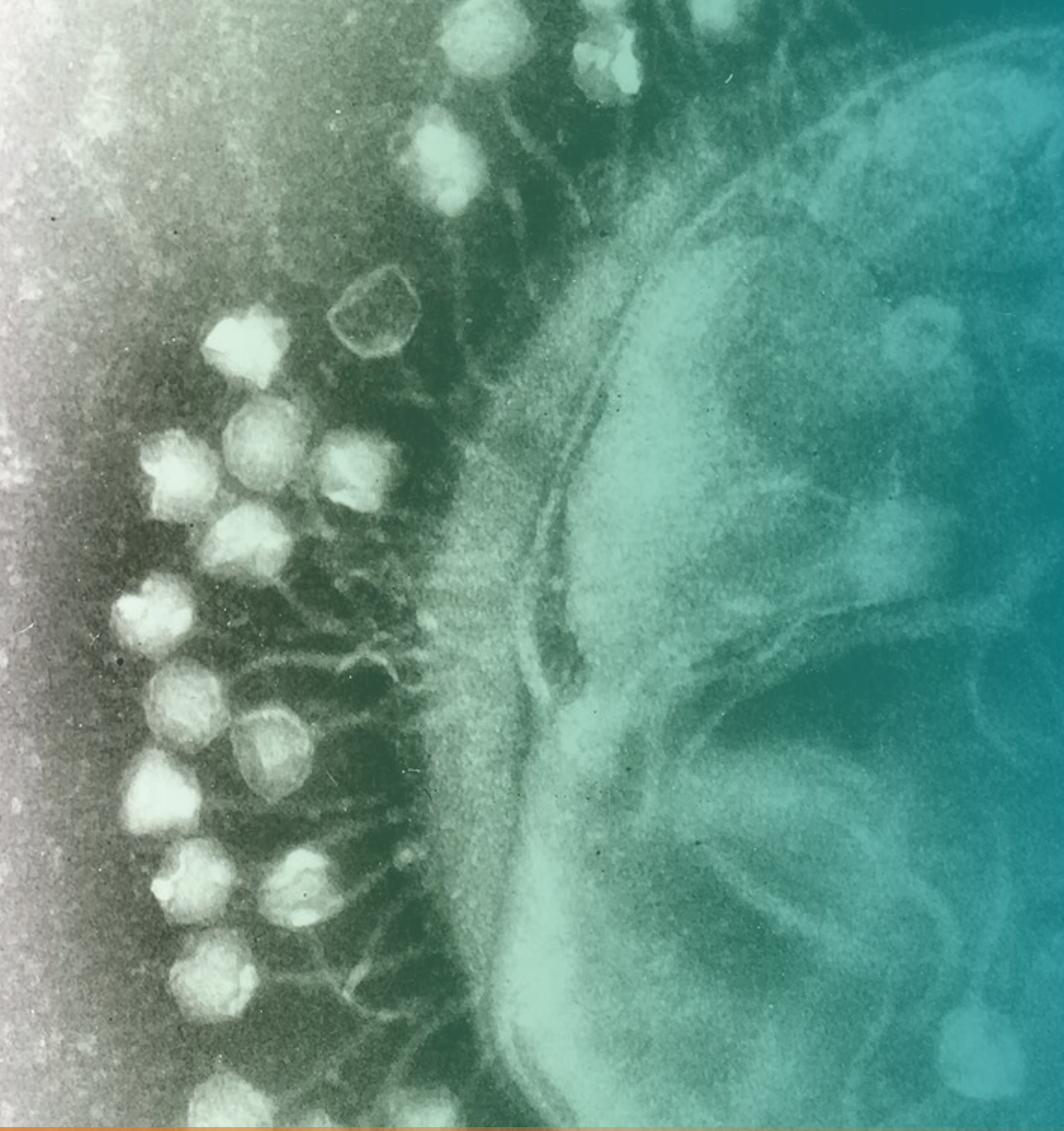
8th Annual SEA-PHAGES Symposium

Ashburn, VA

June 10-12, 2016

Photo Credit: James Kegley



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