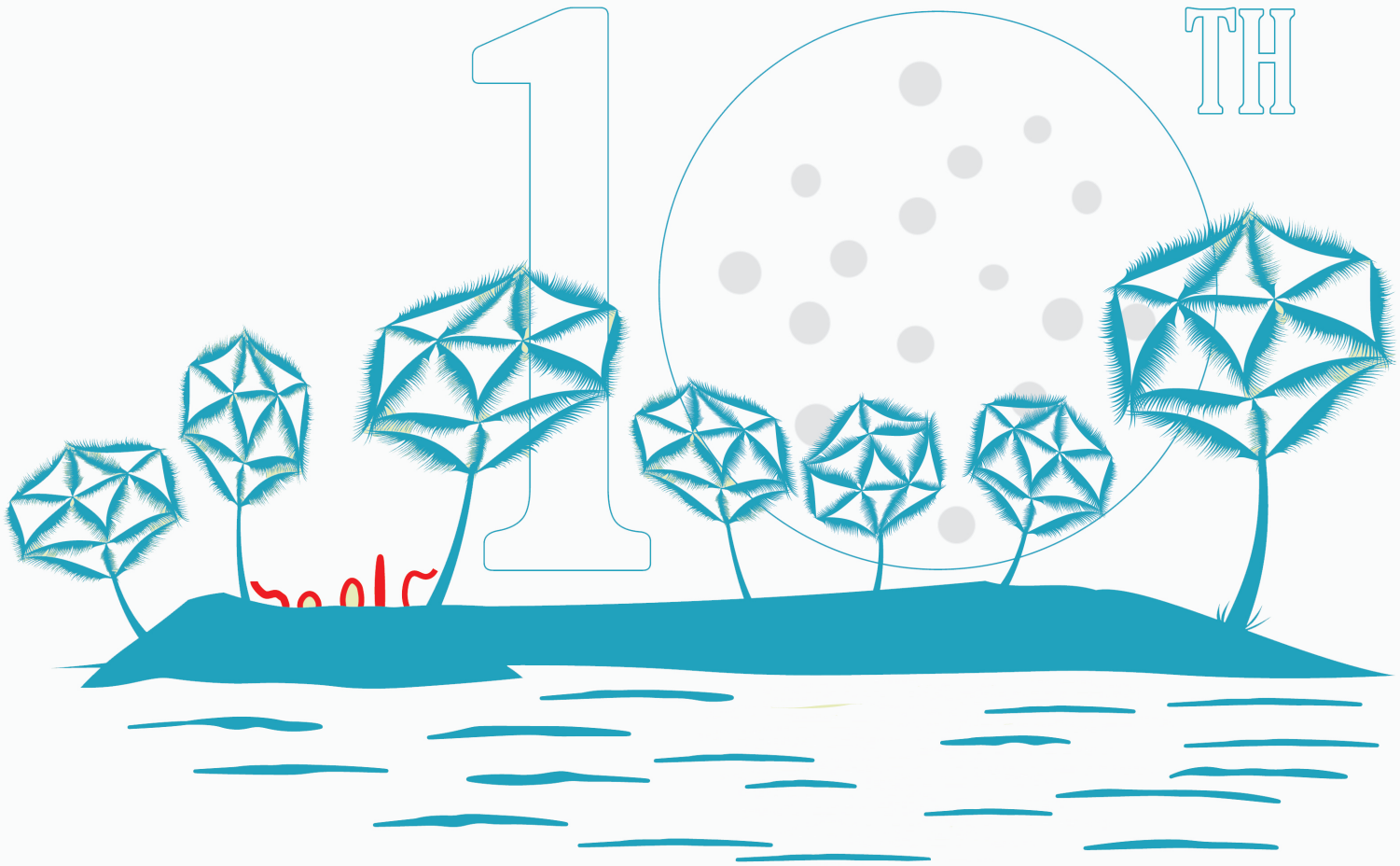
PROGRAM BOOK



SCIENCE EDUCATION ALLIANCE SYMPOSIUM

june 8-10, 2018

hhmi | Howard Hughes Medical Institute

SEA PHAGES

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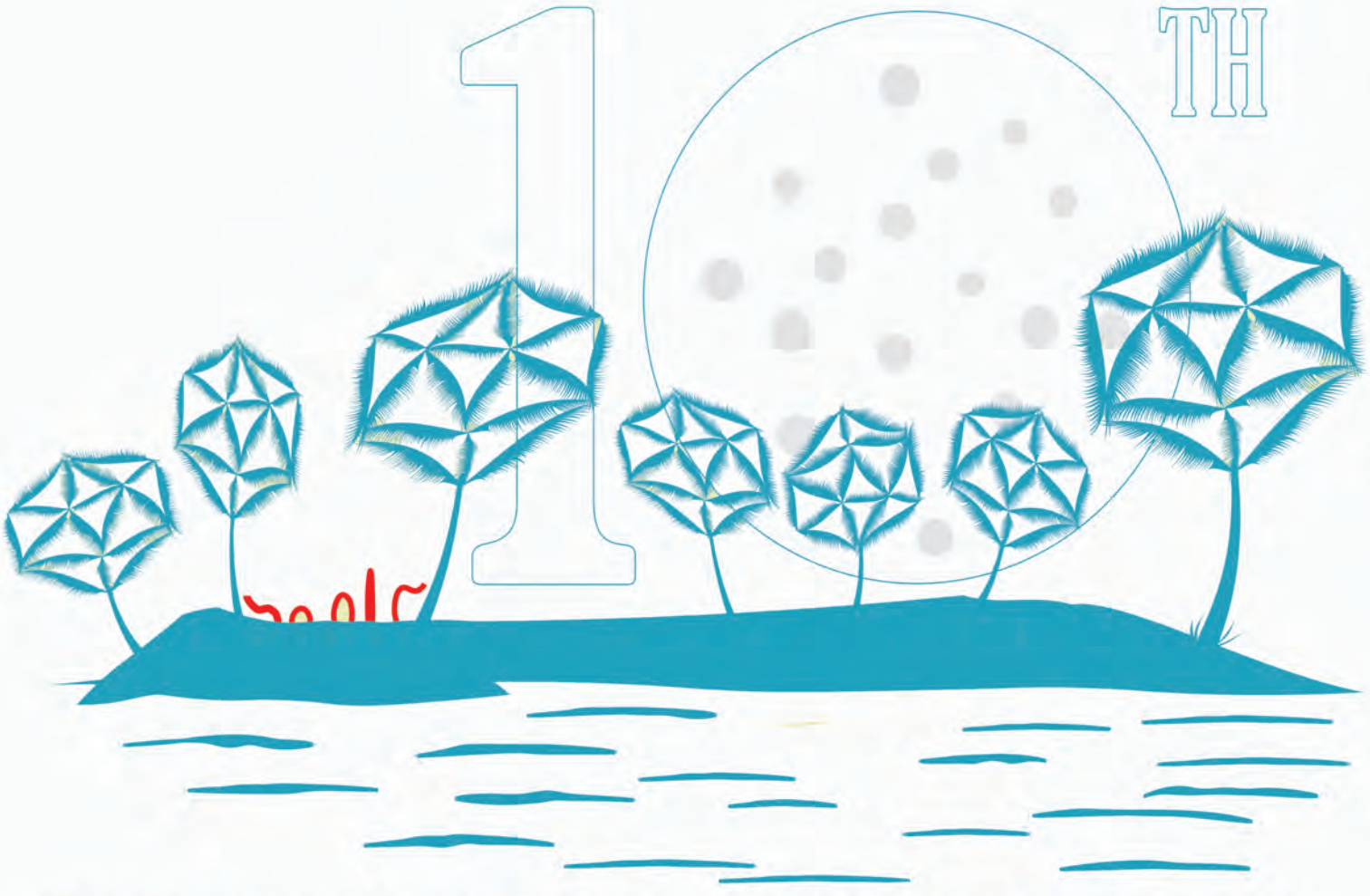

10TH SEA SYMPOSIUM

Friday, June 8, 2018

3:00 PM – 4:30 PM	CHECK-IN POSTER SET-UP STUDENT PRESENTER PRACTICE PERIOD	RECEPTION, THEN GALLERY LOBBY AUDITORIUM
4:45 PM - 6:15 PM	STUDENT DEBRIEFING	AUDITORIUM
5:00 PM - 6:00 PM	FACULTY SESSION	LOBBY
6:00 PM - 7:15PM	DINNER	DINING ROOM
7:15 PM - 7:30 PM	WELCOME AND REMARKS	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM
7:30 PM - 8:45 PM	KEYNOTE I CHEMICAL DISCOVERY IN THE HUMAN MICROBIOTA DR. EMILY BALSKUS HARVARD UNIVERSITY	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM
8:45 PM - 9:30 PM	SOCIAL	LOBBY
9:00 PM – 11:00 PM	HOTEL SHUTTLES – EVERY 30 MINS	DRIVEWAY LOOP ENTRANCE

Hotel guests will be shuttled to their hotels. Shuttles will leave for the hotels every half hour starting at 9:00 PM. The last hotel shuttles run at 11:00 PM.

10TH SEA SYMPOSIUM

Saturday, June 9, 2018

7:00 AM - 8:30 AM	BREAKFAST	DINING ROOM

Hotel guests will be shuttled from their hotels to Janelia Research Campus starting at 6:45 AM. Shuttles run every 15 minutes. The last shuttles run at 8:00 AM.

8:30 AM - 8:45 AM	INTRODUCTORY REMARKS	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM
8:45 AM - 10:00 AM	KEYNOTE II DR. GRAHAM HATFULL UNIVERSITY OF PITTSBURGH	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM
10:00 AM - 10:15 AM	BREAK	LOBBY
10:15 AM - 12:00 PM	POSTER SESSION I | ODD-NUMBERED POSTERS	LOBBY
12:00 PM – 1:15 PM	LUNCH	DINING ROOM
1:15 PM - 2:45PM	STUDENT ORAL PRESENTATIONS I *MODERATOR - PHILIPPOS TSOURKAS*	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM

Investigating Corynebacterium Phages: Similar Yet Very Unique
Mckinley Williams, Ote Staton - University of Alabama at Birmingham

Puzzling Virion Morphology and DNA Characterization Results from Microbacteriophages Discovered at Western Carolina University
Brandon Stamey, Ethan Holcomb - Western Carolina University

Isolation and characterization of actinobacteriophage from the Treasure Coast of Florida
Rhode Dorissaint, Joshua Moreno - Indian River State College

The isolation and genome annotation of Cluster CV phage, Frokostdame
Abigail Moreau, Andrew Wilcox - University of Maine, Farmington

Orphams find a home in OneinaGillian while KaiHaiDragon continues moving forward
Rhiannon Abrahams, Steven Tran - La Sierra University

The Mystery of Jamestown, Virginia (Phages)
Danielle Jones, Hermela Zerihun - College of William & Mary

Saturday, June 9, 2018 (cont'd)

2:45 PM - 3:00 PM	BREAK	LOBBY
3:00 PM – 4:45 PM	POSTER SESSION II | EVEN-NUMBERED POSTERS	LOBBY
4:45 PM – 6:15 PM	STUDENT ORAL PRESENTATIONS II *MODERATOR - RACHEL ARNOLD*	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM

Non-Mycobacterial Actinobacteriophages Expanding Our Knowledge of Phage Biology and Evolution
Sucely Ponce, Christal Rolling - Nyack College

Discovery and sequencing of Gordonia terrae phages reveals insights into prophage-mediated host defenses and phage evolution
Lindsay Ejoh, Alec Kistler - University of Pittsburgh

Host Preference and Recombination in Phages Infecting the Phytopathogen Streptomyces scabiei
Matthew Koert, Courtney Mattson - University of Maryland, Baltimore County

Adventures in phage hunting on new Actinobacteria hosts: Risks and Rewards
Serena Jacob, Evan Ruesch - University of Wisconsin-River Falls

Isolation and characterization of Streptomyces and their Phages from soil samples in the San Diego County
William Bushnell - University of California, San Diego

Biodiversity of Mycobacteriophages Isolated From Soil
Katherine Clarke, Stefanie Moncayo - Dominican College of Blauvelt

6:15 PM - 7:30 PM	DINNER	DINING ROOM AND LOBBY
7:30 PM – 7:45 PM	GROUP PHOTO	FRONT ENTRANCE
7:45 PM - 9:30 PM	SOCIAL	DINING ROOM AND LOBBY
9:00 PM – 11:00 PM	HOTEL SHUTTLES – EVERY 30 MINS	DRIVEWAY LOOP ENTRANCE

Hotel guests will be shuttled to their hotels. Shuttles will leave for the hotels every half hour starting at 9:00 PM. The last hotel shuttles run at 11:00 PM.

10TH SEA SYMPOSIUM

Sunday, June 10, 2018

7:00 AM - 7:30 AM CHECK-OUT

Hotel guests will be shuttled from their hotels. Shuttles will leave for Janelia Research Campus starting at 6:45 AM. The last shuttles run at 8:00 AM. All guests must check out of their rooms before breakfast. Luggage may be stored in the Gallery and Synapse Meeting Room.

7:00 AM - 8:30 AM BREAKFAST DINING ROOM

8:30 AM - 10:00 AM STUDENT ORAL PRESENTATIONS III STUDENTS IN AUDITORIUM
MODERATOR - ELIZABETH RUTLEDGE FACULTY IN SEMINAR ROOM

Extreme Codon Biases in Mycobacteriophage Relative
to Phage Encoded tRNA Genes and Host Codon Biases
Annabelle Malinowski, Kathryn Baker - LeTourneau University

Genomic and proteomic characterization of CR cluster Gordonia phages Marietta and Foxboro
Alex Larson, Samantha Good - College of St. Scholastica

RFLP-Guided DOGEMS and a Class Genome Announcement--Bioinformatics at OBU
Cameron Brownlee - Ouachita Baptist University

Characterization of three uncommon bacteriophages, a global analysis
of Actinobacteriophage predicted transmembrane domain-containing proteins,
and a pan-genome analysis of Microbacteriophages
Ashley Raymond, Maria Lopez - Southern Connecticut State University

Characterization and Genomic Analysis of Mycobacteriophage Wamburgrxpress, Including
the Determination of the Essentiality of the Terminal 5 Kilobase Pairs for Plaque Formation
Riley Hellinger, Hannah Sparks - Montana Tech of the University of Montana

A Story of A4 Bacteriophages: The Split DNA Primase with Dramatic Overlap in Two Different Frames
Ann Le - Worcester Polytechnic Institute

10:00 AM - 10:30 AM BREAK LOBBY

10TH SEA SYMPOSIUM

Sunday, June 10, 2018 (cont'd)

10:30 AM – 11:45 AM	FACULTY PRESENTATIONS *MODERATOR - IMADE NSA*	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM

Finding Arrowsmith: A modern analysis of Sinclair Lewis's 1925 Pulitzer-Prize-winning novel on phage therapy
Daniel Westholm - College of St. Scholastica

PECAAN, a Phage Evidence Collection And Annotation Network
Claire Rinehart - Western Kentucky University

Potential of Mycobacteriophages as Candidates for Phage Therapy
Identification of Phage that Infect Nontuberculous Mycobacteria Pathogens
Christopher Gissendanner - University of Louisiana at Monroe

11:45 AM - 12:00 PM	CLOSING REMARKS	STUDENTS IN AUDITORIUM FACULTY IN SEMINAR ROOM
12:00 PM - 1:30 PM	LUNCH	DINING ROOM
12:00 PM - 1:30 PM	POSTER REMOVAL AND DEPARTURE	LOBBY

All guests will be shuttled to their destination. Shuttles will leave for the airports at 1:00 PM.
The shuttle to HHMI Headquarters for the Faculty Meeting departs at 2:00 PM.

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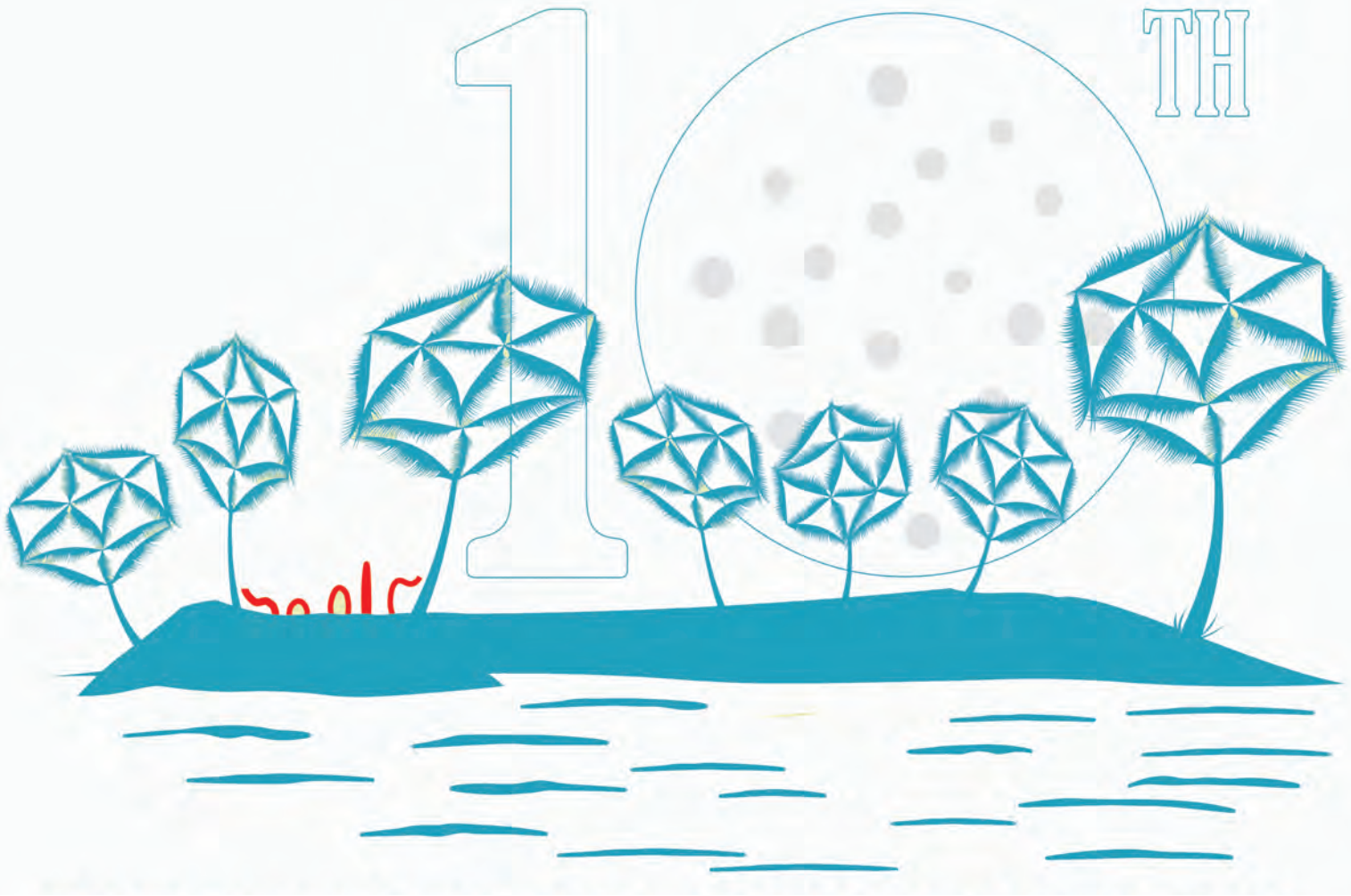

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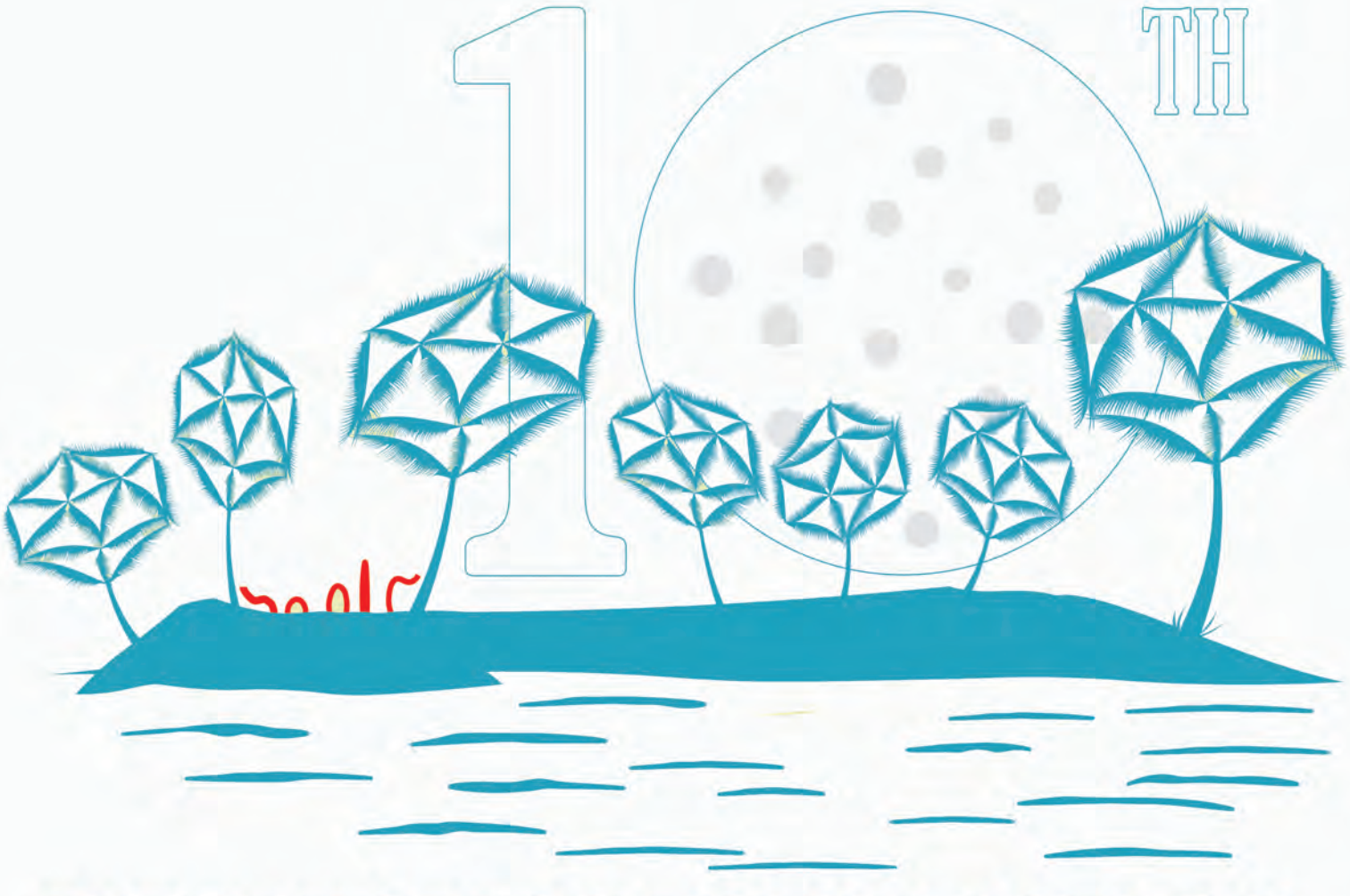


10th Annual SEA-PHAGES Symposium Abstract Poster #102

Aaniiih Nakoda College
Harlem MT
Corresponding Faculty Member: Chelsea Morales (ctmorales@ancollege.edu)

Janine J Ballard

Marcelina Alfaro

Got Phage? The Case of the Very Temperate Mycobacteriophage TwoJumps and the Analysis of Veracruz

Janine J Ballard, **Marcelina Alfaro**, Shelby Denny, Chelsea T Morales, Dan Kinsey

Aaniiih Nakoda College Phage Hunters who participated in the 2017 Fall semester Phage Discovery course (the College's first time to offer the course), collected soil samples from either a demonstration vegetable garden or a medicinal plant garden both located on the College's campus. We were able to discover a novel phage, TwoJumps, which was isolated from the host Mycobacterium smegmatis. The soil sample was collected from a raised bed containing the plant comfry (Symphytum officinale) in the medicinal plant garden. The isolation method was enrichment and the plaques produced were large and cloudy, characterizing TwoJumps as temperate. Unfortunately, once TwoJumps was isolated, purified and lysate was collected, a severe snow storm occurred that resulted in an extended power outage on campus and the campus was closed for several days. Upon return to campus, the students unsuccessfully attempted to continue with calculating the titer of their lysate. Also, the students were unsuccessful in reculturing their phage. It is suspected that the lysate (originally stored in a 4°C refrigerator) degraded during the power outage. After this situation occurred, the students attempted to collect new soil samples and re-isolate Mycobacteriophage, however, by this time of year, the soil in the gardens were frozen. So, the students were unsuccessful in extracting DNA to send off for sequencing. As a result of not having phage DNA sequences to work with for the Phage Bioinformatics course, a request was made to the University of Pittsburg for Mycobacteriophage sequences that could be used by students in the 2018 Spring semester Phage Bioinformatics course. The University of Pittsburg replied with the sequence for Veracruz, a Mycobacteriophage in Cluster A3. Veracruz was isolated from an enriched soil sample at Southern Connecticut State University in 2011. Veracruz displayed small cloudy plaques and is a temperate phage. The genome was annotated using bioinformatics software including DNA Master, NCBI and phagesdb Blastp, Phamerator, Starterator. The length of the genome is 50,062bp and had 85 predicted genes. After annotation, Veracruz genome had 81 genes. The less conservative changes are in genes appear near the end of the genome and likely code for non-essential functions. While the 89 genomes in the A3 cluster are relatively similar to each other, 33 vary largely near the end the genome starting at the RecB-like protein. These 33 genes along with 15 other genomes contain a different minor tail gene than the other phage.

10th Annual SEA-PHAGES Symposium Abstract

Poster #052

Albion College
Albion MI
Corresponding Faculty Member: Ken Saville (ksaville@albion.edu)

Kenzie Bush

Salacia Bacteriophage Annotation

Kenzie Bush, Nicola Milosavljevic, Christopher Reedy, Emma Schlachter,, Katie Wright, Hannah Schoon, Anna Miller, Hernan Rico, Katie Volker, Sadhna Ramanathan, Ken Saville

Bacteriophage are viruses that infect bacteria and use them as hosts. Bacteriophage research is being performed to use phages as a potential treatment of bacterial infection and diseases. The purpose of this project was to examine the genome of the Mycobacteriophage Salacia and to annotate its genome using DNA Master, NCBI BLAST, Phamerator, and other programs used for bioinformatics. Salacia was isolated in the previous semester by the Albion College Virus Hunters class. The annotation process took about five to six weeks to complete. By the end of the project, some genes were added to DNA Master and some genes were removed. The final gene count for Salacia was 259, including 30 tRNAs. Examples of a few specific gene annotations are presented here. Annotation of Salacia could potentially be used to analyze other C1 phages and determine additional protein functions.

10th Annual SEA-PHAGES Symposium Abstract

Poster #059

Austin Community College
Austin TX
Corresponding Faculty Member: Ana Maria Valle-Rivera (anamaria.valle-rivera@austincc.edu)

Isolation and Genome Annotation of mycobacteriophage "RoMag", a Myoviridae Group C Cluster C1 from Austin Community College

Eric A Barnes

It was the second year of submissions of mycobacteriophages to the SEA PHAGE program from the Central Texas area. We were able to discover a novel phage 'RoMag' isolated from infection to the host bacteria *Mycobacterium smegmatis* mc^2155. The isolation method used was enrichment. The plaques produced were of medium size and turbid. Electron microscopy was performed at the University of North Dallas. The electron micrographs
showed that the virus morphology was consistent with the Myoviridae group. The genome of 'RoMag' was sequenced by the Pittsburgh Bacteriophage Institute using Illumina Sequencing. The virus was found to be 156, 329 basepairs in length and characterized as a cluster C1 bacteriophage. The genome was annotated using bioinformatics software including DNA Master, NCBI's Blastp, HHPred, Phamerator and Starterator. The genome had 227 predicted genes mostly forward genes. The genome also had 30 putative tRNA sequences. We found protein functions that match functions for bacteriophages in the national database as well as unknown function proteins.

10th Annual SEA-PHAGES Symposium Abstract

Poster #017

Baylor University
Waco TX
Corresponding Faculty Member: Tammy Adair (tamarah_adair@baylor.edu)

The genomic characterization or Arthrobacter globiformis phage Elesar

Alaina Baird, Jensen Smith

The large majority of phages isolated by students participating in the SEA-PHAGES program have been isolated on *Mycobacterium smegmatis*. These genome annotations have contributed to a vast amount of knowledge concerning phage genes and genomic structure. From the analysis of this data,new questions concerning the interaction of the soil microbial community have developed, such as "how conserved are phage genes across various clusters from different hosts?", and "are phage genomes mosaics of a diverse group of genes from various clusters?". Baylor University has been exploring these questions by focusing on the phages isolated on *Arthrobacter* hosts, most recently *Arthrobacter* phage isolated on *A. globiformis*. The purpose of this poster is to present *Arthrobacter* phage Elesar to the SEA PHAGE community and highlight some of the interesting genomic characteristics in this cluster FF phage. For example, Elesar has a number of orphams and genes only found in the small FF cluster, but it also has a terminase that has high similarity to 101 pham members from phages in clusters isolated across 4 different hosts. The Elesar terminase differs slightly from the 2 other members in its own cluster. Several familiar gene arrangements are found, such as the tRNA, attP site and putative integrase, as well as a few unusual findings, such as a conserved non-coding region in a gap between open reading frames. This evidence supports the conclusion that there is a high level of genetic exchange between phage and their hosts in the soil environment. Continued annotation efforts will lead to more questions about the evolution of the diversity of bacteriophage.

10th Annual SEA-PHAGES Symposium Abstract

Poster #018

Brigham Young University
Provo UT
Corresponding Faculty Member: Julianne H Grose (julianne_grose@byu.edu)

Emily Potts

Searching for Jumbo Bacteriophages that Infect Mycobacterium

Emily Potts, Trever Thurgood, Daniel Thompson, Donald P Breakwell, Julianne H Grose

Jumbo bacteriophages are large phages with extremely large (> 240 kB) genomes. They are often not found using standard isolation procedures due to their inability to move through standard top agar and form visible plaques. The Brigham Young University phage hunters searched for jumbo mycobacteriophages using non-standard top agar including top agar with lower agar concentrations and agarose-based top agar. We will present the jumbo phages found using these techniques and compare them to previously isolated jumbo phages as well as other mycobacteriophages.

10th Annual SEA-PHAGES Symposium Abstract

Poster #011

Bowling Green State University
Bowling Green OH
Corresponding Faculty Member: Jill Zeilstra (jill.zeilstra@gmail.com)

Eleanor M Behling

Eric M Noss

Mycobacterium smegmatis mc^2 155 bacteriophage of Bowling Green, Ohio

Eleanor M Behling, **Eric M Noss**, Tessa Alloy, Anisten Aurand, Jocelyn Botta, Lucia Boulos, Susanta Deka, Katelyn Fletcher, Kristina Gara, Megan Gayer, Melanie Heldman, Miranda Jones, Lauren Lukasko, McKenzie Moss, Julia Orshoski, Landon Rohrer, Rachael Sattler, Jenna Sexton, Katherine Sheetz, Kayla Valente, Rowan Wicks, Natalie Wise, Raymond A Larsen, Vipaporn Phuntumart, Zhaohui Xu, Natalie N Stoian, Jill H Zeilstra-Ryalls

Among a total of 23 bacteriophage isolated on the campus of Bowling Green State University during the fall of 2017, five have been sequenced. For three of the phage, plaque and phage (using TEM) morphologies, together with preliminary sequence data, were used to determine their group memberships. The preliminary sequences of PSullivan indicate its genome is composed of 49,990 bp, and the A1 bacteriophage BBPiebs31 (53,171 bp) and Forsytheast (52,695 bp) are its closest relatives. The nearest relatives of Eugenia, whose genome is predicted to be 69,139 bp in size, are the B1 bacteriophage Serpentine (68,884 bp) and Alex (68,910 bp). Preliminary sequence results for Matalotodo, suggest its genome is 69,232 bp in size, and its closest relatives are the B1 phage Vista (68,484 bp) and Murdoc (68,600 bp). Annotation of the other two sequenced bacteriophage was performed during spring 2018. The first of these, Pita2, is a temperate siphoviridae belonging to the A1 group of bacteriophage. Only 3 other A1 phage besides Pita2 encode a tRNA (for tryptophan). The Pita2 genome also includes an ORPHAM (35960). In addition to annotating its genome, immunity and temperature range of infection studies were undertaken to further characterize this phage. The second phage whose genome was annotated is Godphather, a lytic siphoviridae belonging to the uncommon W group. As is true of two other W phage, Taptic and Megabear, the Godphather genome encodes a glycine tRNA gene. Interestingly, Godphather gene 6 is only present in one other W phage, but it is also present in the genome of a singleton phage that was isolated on Brevibacterium fuscum NRRL B-14687. Likewise, genes 25 and 26, which encode minor tail proteins, belong to phams that are only represented in one other W phage, but are also represented in phages belonging to clusters B, F, N K, and P as well as cluster EG. The latter cluster comprise phage isolated on Microbacterium foliorum NRRL B-24224 SEA. Collectively, these studies of the first Bowling Green phage to be isolated and sequenced suggest that the campus has a diverse phage population. However, since they were all isolated from fertilized soils, the bacteriophage may not be local. Bowling Green is in the region called the Great Black Swamp, which was glacially fed wetland that was drained and settled during the second half of the 18th century. Toward identifying more indigenous phage in future, samples will be taken from areas that are more likely to represent "native" soils.

10th Annual SEA-PHAGES Symposium Abstract

Poster #062

Cabrini University
Radnor PA
Corresponding Faculty Member: Melinda Harrison (mah348@cabrini.edu)

Isabella Romani

Discovery and Annotation of Cluster AN Arthrobacter Phages

Isabella Romani, Andrew Conboy, Sonia Spadafora, Bailey Babarsky, Jenna Bucca, Hunter Angle, Chris Dachowski, Alyson Marshall, Bhumi Patel, Morgan McDonough, Veronica Siko, Adriana Eiwechter, Caroline Germain, Brian Layden, Angelica Valentin, Afua Awuah, Lauren Markowitz, Bianca Santos, Sarah Grant, Alyssa Rothman, Amanda Budzilowicz, Sarah Eplett, Olivia Townshed, Danielle Ayer, Courtney Curcio, Carina Cena, Nikolai Kuchinos, Nicholas Staub, Zachary Rambo, Steve Sampura, Benjamin Giordano, Robert Schmidt, Lisa McKernan, Joseph Kulkosky, Matthew Mastropaolo*, Melinda Harrison

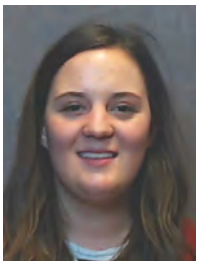
* Neumann University, Aston PA

Bacteriophages are viruses that infect a bacteria host, potentially leading to strategies for treating, preventing, or diagnosing bacterial infections such as tuberculosis that are resistant to conventional antibiotics. We have discovered 20 novel phages from the bacterial host *Arthrobacter* sp.ATCC 21022. The phages were collected from soil and water samples by students from various places around South Eastern PA and Southern NJ. and their genomic DNA isolated. After isolating the genomic DNA, they were photographed through the use of an electron microscope and then the DNA was sequenced. The Phages genomes were then annotated using various bioinformatics tools, such as DNA Master; Phamerator; HHpret and GeneMark to determine gene location and function. Using comparative genomics, unique characteristics of the phage's genome were also explored. This study presents a comparison of seven bacteriophage genomes that were isolated from this research: CGermain, Hunnie, Inspire2; Dewayne; Azathoth; Copper and Ronnie. The genomes of these phages were annotated as a collaborative effort by many students and faculty.

10th Annual SEA-PHAGES Symposium Abstract

Poster #050

Calvin College
Grand Rapids MI
Corresponding Faculty Member: John Wertz (jwertz59@calvin.edu)



Betty Kliewer

Speeding on Kroos Control and We Got GC in Low Places: Gordonia phages Kroos and Tanis

Betty Kliewer, Jesse J Aubin, Jake C Boer, Rebekah J Cross, Laura R Dykstra, Elle D Hazlett, Hanna Jeong, Kelli N Laube, Joshua Y Lee, Haley M Marco, Micah J Meindertsma, Kyle T Musch, Rylan J Shewmaker, Jeremiah D Shultz, Taylor A Ten Pas, Jack B Visser, Bethany R Williams, Mary R Horner-Richardson, Randall J DeJong, John T Wertz

Two new bacteriophage, Kroos and Tanis, were isolated on *Gordonia terrae* 3612. Kroos is a 57,974bp siphoviridae with a 74nm capsid and 315nm tail. It belongs to subcluster DE1, has 85 ORFs, and shares 88% nucleotide identity with Brandonk123. Kroos has a GC content of 68.1%, consistent with its host and the DE cluster. No tRNAs were found but all expected genes were, and function was identified for 28. Unlike most DE phages, Kroos has no reverse genes. In cluster DC, closely related to DE, phages Wizard and Twister6 have 17 phams that have synteny with Kroos. Kroos' genes are generally not shared beyond *Gordonia* phages, as only 5.8% are in phams with 3 or more host species, most commonly *Gordonia*, *Mycobacterium* and *Actinoplanes*. Tanis is a 59,727bp siphoviridae containing 93 genes, of which all are in the forward direction. Tanis has a 55nm capsid and 325nm tail, is in cluster DJ and has 51% GC content - which is consistent with the DJ cluster, but not *G. terrae* (67.8%). Tanis does not contain any tRNA genes that could compensate for this disparity. Tanis shares 95.7% identity with Gravy and Kerry (DJ). In addition to functions shared with Kerry and Gravy, six ORF functions were inferred in Tanis. Interestingly, Tanis contains a major capsid protein/capsid maturation protease, which is consistent within DJ, but not other *Gordonia* clusters. Tanis and the DJ cluster appear to be most closely related to CC, an exclusively *Rhodococcus* cluster. In contrast to Kroos, 31.3% of Tanis' genes are in phams with 3 or more host genera. The most conserved pham (37551) has 346 members across 4 hosts. After *Gordonia* phages, Tanis shares 47.3% of phams with *Rhodococcus*, 29% with *Arthrobacter*, 28% with *Streptomyces*, 16.2% with *Microbacterium* and 12.9% with *Mycobacterium* phages. We wondered whether Tanis' shared genes could be due to frequent host switching, and if that may explain the lower than expected GC content. Actinobacteriophages predicted on PhagesDB to be lytic have a lower GC content (59.6%) than phages predicted to be temperate (64.9%; p=0.001). Predicted lytic phages also have significantly larger genomes than temperate (70,140bp vs 52,832bp; p=0.004). There is no relationship between phage lifestyle, GC content, or genome length and the presence or abundance of tRNA genes. Recent studies show the average GC content of uncultivated actinobacteria is 47%, suggesting cultivation may have led to an inappropriate label of "high GC" to the actinobacteria. Our findings that lytic actinobacteriophages have significantly lower GC content can be explained if lytic phages circulate throughout a population of actinobacteria ranging from mid to high GC content. Temperate phages may circulate less frequently, and our use of high GC hosts may select for high GC temperate phage. Lastly, additional phages were sequenced, representing Gordonia phage clusters CQ, CR, CU, DC, DE, DI, and DJ, and are under annotation.

10th Annual SEA-PHAGES Symposium Abstract

Poster #100

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Tabitha Hudock

John Nykyforuk

Can You C R Gordonia Phages? Discovery and Analysis of KidneyBean (Cluster CR2) and WhoseManz (Cluster CR4)

Tabitha Hudock, **John Nykyforuk**, Vanessa Allanach, Samantha Ardery, Matthew Cootes, Wendy Cruz, Kaitlin Daly, Kevin Dunn, Melanie Gucwa, Brandyn Kirchhoff, Makenzie Mullan, Amy Sorge, Elizabeth Stroh, Isaiah Whitehead, Anna Faust, Caleb Love, Rose Mibus, Emilie Pindras, Camryn Rauen, Tianna Sbarounis, Qinzi Ji, Deborah Tobiason

At Carthage College, 90 bacteriophages were isolated in 2017 using the hosts *Mycobacterium smegmatis* mc2155 and *Gordonia terrae* 3612, over 40 of which infect the bacterial host *Gordonia terrae*. In continuing research, four phages were annotated: KidneyBean (cluster CR2), WhoseManz (cluster CR4), SpikeBT (cluster A1), and GreaseLightnin (cluster P1). Greaselightnin and SpikeBT are temperate mycobacteriophage isolated on Mycobacterium smegmatis, while WhoseManz and KidneyBean are lytic phage isolated on Gordonia terrae. Both of the Gordonia phages lack an integrase gene and are considered to be lytic; however, KidneyBean has a halo plaque morphology with a clear center and a large turbid exterior. Both phages have Siphoviridae morphology, with long, flexible, non-contractile tails. After sequencing and annotation, WhoseManz was found to be in cluster CR4, a cluster with only four members, none of which have yet been submitted to GenBank. Interestingly, WhoseManz was found to have two orphams, one near the beginning and one near the end of the genome. In performing further bioinformatic analysis, we are focusing on Gordonia phages, especially the CR2 and CR4 clusters, which contain KidneyBean and WhoseManz, respectively. This research spans topics from doing whole genome comparisons to focusing on specific genes of interest or regions of interest such as repeated DNA sequences. There is relatively little data available thus far on Gordonia phages; therefore, our research will boost our understanding of Gordonia phages, especially KidneyBean, WhoseManz, and their fellow CR2 and CR4 bacteriophages.

10th Annual SEA-PHAGES Symposium Abstract

Poster #014

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Pedro Safi

Characterization of Novel Bacteriophages that Infect Arthrobacter sp ATCC1022 or Microbacterium foliorum

Kendra Adegbesan, Mabel Bartlett, Carolyn Botz, Cali Colliver, Brooke Dresden, William Fahy, Alejandro Garces, Venkat Gella, Ziyi Guan, Camila Guerrero, Priya Guntur, Wonhee Han, David Hoyos, Meera Krishna, Sydney Lee, Iris Lu, Kiran Mirpuri, Harold Rockwell, **Pedro Safi**, Aria Salyapongse, Emily Schneider, Ariel Uy, Jonathan Jarvik, A. Javier Lopez, Natalie McGuier

Bacteriophages have important ecological roles and applications in medicine, food production and bioremediation. To further the understanding of bacteriophage diversity and evolution, we screened soil samples from diverse locations for bacteriophages that form plaques on lawns of Arthrobacter sp. ATCC1022 or Microbacterioum foliorum. Arthrobacter has potential uses in bioremediation and Microbacterium species can be involved in spoilage of milk products. We identified 14 Arthrobacter phages and 8 Microbacterium phages and we characterized them with respect to plaque morphology and temperature sensitivity, virus morphology, and ability to lysogenize. We purified genomic DNA from 9 Arthrobacter phages and 8 Microbacterium phages and we sequenced the DNA on the Illumina Miseq platform, followed by assembly of the genomes using GS DeNovo Assembler. We obtained complete assemblies for 8Arthrobacter *phages (1 singleton, 2 cluster AK, 1 AN, 1 AR, 2 AU1, 1 FG) and 5 Microbacterium phages (2 EA, 1 EB, 2 EE). We generated finalized annotations for Guntur (Arthrobacter, cluster AN) and Dongwon (Microbacterium, cluster EE), as well as draft genome annotations for all other assembled genomes.*

We found evidence in the Dongwon genome sequence for a programmed translational frameshift to produce a full-length tail assembly chaperone. Two tRNAs were predicted by Aragorn, but they are located in regions of strong coding potential within the tapemeasure protein ORF, suggesting that they are not expressed tRNAs. They are also predicted, in different combinations, within some but not all EE phages. To investigate whether these sequences represent ancestral insertions of tRNA genes that were modified by mutation and coopted to encode portions of the tapemeasure gene, we performed a comparative phylogenetic analysis of the tapemeasure gene and flanking genes, including a minor tail protein gene that is represented by different Phams within the EE phages.

We also analyzed in detail the sequence and conservation of Guntur gene 23 ORF, more than half of which exhibits very poor coding potential. The N-terminal portion (strong coding potential) encodes an HTH domain with similarity to Bacillus DNA-D, an origin recognition protein (HHPred analysis). The poor-coding potential region contains a series of inverted and direct repeats that are more highly conserved than the HTH region, as well a relatively AT-rich region. We suggest that Gene 23 may contain an origin of replication and also encodes a protein that recognizes it.

We isolated an interesting derivative of Arthrobacter sp ATCC1022 that is partially resistant to bacteriophage Wyth and exhibits different phage-specific modifications in plaque morphology with a range of our Arthrobacter phages. Although this strain was initially isolated as a survivor or Wyth infection, we have not been able to purify and sequence Wyth DNA so we cannot yet determine whether it is a lysogen or a bacterial mutant.

10th Annual SEA-PHAGES Symposium Abstract

Poster #051

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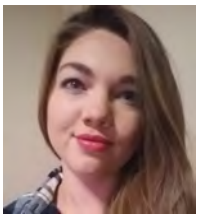

Mary Akers

Kelsey Largen

Comparative Analysis to Assess Hangman's Potential for Phage Therapy

Mary Akers, **Kelsey Largen**, Megan Cevasco, Daniel Williams

Phage therapy can be used to combat bacterial diseases by treating patients with phages that lyse infectious bacteria. Because antibiotic resistant bacteria are on the rise, phage therapy is currently being explored as an alternative to antibiotics. Comparative analysis can be used to identify phages or genes within phages that have therapeutic potential. Using *Mycobacterium smegmatis* as a host, Hangman was isolated at Coastal Carolina University, South Carolina in the fall of 2017 and belongs to subcluster B4. During annotation of Hangman, we discovered lysin A belongs to a pham that includes lysin A genes from cluster K phages, some of which infect *Mycobacterium tuberculosi*s. We performed comparative and phylogenetic analysis of Hangman with other phages that show broad host specificity. Our results will be used to assess the potential of Hangman for phage therapy.

10th Annual SEA-PHAGES Symposium Abstract

Poster #071

College of Charleston
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Hailey Kinsland

Characterization of Eight New Actinobacteriophages from Charleston, South Carolina

Hailey Kinsland, Christine Byrum

In Spring 2018, 44 students in the College of Charleston Molecular Biology labs collected environmental samples and attempted to isolate bacteriophages that attack the host bacterium Mycobacterium smegmatis. Using direct isolation or enrichment techniques, members of the class obtained high titer lysates for eight new bacteriophages. After genomic DNA was extracted from each of these viruses, restriction analysis and PCR-based cluster analysis were performed to better characterize each. Students also examined the morphology of these actinobacteriophages by transmission electron microscopy and data collected for each virus was entered in the Actinobacteriophage Database at PhagesDB.org.
The eight new bacteriophages isolated include: Awendaw, Carmen, Cashua, Guwop3, Hacker94, Lolalove, Luna22, and Revamp. Based on the preliminary PCR analysis of four bacteriophages tested, the class hypothesized that Awendaw, Cashua, and Hacker94 are all members of the A1 cluster and that Luna22 is not a member of the clusters evaluated (A1, A2, B1, B2, B3, C1, C2, D, E, F, H1, H2, or I). The class also determined which of six restriction enzymes (BamHI, ClaI, EcoRI, HaeIII, HindIII, or SalI) cut each actinobacteriophage genome. Genomes were cut by the indicated restriction enzymes: Awendaw and Cashua (only HaeIII), Carmen (ClaI, EcoRI, HaeIII, SalI), Guwop3 (None cut; may need to retest), Hacker94 (ClaI, HaeIII), Lolalove (None cut; ClaI and HaeIII untested), Luna22 (BamHI, ClaI, HaeIII, SalI), Revamp (BamHI; ClaI, EcoRI and HaeIII untested). Finally, a comparison of the bacteriophage morphologies is also presented. Based on their appearances and the restriction analysis results, we hypothesize that all eight bacteriophages are distinct members of the Siphoviridae. Each of the viruses has been prepared for archiving and will be submitted to the University of Pittsburgh in Fall 2018.

10th Annual SEA-PHAGES Symposium Abstract

Poster #008

College of Idaho
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Alexandra Fisher

Pedro Galvan

A Comparison of Rickmore and Duffington, two similar Gordonia phages from Idaho

Alexandra Fisher, **Pedro Galvan**, Aleah Mendiola, Suman Dhungana, Ann Koga

In the fall of 2017, our BIO 210 class isolated eight bacteriophages that infected *Gordonia terrae*. We selected two phages named Rickmore and Duffington for sequencing. Despite the enormous diversity in bacteriophage genomes, Rickmore and Duffington were found to have 95% nucleotide identity, joining five other Gordonia phages to form Cluster DJ. These two phages also shared similar physical characteristics, such as tail length and capsid size. Considering the two phage genomes are almost identical, we were curious to see if they were similar in functional characteristics as well. Cluster DJ phages are lytic and have a GC content that differs greatly from that of *Gordonia terrae* (67.8% for *G. terrae* vs 50.5% and 51.4% for Rickmore and Duffington, respectively), suggesting that these phages may be able to infect other bacteria. Thus, we decided to test the host range of Duffington and Rickmore. Preliminary findings indicate that Duffington may have a broader host range than Rickmore. It is clear from comparisons of the DJ phages that Duffington and Rickmore are very closely related to another DJ phage, AlainaMarie (all are 95-97% identical to each other) while not as closely related to the other four DJ phages (<90% identity). Perhaps these two groups of phages will form 2 separate subclusters within the DJ cluster.

10th Annual SEA-PHAGES Symposium Abstract

Poster #077

College of St. Scholastica
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Alex Larson

Sammantha Good

Genomic and proteomic characterization of CR cluster Gordonia phages Marietta and Foxboro

Alex Larson, **Sammantha Good**, Erin Burke, Jolene Duda, Eric Jeffords, Sadie Nowak, Mariah Pearson, Mariah Ricci, Anna Sohlstrom, Daniel Westholm

Cluster CR bacteriophages Marietta and Foxboro were isolated using *Gordonia terrae* CAG3 as part of the SEA-PHAGES program at The College of Saint Scholastica. Genome sequencing of Marietta revealed a 64,370 base pair genome with a 66.1% GC content and CR4 subcluster designation, while genome sequencing of Foxboro revealed a 67,773 base pair genome with a 65.8% GC content and CR2 subcluster designation. Phylogenetic analysis of over 20 actinobacteriophages using MEGA software indicated the both Marietta and Foxboro were most closely related to other phages that infect *G. terrae*, although GRU1 isolated with *G. rubripertincta* forms a unique clade with CR1 phages. Marietta and Foxboro are lytic phages, as both produced clear plaques on lawns of *G. terrae* and genome annotation did not identify lysogeny related genes. Promoter scanning of Foxboro identified 12 putative promoters scattered throughout the intergenic regions of the genome. However, an additional 15 putative promoters were identified between genes 90 and 91, the last two genes on the genome. This is likely an artifact of the unusually high AT rich character of this region, but there is a possibility that multiple promoters exist between these genes. In the Marietta genome, low complexity BLAST searches, along with the DNA Master scan tool and Phamerator, identified a sequence repeat near the 3' end of the genome. Weblogo analysis of this repeat identified a consensus sequence of ACTTGTCACGGTTAACGTCATCNCGTAACGTCNGCCTCA that occurred 5 times in intergenic regions within the last 10,000 base pairs of the genome. Interestingly, this sequence appears to be a defining feature of the CR4 subcluster, as the repeat is found in all CR4 phages including SuperSulley, BiPaunetto, IDyn, and WhoseManz. Foxboro, a CR2 phage, appears to contain 2 instances of this sequence repeat. This feature is visible with Gepard dotplot analysis and Phamerator comparison maps. A TomTom DNA motif analysis failed to detect a known function of this repeat, although promoter scans detected promoter sequences embedded within the repeat sequence. Finally, both Marietta and Foxboro infections were subjected to tandem mass spectrometry analysis. Liquid infections using each phage were Infections were carried out for 4 hours, then pelleted and subjected to LC-MS/MS analysis. Final sample analysis was not completed in time for abstract submission, but will be ready for presentation at the symposium.

10th Annual SEA-PHAGES Symposium Abstract

Poster #086

College of William & Mary
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Danielle D Jones

The Mystery of Jamestown, Virginia (Phages)

Danielle D Jones, Meredith Andersen, Sarah Belay, Yessica A Bonilla, Alyssa Costello, Mandi S Cotttrill, Jonathan Diaz-Ramos, Elizabeth N Do, Sunil Fontaine-Rasaiah, Langston N Forbes-Jackson, Victoria Guillen, Kylee L Hartman-Cabellero, James Hill, Alexandra A Huang-Queiroz, Angelica M Johnson, Ryan Lauzardo, Autumn Liu, Sara E Martinez, Sarah E Modlin, Camille C Okonkwo, Tana V Palomino, Sudip Paudel, Lizzie P Peteraf, Tatiana M Prioleau, Khalil Russell, Ryan T Shipman, Sarah H Thompson, Hermela Zerihun, Mark H Forsyth, Margaret S Saha

In an attempt to expand the diversity of Actinobacteriophage discovery, the 21 freshmen that comprised the William and Mary 2017-18 SEAPHAGES Lab attempted to identify phages that infected Corynebacterium glutamicum, and Tsukamurella paurometabola. After eight weeks and several hundred enrichment attempts no phage were isolated. Nearing November (and desperation) we turned to two Mycobacteria hosts: M. neoaurum and M. aichiense. Following over 50 enrichments, while no plaques were obtained from M. neoaurum, phage that infected M. aichiense were isolated by a single student and from the instructor's "magic compost." Despite apparent success with a novel host, in order to meet the sequencing deadline, William and Mary submitted Gorge, a phage isolated by our community college colleagues at the Colonial Williamsburg campus of Thomas Nelson Community College with whom we have collaborated for the past decade. Using M. smegmatis as a host, Gorge belongs to the F1 subcluster with high similarity to Saal. Concurrently with annotating Gorge, we continued to pursue our M. aichiense phage. Strikingly, in contrast to the rates of obtaining M. aichiense plaques from our SEAPHAGES class, in three separate high school outreach events during winter 2018, over half of the 66 high school students from Jamestown High school who conducted a phage discovery experiments at William and Mary obtained phage in their enrichment plates. These phage consistently show extremely low titers that decline quickly over time; moreover, even using three different methods of DNA extraction, we have not obtained significant amounts of DNA for sequencing although positive M. smegmatis and G. terrae lysates from previous years worked perfectly. For M. aichiense, all DNA was degraded following the DNAse step. We therefore performed shotgun cloning of non-DNAsed sample; while bacterial debris sequences comprised about 50% of the sequences, another percentage were phage sequences that showed some albeit low homology over very short stretches to F subcluster phages. These phages do not plaque on M. smegmatis. We are continuing to characterize these interesting and apparently novel phages along with the other 30 isolates from Jamestown, Virginia, using a variety of different approaches.

10th Annual SEA-PHAGES Symposium Abstract

Poster #083

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Marissa Franco

Caroline Long

Exploring the Genome and Phamily of the Mycobacteriophage Amochick

Marissa Franco, **Caroline Long**, Matthew Carrell, Margaret Dickel, Justin Graham, Hassina Hamzaoui, Khoi Ho, Edinah Nyagwencha, Sophia Hines, Carole Twichell, Jonathan Lawson, Bridgette Kirkpatrick

The Mycobacteriophage (phage) Amochick was discovered in 2017 by students at Collin College in a soil sample collected from a chicken yard in Farmersville, Texas. Amochick was determined to be a siphoviridae phage that infects the host *Mycobacterium smegmatis* mc^2155. After sequencing by the Pittsburgh Bacteriophage Institute, it was found that Amochick has a genome length of 54,145 base pairs and is one of 12 phages in Cluster Q. Cluster Q phages are temperate, meaning they form lysogens, which is also true for Amochick. Sequencing revealed that Amochick has a 14 base pair overhang on the 3' end of its genome. BLAST results revealed the most closely related annotated phages are Giles and Evanesce with an identity of 98% and draft phage Deagal_Draft_67 with an identity of 99%. There are three major differences between Amochick and other Cluster Q phages: Gene 66 (Pham 4300), Gene 72 (Pham 2661) and Gene 87 (Pham 37796). Gene 66 is only found in one other draft phage in our cluster, Daegal_Draft_67, with a 100% identity. While there are some phages in Cluster R which have a gene similar to Amochick Gene 66, the identity is low at 54%. The product of this gene is structurally similar to a transferase from the bacteria, *Lactobacillus helveticus*, suggesting that Amochick may have acquired this gene from a previous host. Gene 72 stands out in its phamily because it has a 580 bp insert which has partial gene identity to a 50S ribosomal protein L22 inserted into the gene. OBUpride is the only other annotated phage with this insertion with a 99% identity; the drafts of Gancho and Daegal also include the insertion. Gene 87 of Amochick is not found in any other sequenced phage. This unique gene was not initially called by Glimmer or Starterator, however it does demonstrate coding potential using Genemark when trained with self, M smegmatis and M tuberculosis. This gene is a reverse gene of 120 bp which requires further functional validation. The goal of annotating phages like Amochick is to be able to better understand phage and how they may be used to combat antibiotic resistant bacteria.

10th Annual SEA-PHAGES Symposium Abstract

Poster #022

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Kyle M Strange

Isolation and Characterization of the Bacteriophage Jaykayelowell

Taylor L Barber, Cody A McClain, Dylan J Melton, Cheyenne L Roberts, **Kyle M Strange**, Muhammad E Seegulam

The goal of this experiment was to isolate and characterize a novel bacteriophage from an environmental soil sample. A soil sample from the Northeast Missouri region containing a putative phage was collected, amplified using the bacterial host M. smegmatis mc2155, and subjected to several rounds of purification in order to identify a novel phage. The formation of plaques on bacterial lawns infected with phage samples indicated the presence of bacteriophage in the sample. Plaque morphology appeared large, circular, and lytic but decreased in size and increased in number with repeated infections and plating. Bacteriophage Jaykayelowell isolated by the 2015 cohort was analyzed. The Jaykayelowell genome was found to be 51,367 base pairs in length and was assigned to cluster A subcluster A4. Among the 85 genes present in the Jaykayelowell genome, several encoded known products including minor tail protein, capsid maturation protease, and membrane domain protein, while 36 of the genes were found to have no known function.The programs used for annotation include DNA Master, Phamerator, Starterator, HHpred, NCBI BLAST and PECAAN.

10th Annual SEA-PHAGES Symposium Abstract

Poster #045

Del Mar College
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Danial N Azadani

Daisy Zhang

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Jeffrey Turner

Characterization and complete genome sequence of a lytic bacteriophage infecting antibiotic resistance Enterococcus faecalis

Danial N Azadani, Daisy Zhang, J. Robert Hatherill, Jeffrey Turner*

* Texas A&M, College Station TX

Enterococcus faecalis is a Gram-positive bacterium that commonly inhabits the gastrointestinal tracks of humans and animals. A minority of strains are pathogenic and the emergence of drug-resistant strains is a growing health crisis. To address this crisis, the search for alternative treatments like bacteriophage (phage) therapy has been revitalized. In this study, a lytic E. faecalis bacteriophage was isolated from an in-flow water sample collected at a local wastewater treatment plant (WWTP). Transmission electron microscopy (TEM) and whole-genome sequencing showed that enterophage phiNASRA1 belongs to the Siphoviridae family of double-stranded DNA viruses. The phage is approximately 250 nm in length and its complete genome (40,139 bp, 34.77% GC) encodes 60 putative proteins. It demonstrated a short latent period (~20 min) and a high lytic efficiency (97.52%) using a streptomycin-resistant E. faecalis as the host. A phylogenetic comparison of phiNASRA1 with 13 closely related Enterococcus phages, from wastewater and sewage collected from municipal, hospital and agricultural environments spanning five countries, indicated that these phages were widely distributed and recalcitrant to spatial and temporal turnover. The phage's narrow host range, short latent period and high lytic efficiency suggest that phiNASRA1 would be a promising candidate for the development of a phage therapy.

10th Annual SEA-PHAGES Symposium Abstract

Poster #058

Doane University
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Danielle Schreiber

Discovery and genome annotation of Bread and Fudgetart, two cluster C1 Mycobacteriophages

Danielle Schreiber, Grace Su, Brea D Murnan, Kaitlyn Mahnke, Jason T Iltz, Alexandria M Osborn, Teryn M Koch, Kate J Grint, William M Herron, Jaime G Troester, Brandon W Gannon, Dane M Bowder, Erin L Doyle

Many mycobacteriophages were isolated from soil samples collected around the Doane University Crete campus in August of 2017 using Mycobacterium smegmatis as the host bacteria. The phages were isolated from enriched soil samples. Harvested lysates were used for TEM imaging and DNA extraction. TEM imaging showed a high percentage of myoviridae. Two phages, Bread and FudgeTart, were selected for DNA sequencing based on morphology. Sequencing confirmed that both Bread and Fudgetart were lytic phages belonging to the C1 subcluster.

Bread's genome is 153,796 bp in length with 225 ORFs, 79 of which have identified functions, and 32 tRNAs. Fudgetart's genome is 154,658 bp in length with 228 ORFs, 78 of which have identified functions, and 30 tRNAs. Both genomes also contained a single tmRNA.

10th Annual SEA-PHAGES Symposium Abstract

Poster #031

Dominican College of Blauvelt
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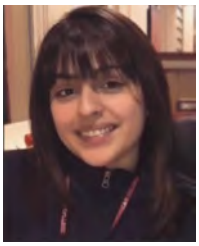
Katherine Clarke



Stefanie Moncayo

Biodiversity of Mycobacteriophages Isolated From Soil

Katherine Clarke, **Stefanie Moncayo**, Bernadette Connors, Regina Alvarez

In the current study, five mycobacteriophages from the soil were isolated and their genomes were sequenced, and subsequently annotated. Mycobacteriophage are viruses that infect members of the genus Mycobacterium, and are commonly found in soil from myriad environments. Soil samples from Rockland and Bergen Counties were used as the source for these isolations, and all bacteriophage were obtained through direct isolation. The isolated phages were characterized using restriction endonuclease digestion followed by gel electrophoresis. Six genomic DNA samples were submitted for sequencing to the Pittsburgh Bacteriophage Institute for sequencing. The phages discovered by students at Dominican College included cluster J (Zelink) and subclusters B1 (Gophee), B3 (Tydolla), F1 (Spikelee), and K4 (Patt) phages. Genome annotations were completed for all of the five isolates using DNA Master, Glimmer, GeneMark, BLAST, HHPred, Starterator, and Phamerator to identify start sites and assign putative functions. The proportion of the genes with function was determined, and the gene content similarity was analyzed. For Spikelee, 28.83% of the genes were found to have a function. Gophee and Tydolla, on the contrary, had 96.12% and 92.16% of genes with no known function. Gophee (B1) and Tydolla (B3) displayed a significant amount of similarity in terms of gene content
(29.3%), while gene content similarities between F1 phage found by students from 2016-2018 at Dominican College (EleanorGeorge, Mattes, Spikelee) ranged from 42-50%. This is a relatively narrow range of similarities compared with that of a random sample of 10 phages on the Actinobacteriophage Database (29-64%). Immunity testing with the Xeno lysogen and experimental analysis of infectivity in various concentrations of CaCl2 (0.1-10mM) and incubation in a range of temperatures (22-42ºC) revealed a variety of phenotypes related to these environmental factors, which may be related to these genetic differences. As a result of researching bacteriophages, we have been able to make observations about the biodiversity of acellular life, including variations in life cycle and speed of reproduction amongst bacteriophages, genomic architecture, and phenotypes. In addition to this, we studied the practical qualities of bacteriophages, which included adaptations and the ability to infect bacteria. We also found that the successful development of bacteriophages required ideal temperature and CaCl2. Further research continues to be conducted on these bacteriophage, including an analysis of their ability to migrate antibiotic resistance genes between host cells.

10th Annual SEA-PHAGES Symposium Abstract

Poster #073

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Apollonia Quiros

Analysis of Four F1 Cluster Mycobacteriophages: A True Phage Phrenzy!

Apollonia Quiros, Matan Ben Abou, Abhinaya Bharath, Akash Bhat, Ishaan Bhatt, Praneetha Bheemarasetty, Madison Bockol, Meredith Anne Capuli, Zachary Cataline, Upoma Chakraborty, Patrick Chang, Jessica Chase, Amanda Chen, Jackie Chen, Nina Cheng, Hannah Cherusseril, Justin Cicarelli, Caitlin Clifford, Cara DePiano, Nihar Desai, Leah Dobossy, Skylar Driscoll, Jinie Eom, Rahul Gandhi, Jakub Gocal, Saksham Goel, Alvin Guan, Zachary Gulgule, Natalie Gundling, Selam Haile, Ashley Harkins, Lucy Helgren, Samir Jambhekar, Hannah Johnson, Rohan Joshi, Shraddha Kamat, Akriti Keswani, Jonathan Khoo, Un Bi Kim, Victoria Koa, Merlin Kochunilathil, Hannah Kostan, Kaylee Krapp, Prem Krishnaprasad, Zhuo Kuang, Sophie Lawrence, Hannah Lee, Katherine Li, Angelo Lodato, Richard Lu, Gabriella Macera, My Mai, Shreya Mandloi, Rohil Mediratta, Sreya Muchivolu, Tania Mulherkar, Mekha Nair, Emily Navarreto, Chandler Olson, Angele Oye-Mba, Victoria Palochik, Chanyeong Park, Dhwanil Patel, Radhika Patel, Riya Patel, Kenil Patel, Uyen Phan, Neha Puttagunta, Natasha Reddy, Joy Rosenberg, Megha Sangam, Mitali Shah, Saloni Shah, Alexandra Silvestri, Maria Sims, Shannon Smith, Allison Tran, Alyssa Truxon, Najia Uddin, Serin Varughese, Shannon Smith, Allison Tran, Alyssa Truxon, Najia Uddin, Serin Varughese, Kanyinsola Yoloye, Nari Yoon, Eleanor Young, Justin Yuen, Nathan Zhang, Arden Edgerton, Christine Tang, Ritu Dalia, Susan Gurney, Alison Moyer

Drexel University undergraduates isolated and purified 89 unique bacteriophages that infect *Mycobacterium smegmatis* mc^2 155 in the Fall of 2017; and have successfully completed a third year in the SEA PHAGES program. From these, eight phage genomes were sequenced, and represent three different clusters and subclusters, including four F1 phages: JoeyJr, Nimbo, Phappiness, and QuickMath. These sequenced genomes were annotated throughout the Winter quarter. Genomic comparisons with other F1 phages indicate that QuickMath has the highest level of diversity compared to JoeyJr, Nimbo and PHappiness. The highest sequence similarity observed for QuickMath is 83% coverage with phage Kimberlium. In the Spring quarter students conducted independent wet lab research projects to characterize the growth patterns of these F1 phages under varying conditions. Experimentation involved testing differing growth variables such as incubation temperature, pH, ion source in phage buffer, exposure to different proteins during infection, and growth in the presence of various sugars. In addition, because F1 cluster phages contain the gene encoding for integrase, they can potentially form stable lysogens. Therefore, creating and testing lysogens was explored.

10th Annual SEA-PHAGES Symposium Abstract

Poster #029

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Betiel M Amanuel

Timothy F Hall Jr.

All the Phuss about Gordonia Phages: Exploration of Three Novel Gordonia terrae Bacteriophages: Horus, Octobien14 and IDyn

Betiel M Amanuel, **Timothy F Hall Jr.**, Charles J Anspach, Rebekah J Chiquito, Jailyn M Gales, Khadijah Hotaki, Brenda Lozano, Peter Said, Steven A Leadon, Marie P Fogarty

Bacteriophages are viruses that infect bacteria and in most cases cause lysis by disrupting host metabolism. This year Durham Tech collected three bacteriophages capable of infecting *Gordonia terrae* 3612 - Octobien14, IDyn and Horus. Octobien14, a singleton, was found by direct isolation and contains 132 genes. Horus is assigned to subcluster DN1 and contains 105 genes and IDyn is a member of subcluster CR4 and contains 89 genes. Horus and IDyn were both found by enrichment isolation. Genome annotations were carried out using DNA Master and PECAAN. Following genome annotation, some additional biological and bioinformatics experiments were carried out. In an effort to improve bacteriophage DNA yields for future semesters, we compared DNA yield from phenol:chloroform extraction with the Promega Wizard® Clean-Up Kit method. Phenol-chloroform extraction yielded a three-fold higher DNA concentration and improved purity and integrity. Phage survival following dehydration in the absence of host bacteria for up to three weeks indicated that plaque formation for IDyn dropped 10^6-fold, while Octobien14 was reduced 10^2-fold suggesting that Octobien14 is more resilient under these conditions. The presence of the integrase gene in Octobien14 and Horus prompted us to test ability of these phages to form lysogens and to search for the integration sites (attP sites) in their genomes. Results from patch assays and liquid phage release assays suggest that Horus and Octobien14 are capable of creating stable lysogens. Initial sensitivity assays demonstrated that Octobien14 and Horus are unable to infect their own respective lysogens. Regions of homology between phage and host were identified for Octobien14 at 60989-61699 bp and between 32484-32536 bp in Horus. For Horus, this region is adjacent to the integrase gene and shows homology with a region in between (but not within) two tRNAs in the host genome. tRNA enumeration revealed that Octobien14 had six, Horus had one, and IDyn had no annotated tRNAs. Bioinformatics analysis included investigation of possible promoters and terminators, and identification of transmembrane proteins. Using the program SOSUI, we predicted that IDyn had nine transmembrane proteins, two with assigned functions of Holin and Tape Measure and five with unknown function. Seventeen transmembrane proteins were identified for Horus, with three having assigned functions of Holin, Tape Measure, and Membrane Protein Band-7 Like, and the remaining fourteen lacking an annotated function. The transmembrane helices for these proteins were confirmed using TMHMM (through PECAAN). Indication of transmembrane proteins provides some functional insight for genes with unknown function. The exploration of phages Horus, Octobien14, and IDyn increases our knowledge about Gordonia terrae bacteriophages and their host, which may ultimately be useful in a multitude of applications such as phage therapy and genetic engineering.

10th Annual SEA-PHAGES Symposium Abstract

Poster #085

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Isolation and Characterization of Four Novel Mycobacterium Phages from the Sandhills Region of North Carolina

Lorette Rivera-Lugo, Wendy Adams, Brittany Anderson, Korina Baldazotes, Tameka Colden, Chaselyn Ladd, Christopher Livingston, Jamahl Martin, Troi Miller, Kelsey Mohr, Gorpu Ngumah, Naimah Page, Daniya Pate, Loletha Porter, Tilar Stanford, Sangita Subedi, Jza-Qera Thompson, Nickolas Torres, Cynthia Waller, Ariana Wilson, Kristen Delaney Nguyen

Bacteriophages are the most numerous microbes in the biome and display massive genetic diversity. Thirteen phages capable of replication in Mycobacterium smegmatis mc2155 were isolated in the Sandhills region of North Carolina. Four phages (Datway, FrayBell, Leogania, and Lephleur) were sequenced at the Pittsburgh Bacteriophage Institute. All four of the sequenced phages were novel. Fraybell demonstrated myoviridae morphology and Leogania was siphoviridae. Datway and Leogania appear to be temperate phages due to their turbid plaques and the presence of lysogeny-associated genes such as integrase and immunity repressor. Continuing work will involve seeking related phages from the same geographic region using Datway and Leogania lysogens.

10th Annual SEA-PHAGES Symposium Abstract

Poster #005

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IDENTIFICATION OF POSSIBLE PHAGE QUORUM SENSING SYSTEMS

Shane Stober, Dana Indihar, Davidson Nougues, Kaylee Clifford, Frances Elmore, Montana Knight, Kimchi Le, Debora Lobaina, Amy Salema, Kenneth Sweeney, Thomas Truong, Sharon Isern, Scott Michael

Quorum sensing is the production of group behavior in bacterial cells through the release of extra-cellular chemical autoinducers. When autoinducers reach a certain concentration, cells will sense this and engage in coordinated gene expression resulting in a change in group behavior. Well known examples include the production of flagella, toxins, and formation of biofilms. In a recently published paper, it was reported that bacteriophage also communicate with each other using quorum sensing systems to regulate phage lysis versus lysogeny (Erez et al, 2017). The authors named these "Arbitrium" systems in bacteriophage and described the key genetic elements involved in the Bacillus phage Phi3T. The authors showed that the gene for a small, secreted protein, arbitriumP (aimP) that functions as the extra-cellular autoinducer, follows the gene for an intracellular peptide receptor helix-turn-helix (HTH) protein (aimR). High concentrations of aimP cause aimR to dimerize and bind to a DNA regulatory site just downstream of the aimR gene, resulting in increased transcription of a downstream aimX protein, which down regulates lysis through an unknown mechanism. To find similar genetic elements in *Actinobacteria* phage, we searched annotated genomes in PhagesDB looking for HTH protein genes followed by a gap region. We hypothesized that the small aimP protein genes may not have been annotated, but might be found in the gap region along with the DNA regulatory sites for aimR binding, followed by a putative aimX gene. Most phage did not have the genetic pattern described. However, of the 50 *M. smegmatis* cluster C1 phage (all myoviruses) currently in GenBank, all 50 contain HTH protein genes followed by a gap containing one or more small, un-annotated ORFS with strong N-terminal secretory signals. Further, we identified 33 out of 70 phage in the Bacillus cluster C (myoviruses) with similar HTH/gap patterns, containing small, un-annotated ORFS in the gap regions. Curiously, none of the *Bacillus* phage small ORFS had N-terminal secretory signals, but instead had internal type VII secretory signals. No type VII secretory signals were found in the *M. smegmatis* cluster C1 phage small ORFS. We speculate that these may represent phage-encoded quorum sensing genes.

10th Annual SEA-PHAGES Symposium Abstract

Poster #033

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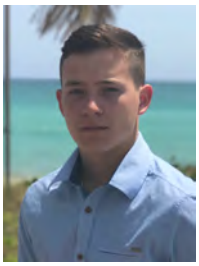

Carlos Sandoval

Juan Santana

Comparison of Cas4 homologs and genome architecture patterns in two Bacillus bacteriophages

Carlos Sandoval, **Juan Santana**, Joseph Ahrens, Katherine Dougan, Mauricio Rodriguez-Lanetty

Bacillus cereus is a Gram-positive soil bacterium, commonly known for causing foodborne illness in humans as well as its occasional use in probiotics for animals. Research has revealed extensive evidence of horizontal transfer among bacteriophage and host genes, mediated by mechanisms such as transduction, resulting in mosaic phage genome architectures. Here, we evaluate the architectures and specific gene content of two *Bacillus* phage genomes, Rocco and Phamous, using conventional analytical tools such as DNAMaster for functional gene annotation, PHASTER for genome architecture analyses, and MEGA for aligning homologous sequences and constructing phylogenetic trees. Comparative genomics between the two *Bacillus* phages not only reveal similarities but also differences, such as two intact prophage regions in Rocco as opposed to one region in Phamous. Additionally, open reading frame (ORF) 64 in Phamous can be classified as a putative homolog of Cas4 as demonstrated in both a Bayesian inference and maximum likelihood tree. Phylogenetic data indicates ORF 64 is more closely related to bacterial homologs than phage homologs which can be regarded as evidence of horizontal transfer. This possibility is further supported by research indicating that the Cas4 gene is mobile. Future research can focus on the expansion of phage space of firmicute hosts and further investigation of the evolution of the CRISPR-Cas complex in bacteria and phages.

10th Annual SEA-PHAGES Symposium Abstract

Poster #106

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Brian Nguyen

Isolation and Characterization of Mycobacteriophages Podrick and Pivoine

Mohamad Alayouni, Christina Baker, Sianna Burnett, Yordanos Degefu, Erik Escobar, Baharak Farzamnia, Joseph Glapion, Mahra Jabeen, Briana Marcy, Fabio Martinez, Karina Mejia-Cardenas, **Brian Nguyen**, Noushin Noursalehi, Chau Pham, Edwin Portillo, Haris Sekandiri, Tamia Shazer, Alan Tu, Jacqueline Woods, Zelikha Yosufzai, Yasir Zafar, Jen Jones, Bonnie Madden, Anne Scherer

Here we describe two novel bacteriophages that were isolated in Fairfax, VA using Mycobacterium smegmatis mc2155 as the host bacterium. Both genomes were sequenced using Illumina sequencing at the Pittsburgh Bacteriophage Institute.

One of the phages isolated, Podrick (GoT character), is a novel Siphoviridae mycobacteriophage that was isolated from a mulch sample using the enrichment method. Podrick, a subcluster B1 phage, has a 68,406 bp genome with a GC content of 66.4%. Podrick contains a putative RuvC-like Resolvase, which potentially functions in the resolution of both Holliday and branched DNA junctions during DNA recombination. We also found a 2 putative Helicase subunits and a Primase, which function during DNA replication. We will also present a comparison the Podrick and Phareon genomes. Phareon is a B1 cluster mycobacteriophage isolated last year from the same flower bed.

Our second phage, Pivoine (French for Peony), is a novel Myoviridae mycobacteriophage that was isolated from a residential flower bed using the enrichment method. Pivoine, a subcluster C1 phage, has 155,256 bp genome with a GC content of 64.7%. The genome of Pivoine contains many genes with assumed functions including: DNA primase, Helicase, Resolvases, ssDNA Binding Proteins, Kinases, DnaJ-like Chaperonin, DNA-directed RNA Polymerase and many more! We will also describe a -1 frameshift present in the tail assembly chaperone gene preceding the tapemeasure gene.

10th Annual SEA-PHAGES Symposium Abstract

Poster #041

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Joseph Nichols

Rachael Schoonover

The isolation of 205 bacteriophages on Microbacterium foliorum: Some struggles and some successes

Joseph Nichols, **Rachael Schoonover**, 396 students in the BIOL 105L lab course, Kirk Anders, Ann-Scott Ettinger, Marianne Poxleitner, Amanda Braley

With the hope of discovering new bacteriophages and expanding our understanding of phage diversity, we searched for phages that could grow on *Microbacterium foliorum* NRRL B-24224 SEA in the Phage Discovery Lab course, BIOL 105L, at Gonzaga University during the academic year 2017-2018. We purified and characterized 205 phage isolates from enrichment cultures of soil, grass, acorn, moss, bark and water samples. The phages were propagated at room temperature (22-25 deg C) during isolation, purification, and amplification. At this temperature, lawns and plaques could be observed after 24 hours, but plaques continued to grow in size over the next 3-4 days. Compared to previous students' experiences with *M. smegmatis* phages, we found that a higher percentage of isolates were difficult to work with. A number of us found it difficult to prepare a lysate with a titer greater than 10^8 pfu/ml, a number of phages appeared distorted in TEM images, and a large percentage of lysates produced DNA that appeared to be fragmented or degraded. We report here on our experiences with these experiments, and our efforts to troubleshoot working with *M. foliorum* phages.

10th Annual SEA-PHAGES Symposium Abstract

Poster #108

Howard Hughes Medical Insitute
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Gene Detectives: Exploring Phage Gene Function

Danielle Heller, Ilzat Ali, Aleem Mohamed, Padraig Deighan, Maria Gainey, Dmitri Mavrodi, Jamie Wallen, Viknesh Sivanathan

In the past decade, the SEA-PHAGES program has isolated and characterized thousands of phages, providing a wealth of genetic data to the broader scientific community. These data have already furthered our understanding of the diversity and evolutionary history of phage populations; they have also revealed the vast unknown contained within phage genomes. In their 2015 comparative genomic analysis, Pope et al. reported that out of the 5,205 gene phamilies found in 627 mycobacteriophage genomes, approximately 75% have no assignable function. Characterizing the functions of these phage genes is an important endeavor that will provide greater insight into phage-host and phage-phage dynamics and potentially inspire advances in therapeutics and molecular technologies.

The SEA is currently developing research tools and methods that will allow the SEA community to utilize this expanse of genetic data and investigate phage gene functions. These include:

1) A workflow for the construction of phage gene expression libraries that can be utilized in various bacterial host expression assays. This workflow was successfully implemented as "pilot projects" at three undergraduate institutions, generating four phage gene libraries that were then systematically analyzed to identify a subset of genes that inhibit M. smegmatis growth.

2) A bacterial two-hybrid selection platform that allows for the rapid testing of over a million pairwise interactions between a phage protein of interest and a library of M. smegmatis protein fragments, thus enabling the identification of putative phage-host interactions using straightforward yet powerful genetic techniques.

Part of this workflow was successfully implemented as "pilot projects" at three undergraduate institutions, generating four phage gene libraries that were then systematically analyzed to identify a subset of genes that inhibit M. smegmatis growth. Together, these phenotypic and interactome analyses can open the door to new hypotheses and mechanistic questions for the community to explore. We believe that by collecting evidence piece-by-piece, the SEA can be an important driver in the elucidation of phage gene function.

10th Annual SEA-PHAGES Symposium Abstract

Poster #109

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A Workflow for Microbial Community Analysis

Joslynn Lee, Priscilla Kobi, Emily Davis, Danielle Heller, Viknesh Sivanathan

The development of the course-based research experience (CRE) aims to increase undergraduate interest and retention in the biological sciences through immediate immersion in authentic, valuable, yet accessible research. Working with HHMI's SEA, I am developing a microbiome CRE to enable undergraduate researchers to study the microbial community of environmental samples using 16S rRNA amplicon sequencing. This one-semester course is designed in modules for students to start by simply sample collection but advance to running a microbiome analysis pipeline. Presented in the pilot study to profile the microbial community of commercial soils, mulch, garden bed and non-garden bed samples. The microbiome workflow presented can be used to both develop, test, and support hypotheses, while provide undergraduate researchers important microbiology and computational biology skills.

10th Annual SEA-PHAGES Symposium Abstract

Poster #112

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New Actinobacterial Hosts & A New DNA Isolation Protocol

Emily Davis, Priscilla Kobi, Aleem Mohamed, Viknesh Sivanathan

New Actinobacterial Hosts: The scientific goal of SEA-PHAGES is to explore the biodiversity of actinobacteriophages. As we expand from isolating and studying mycobacteriophages to actinobacteriophages, the careful "piloting" of new actinobacterial hosts can facilitate this process. Here, we present data from piloting several actinobacterial hosts, the most recent being Gordonia rubripertincta and Microbacterium testaceum. This data is presented in contrast to working with Mycobacterium smegmatis. Come see why making the switch to working with Actinobacteria other than M. smegmatis is easy, cheap, and scientifically rewarding.

New DNA Isolation Protocol: When a titer greater than 10^9 pfu/ml cannot be obtained for a phage lysate, the lysate can be concentrated by precipitating phage. This is typically done using PEG. Here, we compare and contrast an adapted protocol for precipitating phage using zinc chloride to that using PEG. Phage precipitation with zinc chloride is faster and less cumbersome than when using PEG, but requires some tricks. Come to our poster to learn more.

10th Annual SEA-PHAGES Symposium Abstract

Poster #027

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Boluwatito Oladeinde

Isolation and Characterization of Fourteen Siphoviridae and Myoviridae Isolated from The Campus of Howard University

Boluwatito Oladeinde, Jalen Jean -Baptist , Kennis Booker, Naji Cameron, Adaora Ekwunife, Ehab Elhag, Kiana Fludd, SeighViance Givens, Quinlyn Highsmith, NyAsia Kelley , Camryn Lam, Briana Louis, Chidera Nwude-Jacobs, Ebonee Major, Charity Onwuchekwa, Students of HU- PHAGES 2017-2018, Kristodea Boadum, Michelle Lourds Fernando, Madison Moore, Jerome Oliver, Adebiyi Sobitan, Michael Smith, Roy Swagota, Adrian Allen, Mary Ayuk, Leon Dickson, Somiranjan Ghosh, Ayele Gugssa, , Hemayet Ullah, Winston Anderson, Courtney Robinson

Bacteriophages, or phages, may be utilized to study viral ecology, epidemiology, and enhance the development of therapeutics. In the current study, enrichment and direct culturing methods, in conjunction with host Mycobacterium smegmatis MC^2155, were used to elucidate phage diversity in soil samples collected from the campus of Howard University. Over 100 phages were purified using standard isolation protocols. Subsequently, lysates were generated, and DNA was extracted. Fourteen genomes were sequenced (Pittsburgh Bacteriophage Institute), using Illumina Sequencing. This revealed that the sample phages belong to four clusters: A (phage Naji); B (phages Bishoperium, CamL, Mecca, Samaymay, Omniscient, QueenBeane and West99); C (Basquiat, Chargie21, Fludd, JustHall and Naija), and Q (DeepSoil15). The genome sizes range from 53,746 to 154,456 bp with G+C contents ranging from 63.5 to 68.9%. Morphotypes were identified as Siphoviridae and Myoviridae. Annotation of the genomes is currently underway. Additionally, all of phages in the 2017-2018 collection are undergoing assays to determine patterns of phage sensitivity/insensitivity—immunity using wildtype *M. smegmatis* MC^2155 and lysogens generated from the collection.

Key words: Mycobacteriophages, Soil, *Mycobacterium smegmatis*, Siphoviridae and Myoviridae

10th Annual SEA-PHAGES Symposium Abstract

Poster #090

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Rosemary Josenkoski

So a J Phage, a B Phage, and a Couple of Rhodobacter Phages Walk Into a Plaque Assay….

Rosemary Josenkoski, Julie Xu, Jory Vance, Andrew Runkle, Ria Patel, Sydney Longfellow, Gregory James, Carlie Haagen, Lilia Garcia, Megan Frederick, Brooke Dominski, Jaeden Danko, Julia Chen, Meghan Bowler, Zaain Ahmad, David Bollivar, Richard Alvey

During the fall semester of 2017, students at Illinois Wesleyan University were tasked with the important mission of extracting bacteriophages from soil and water samples. Once samples were gathered, the phage expedition then centered on isolating and clustering these bacteriophages. Attempting to cluster the phages included methods of lysogen testing and polymerase chain reaction, along with qualitative comparisons of plaque assays and transmission electron microscope morphologies. Ultimately, the phages we found to be the most interesting were submitted for genomic sequencing. The two sequenced phages that infected *Mycobacterium smegmatis* were Constella and Doddsville. Constella was clustered as a J phage, a rare phage containing a large genome of about 110 kilobase pairs. Constella is a *Siphoviridae* that produces small, turbid plaques, an indication that it is capable of producing a lysogen. Doddsville was discovered to be a B1 phage, a more common phage having a genome about 68,000 base pairs in length. Doddsville is also a *Siphoviridae* but yields small, clear plaques on a plaque assay, suggesting that it is virulent. Both Doddsville and Constella had their genomes annotated with programs including DNA Master and PECAAN. Additional work focused on isolating *Rhodobacter capsulatus* phages, which have only been discovered in aquatic locations. Phages that infect *R.capsulatus* were hunted for in an attempt to possibly find more unique phages through the use of this alternative bacterial host. Indeed, this goal was accomplished by the discovery of SchulyerLagoon, a potentially single-stranded DNA *Microviridae* phage. Additionally, the finding of Xuper, a *R.capsulatus* phage with a prolate head and extraordinarily long tail of approximately 300 nm and capsid diameter length-wise of about 150 nm, was noteworthy. Xuper was sent for genomic sequencing where it was clustered as a singleton and subsequently had its genome annotated.

10th Annual SEA-PHAGES Symposium Abstract

Poster#053

Indian River State College
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Rhode Dorissaint

Joshua Moreno

Isolation and characterization of actinobacteriophage from the Treasure Coast of Florida

Rhode Dorissaint, **Joshua Moreno**, Brandon Bryant, Matthew Parks, Jeffrey Garofalo, Samuel Marcillion, Jordan Norus, Jacyln Kuiack, John Peroza, Ariana Burch, Kelly Wilse, Tom D'Elia, Helen Wiersma-Koch

During the 2017-2018 academic year, Indian River State College (IRSC) students successfully isolated 11 bacteriophage using Mycobacterium smegmatis mc2 155 as a host and one bacteriophage using Microbacterium folorarium as a host. Four phage genomes were sequenced. These four phage were the most diverse group of phage that has ever been isolated at IRSC. Mycobacteriophage Fowlmouth, Lizziana, and BoostSeason belong to clusters AC, K, and F respectively while Microbacteriophage Noelani belongs to cluster EE. Phage Fowlmouth was isolated from soil found in a chicken coop. It belongs to cluster AC, of which there is only one other member, Cuke. Phylogenetic analysis of the tapemeasure protein shows that the AC cluster is most closely related to cluster R. The resolvase gene was selected for further study. This gene has an unusual evolutionary history, illustrating the mosaic nature of phage genomes. Phage Lizziana was found to be in subcluster F1. This phage is most closely related to phage ShiLan. The immunity cassette was selected for further analysis and it was found that Lizziana had a Y-integrase and repressor, but lacked the antirepressor found in other F1 phage. Phage BoostSeason is one of nine phage in the K2 subcluster of phages and shares high similarity to phage Mufasa. K2 cluster phages are unique in their ability to infect both M. smegmatis and M. tuberculosis. Conserved regions in Lysin A, Lysin B, Holin, and the minor tail proteins allow for this cross-infectivity. These proteins in BoostSeason, other K2 phage, and A phage were investigated for evolutionary relationships. Finally, the genome of Microbacteriophage Noelani was sequenced. This phage belongs to the EE cluster, along with 11 other phage. Noelani's genome is small, having only 24 ORFS, eight of which have a known function. One protein of particular interest was the LSR2 DNA binding protein. Homology modeling predicted that this protein is a zinc-finger. Since the phage isolated belong to clusters with few members, their characterization will help us better understand the diversity of actinobacteriophage and provide a valuable resource for comparative analysis.

10th Annual SEA-PHAGES Symposium Abstract

Poster #019

Indiana University of Pennsylvania
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Maroc7: a Mycobacterium phage from Utah

Tori Swartz, **Sarah Shiber**, Carl Luciano, Seema Bharathan, Cuong Q Diep

Bacteriophages are viruses that infect bacteria. Understanding their biology and diversity could lead to treatments for antibiotics resistance and diseases such as cystic fibrosis. Phages infect very specific hosts, which reduces side effects for any potential therapy. Here, we annotate a Mycobacterium smegmatis temperate phage called Maroc7, which was isolated in 2010 by students at Brigham Young University. Maroc7 belongs to the Subcluster A1, which has a -1 frameshift in the tail assembly chaperone (gp21-22). Interestingly, gp34 (minor tail protein) has a cytosine insertion that resulted in a premature stop codon in the middle of the gene. Therefore, the second half of the gene (gp35) was called as a new gene with a 74 bp overlap with gp34. This suggests that gp34 is a nonessential gene. It would be interesting to determine experimentally whether the truncated protein is produced or not.

10th Annual SEA-PHAGES Symposium Abstract

Poster #094

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Inna Couri

Gretchen Swift

Identification and Characterization of mycobacteriophage Olga

Inna Couri, **Gretchen Swift**, Nick Peters, Nancy Noury

Olga was isolated by Inna Couri, and is a cluster G1 phage. It was discovered in a flower pot on campus at Iowa State University by direct isolation using *Mycobacterium smegmatis* as a host. This phage has a genome that is 41902 bases in length and a CG content of 66.6%, and transmission electron microscopy revealed the morphotype as Siphoviridae. Initial Blast analysis of the genome showed that phages Annihilator and Avrafan were most similar. Annotation of Olga's genome indicated that there were 62 genes and no transfer RNAs. While working with the phage it explicitly expressed the lytic life cycle, but as the genome was annotated we found that under certain conditions this phage could possibly express a lysogenic cycle due to genes particular to the lysogenic process. There were also additional genes that made Olga unique from its closest relatives, which revealed some potentially interesting evolutionary events in progress for this phage. Upon completion of annotation we were not able to assign a function to every gene that we identified, which is typical for phage genomes. Though this phage has many close relatives found in the database, here we describe some interesting elements that set Olga apart from its relatives.

10th Annual SEA-PHAGES Symposium Abstract

Poster #081

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Emily Bordelon

Ryan Showalter

The Great Phage Escape

Imraan Adat, Brian Anderson, **Emily Bordelon**, Justin Herman, Akaash Patel, Claire Ravenburg, Kyle Rubino, **Ryan Showalter**, Ethan Tyler, Chih Hao Huang, Daniel Mingo

Phage therapy is coming to the forefront as a resource to combat the crisis of intractable diseases caused by antibiotic resistant bacteria. Two important genera receiving attention are *Pseudomonas, Bacillus,* and *Staphyloccocus*. We used surrogates of the pathogens, *P. aeruginosa*, *B. cereus* and *S. aureus* as hosts for our viral discovery classes' phage hunting, which yielded 12, 1, and 6 phages on their respective hosts. The genomes of three bacteriophages were analyzed with a specific focus on their lysis genes. After isolating and purifying these phages from soil in Rockingham County, VA, the DNA was sequenced and analyzed using standard bioinformatics programs. Waldo5, infecting *P. putida*, had a genome of 41,195 bases, including a 219 base-pair direct terminal repeat, while Ray17, infecting *B. amyloliquifasciens* spp EMHS, had a genome of 43,733 bases and was circularly permuted. BruegS6, infecting *S. scuiri* spp 203, was a small podovirus with genome of 18,368 bases and defined ends with attached proteins. Holin and endolysin genes were identified in these phages, though their gene arrangements varied. In Ray17, the two genes were found next to each other, a common arrangement in phages, but in Waldo5, there were 30 genes between the holin and the endolysin. In BruegS5, the presumed endolysin and holin genes were immediately adjacent to the tail fiber and tail lysin genes. We are particularly interested in the endolysins as potential antibiotics that kill bacteria from the outside. Future work will include cloning and expressing the endolysin genes and testing for lysis on bacteria.

10th Annual SEA-PHAGES Symposium Abstract

Poster #097

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Andrew J Lopuch

Isolation and Genomic Characterization of Streptomyces griseus phage TomSawyer

Andrew J Lopuch, Steven G Cresawn

Our study involves bacteriophages found in soil and their interaction with *Streptomyces griseus*, the host bacterium chosen for our study. Relative to many other host bacteria in the SEA-PHAGES program, a small number of *S. griseus* phages have been sequenced. Additionally, a high proportion of these phages are singletons, which increased our chances of finding a substantially novel phage. We isolated two phages, TomSawyer and Wipeout, whose genomes were sequenced and then annotated. Genome annotation involved use of DNA Master, Phamerator.org, PhagesDB.org, GeneMark, and HHPred.

TomSawyer and Wipeout both belong to the BE2 subcluster and have genome lengths of 133,961 bp and 132,935 bp, respectively. They are approximately 99% identical to one another. The genome of each of these phages has a GC content of approximately 49.3%, which is markedly different than the host's 72% GC content. The reason for this is unclear, though we hypothesize that the 43 predicted phage-encoded tRNA genes may allow for productive translation within a cell with dissimilar codon bias relative to that of the phages.

All BE2 subcluster phages possess long direct terminal repeats (12,182 bp in TomSawyer and 12,204 bp in Wipeout), and both TomSawyer and Wipeout are closely related to a likely prophage found in a strain of our host bacterium, *S. griseus* JV178. We speculate that our phages may be temperate as well, although we have thus far been unable to locate integrase or immunity repressor genes in either genome. Other phages that are closely related to TomSawyer are Mildred (78% identity), Parradiddles (77% identity), and Warpy (77% identity).

10th Annual SEA-PHAGES Symposium Abstract

Poster #074

Johns Hopkins University
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Corresponding Faculty Member: Emily Fisher

Johns Hopkins Blue J(cluster)s: Ejimix and Shiloh(s) expand the diversity of mycobacteriophages on campus.

Taskinuddin Forkan, Emily J Fisher, Jaemie Bennett, JHU PhageHunters

Since 2008, the J cluster has grown to include 34 sequenced phages including 4 new genomes in the last year. One of these new J-cluster genomes is that of Ejimix, a temperate siphoviridae isolated on the campus of Johns Hopkins University. Like other J-cluster genomes, the Ejimix genome is nearly twice as long as the average siphoviridae genome, comprised of 111,924 base pairs and 242 genes. Ejimix shows significant homology and synteny with other J-cluster phages BAKA and Wanda and has the programmed frameshift and intron found in other genomes in the J cluster. We highlight several regions in which the Ejimix genome departs from synteny with these related genomes. We also examine several instances in which Glimmer and GeneMark called for overlapping forward and reverse genes and present our determinations. Ejimix is a temperate phage, which distinguishes it from Shiloh-1 and -2, J-cluster phages isolated together at JHU in 2016. This work adds to our understanding of conservation among these surprisingly long siphoviridae mycobacteriophage genomes and offers guidance for future J-cluster annotation projects.

10th Annual SEA-PHAGES Symposium Abstract

Poster #040

Kansas State University
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Flint Hills Phages – Netting the Jumping Gene of Pollywog and other F1 Mycobacteriophages

Ayanna M Castro-Ross, Lucas G Ernzen, Victoria D Dorman, Connor M Horn, Grant R Hughes, Benjamin D Hulsing, Ana C Martinez, Adrienne E Pohl, Martha Smith Caldas, Christopher D Herren

For 3 years, Kansas State University has been isolating mycobacteriophages from enriched soil samples in Mycobacterium smegmatis strain mc2155 at 37°C. This year's project isolated Pollywog, an F1 subcluster mycobacteriophage. Currently there are 146 subcluster F1 phages that average 104 genes over 57,428 nucleotides with no encoded tRNA genes. Pollywog encodes 104 genes over 58,397 base pairs with no encoded tRNAs. In annotation of Pollywog, it was noticed that Pollywog encodes an appropriately located integrase gene (Y-int) for lysogeny and an additional transposase gene. It is currently not hypothesized that mycobacteriophages utilize transposase mechanisms for chromosomal integration, as seen in Mu phage infection (1), but instead rely on integrase and att sites to establish lysogeny (2). Upon further investigation, it was determined that a total of 21 subcluster F1 phages (14% of all F1 phages), but no other subcluster of mycobacteriophages, contain this Pham of transposase gene. Genomic location of transposase corresponds to one of 2 sites found in 20 of the 21 identified F1 phages and a third unique site found in only one of the F1 phages. In 11 of the F1 phages, transposase is found in the reverse orientation 0-1 gene 5' to the usual integrase gene. In 9 other F1 phages, transposase in located in the reverse orientation immediately 3' to the tapemeasure gene on the left arm of the genome. In a single F1 phage, transposase is located on the far right arm in the forward orientation. Phamerator, Clustal Omega and other gene alignment tools were used to both map the ends of the transposons in each of the identified F1 phages, including Pollywog, and to compare differences within the transposase genes themselves.

10th Annual SEA-PHAGES Symposium Abstract

Poster #016

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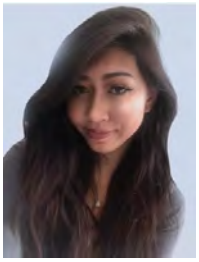

Krystal Thach

Cherrybomb426 is a New Member of the G1 Subcluster of Siphoviridae Phage

Krystal Thach, Sarah Devine*, Lina M Barrio, Taj Boynton, Andrew M Ciliberto, Courtney A Elliott, Ryan J Guckin, Jay T Jennings, Connor F Merrill, Keyla N Peralta, Christoff P Ras, Christina M Rice, Madeline K Rodahaver, Zachary T Salvatore, Vanessa Ulysse, Mi'Asia S Underwood, Zaire O Whaley, Molly L Wible, Jennifer Chau, Fabian A Bergman, Nancy L Jones, Jason Diaz [* Smith College, Northampton MA]

Antibiotic resistance is a growing crisis in managing bacterial infections. Bacteriophage are a promising alternative as they are uniquely specific to their host and can rapidly evolve in response to bacterial adaptation. Cherrybomb426 is a phage of the Siphoviridae family and infects *Mycobacterium smegmatis*. It was isolated from an enrichment sample by Sarah Devine in 2011. It is a temperate phage, which is different than the lytic phage, because the injected DNA will stay in the bacterium's genome for an extended period of time, and eventually enter the lytic cycle. Students from La Salle University in the Integrated Science, Business, and Technology Department annotated Cherrybomb426. Programs used in this process included DNA Master, Phamerator, Starterator, Genemark, HHPred, and NCBI Blast. Cherrybomb426 is in the G cluster (G1 subcluster). Phamerator was used to compare the sequence of Cherrybomb426 with other similar phages. One phage in particular was LouisV14 which was found by Jordan Hagerty, another La Salle ISBT student. Cherrybomb426 consists of 60 genes and its genome has a length of 41456 base pairs, and an overhang sequence of CCCCATGGCAT. The GC content is 66.7%. Students worked together to tackle issues such as addressing reverse genes and start sites that were not initially agreed upon in the results from the auto-annotation. Our class determined that Cherrybomb426 has 3 reversed genes 32, 33, and 59. According to BLAST, Gene 32 codes for an integrase protein which produces an enzyme that permits the genetic material to be injected into the DNA of the host. Gene 33 codes for an immunity repressor protein that binds to a short specific DNA sequence and regulates the expression of a host gene. Gene 59's authenticity was up for debate for multiple reasons. The gene was found in the reverse direction, which is uncommon but when it does occur, is often accompanied by other genes also going in the reverse direction. Gene 59 was not accompanied by any others and overlapped with the entirety of Gene 60, which is incredibly rare. Gene 59 was blasted on multiple programs and was compared with Phamerator to other phages, and it was finally decided that it was not a real gene. After rounds of BLASTing and comparing the sequence of Cherrybomb426 with similar bacteriophages in the G1 subcluster, we deleted gene 59 and added forward gene 61, which matches genes predicted in this region in other Phamily members. Genes 30 and 3 represented another challenge as Glimmer, GeneMark and Starterator had different start-site predictions. Our BLAST results supported the Starterator start as the best one. The majority of genes have only hypothetical functions; however, for some genes, functions have been established such as minor-tail protein, head to tail connector, major capsid protein, and terminase, among others. The annotation of Cherrybomb426 also includes a programmed translational frameshift.

10th Annual SEA-PHAGES Symposium Abstract

Poster #012

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Rhiannon Abrahams

Steven Tran

Orphams find a home in OneinaGillian while KaiHaiDragon continues moving forward

Rhiannon Abrahams, **Steven Tran**, Daniel Bazan, Bryceson Beglau, Ethan Blaylock, Jessica Choi, Shannon Grewal, Elmira Hernandez, Daniel Kim, Kay Kim, Yumin Lee, Michael Linde, Meagan Lopez, Gillian Miller, Emily Pangalila, Markell Parker, Rachel Specht, Ming Chung Teng, Benjamin Toledo, Houting Yu, Natasha Dean, Arun Muthiah, Arturo Diaz

Undergraduates enrolled in the SEA-PHAGES program at La Sierra University obtained a total of twenty bacteriophages infecting *Microbacterium foliorum*; sixteen phages were found from direct isolation and four were found through enriched isolation. Two direct isolates, KaiHaiDragon and OneinaGillian, were sequenced and annotated.

KaiHaiDragon has a capsid diameter and tail length of 72.2 nm and 124.4 nm, respectively. It was assigned to cluster EC and was experimentally found to be lytic as are all other phages in that cluster. The plaques were clear and 0.1mm in diameter and no integrases were identified during the annotation.The genome is 52,992 base pairs long and 90 genes were indentified. The GC content is 68.9%, which is similar to the GC content of the other phages in that cluster. Of note, all 90 ORFs are transcribed in the forward direction.

OneinaGillian was assigned to cluster EG. Interestingly, OneinaGillian is only one of three phages assigned to that cluster. It has a capsid diameter of 57 nm and tail length of 143 nm. OneinaGillian is 61,703 base pairs long and 101 genes were identified, including a Tyrosine tRNA. 66 genes are transcribed in the reverse direction while the remaining 34 are in the forward direction. 42 genes were orphams, while 58 genes were assigned to a pham when compared to similar genomes.
None of the twenty phages were able to infect Mycobacterium smegmatis. A host range assay is currently being done with three other Actinobacteria species. Moreover, attempts to isolate lysogens were unsuccessful.

10th Annual SEA-PHAGES Symposium Abstract

Poster #020

Lafayette College
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Kimberly Manalang

The Corazón of Lafayette College: An Analysis of Genes 32, DNA methylase, and 35, Terminase

Kimberly Manalang, Driss Camara, Dominique Gordy, Eric Ho, Driss Camara, Dominique Gordy, Eric Ho

Mycobacterial phage, Corazón, was isolated from soil on Lafayette College, Easton PA, campus using the direct method of isolation. This phage was named Corazón, which means 'heart' in Spanish, because students put their 'heart' into this project. Corazón, a Siphoviridae, is a Cluster S mycobacteriophage, one of only 11 members of this group. It has a genomic length of 64,931 base pairs and 63.4% GC content. We analyzed two genes – gene 32, a DNA methylase, and gene 35, a Terminase – to determine their evolutionary lineages and the role of each in the bacteriophage life cycle. The protein sequence of each gene was compared to its homologs in the NCBI Protein database by CLUSTAL OMEGA. Alignment results showed that the DNA methylase protein was only conserved in two regions, whereas the Terminase protein contained 12 conserved regions. There were fewer bacteriophage genomes with a DNA methylase compared to the number of bacteriophage genomes containing a Terminase gene. Why is Terminase more common in bacteriophages than DNA methylase? We hypothesize that the Terminase gene is more common due to its crucial biological function of recognizing newly replicated viral genomes and packaging the genome into the phage head whereas the function of DNA methylase is to protect against additional invading viruses, rather than benefiting the phage.

10th Annual SEA-PHAGES Symposium Abstract

Poster #075

Lehigh University
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Alecia B Rokes

Ryan A Seth

Exploring a SEA of Phages: Insights into Host-Phage Interactions

Alecia B Rokes, **Ryan A Seth**, Russell A Caratenuto III, Cassidy L Drost, Nicholas C Kahler, Nicole A Kirven, Julia C Melehani, Krishna Patel, Matthew C West, Catherine M Mageeney, Hamidu T Mohammed, Margaret A Kenna, Vassie C Ware

Lehigh's SEA-PHAGES program continues as a collaborative research enterprise for first year and advanced undergraduates who focus on isolating and characterizing Actinobacter phages to gain a better understanding of phage genome structure, gene function, and phage biology in general. Of special interest to our group are mycobacteriophages from Cluster N – a group of temperate phages characterized by relatively small genomes of average size 43,111bp and a highly variable region centrally positioned within the genome. Recently, a novel mechanism of prophage-mediated immunity was uncovered for Cluster N lysogens that provides defense against attack by variable groups of heterotypic mycobacteriophages and appears dependent on genes in the variable region of Cluster N genomes (Dedrick *et al*., 2017). We report on progress on several projects. **I**. In addition to uncovering 16 new phages infecting *Mycobacterium smegmatis* including two new Cluster N phages, Parmesanjohn and Spongebob, our 9th year cohort of students focused on testing infectivity of newly isolated phages on Cluster N Xeno lysogen lawns, and discovered three Cluster A11 phages with reduced infectivity compared to infectivity on *M. smegmatis* lawns. Isolation of defense escape mutants is currently in progress. **II**. An estimate of cluster diversity was obtained from DOGEMS analysis and annotation of two Cluster N phages (Spongebob Parmesanjohn) and 3 Cluster A11 phages (Munch, Bowtie and Bud). **III**. We used RNAseq to explore changes in host gene expression following early and late lytic infection (by a Cluster N phage). Additionally, we examined transcription patterns in a Cluster N lysogen and a putative Cluster W lysogen. Preliminary data show patterns of *M. smegmatis* gene expression vary depending on phage type. Collectively, these experiments provide further insights into M. smegmatis host-phage interactions affecting host defense against viral attack and host gene expression.

10th Annual SEA-PHAGES Symposium Abstract

Poster #004

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Annabelle Malinowski

Kathryn Baker

Extreme Codon Biases in Mycobacteriophage Relative to Phage Encoded tRNA Genes and Host Codon Biases

Annabelle E Malinowski, **Kathryn B Baker**, Bree E Mask, Payton N Pierce, Frederick N Baliraine, Gregory D Frederick

Life of a bacteriophage in the wild is treacherous at minimum. In order to persist in nature, a phage must encounter a potential host cell, successfully adsorb onto the cell, create an entry portal, and introduce its genome into the potential host cell. Each of these processes entails unique challenges. Bacteria in the environment are numerous and diverse. Successful introduction of the phage genome does not ensure phage survival and replication. There are many additional barriers such has restriction systems, CRISPR/CAS systems, and prophage induced immunity that impede the continuation of the phage life cycle.

Assuming evasion of the aforementioned defense systems, as phages search out their next host in nature, there is yet another significant factor that potentially limits their 'fitness' in the newly infected host cell, specifically host and phage codon biases.

Many Mycobacteriophage genomes encode tRNA genes. tRNA genes are most abundant in the phage genomes of clusters C, L, M, and V. Only about half the members of cluster A have at least one tRNA gene. However, there are at least 10 clusters in which no member encodes tRNA genes. While tRNA genes are inherently short, accumulating and retaining these genes within the genome undoubtedly provides phage with some selective advantage. Two independent selective pressures potentially drive accumulation and retention of tRNA genes within phage genomes: the first being extreme codon bias within the phage genome and the second being extreme codon bias within potential or previous hosts. It is imperative that viruses adapt to the cellular conditions of as many potential hosts as possible for long term survival. Phage tRNA genes have been acquired from a variety of previous bacterial hosts, thus increasing their fitness for evolutionary survival and propagation of their genes.

In this study, tRNA genes in phage members of clusters A, G, K, L, M, N, and P isolated in Longivew, TX are analyzed. The encoded tRNA anticodons are compared to the codon usage of the phage, the isolating host, Mycobacterium smegmatis mc2155, and a number of other potential environmental bacterial hosts identified through annotation analysis in Blast results. The phage examined include Bombshell (A4), Dublin (A5), Pmask (A6), Wunderphul (A6), Arrostook (G1), Gideon (G1), Peanam (K1), Tyson (L1), Reindeer (M1), Andies (N), Glaske (P1) and Willsammy (P1).

A number of extreme and moderate codon biases corresponding to phage encoded tRNA genes are reported. Considerations and implications relative to probable host codon usage will also be discussed.

10th Annual SEA-PHAGES Symposium Abstract

Poster #066

Lincoln University
Lincoln University PA
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Davisha A Brown

Ohfah, a newly annotated A4 subcluster mycobacteriophage

Davisha A Brown, Charlene L Combs, Seth Donnelley, Saran A Hendrickson, Chiamaka A Ihejirika, Ramona A Plummer, David F Royer

Ohfah, an A4 subcluster temperate bacteriophage, was isolated from a soil sample collected on the campus of Lincoln University as part of a Phage Hunters class offered during the 2017-18 academic year. Ohfah is similar to other A4 subcluster phages in genome length, GC content and number of genes. Genome wide comparisons using the Phamerator program and BLAST revealed that Ohfah is most closely related to members of the A4 subcluster of mycobacteriophages. The greatest similarity is to A4 phages BellusTerra (Gettysburg, PA), Peaches (Monroe. LA), Maverick (Richmond, VA) and Kampy (Williamsburg, VA). It is a member of the Siphoviridae with a characteristic long tail. The annotation revealed 83 genes, of which 51 were assigned a putative function based on comparisons with other A4 mycobacteriophages using BLASTP assignments from both phagesdb.org and GenBank. Of the 51 genes assigned a function, 17 coded for structural proteins and 34 coded for functional proteins. A programmed translational frameshift involving genes 23 and 24, tail assembly chaperones, was identified.

10th Annual SEA-PHAGES Symposium Abstract

Poster #037

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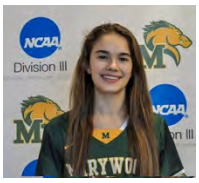

Lilli Foote

The "Twins" Marley 1013 and Morty007: Fighting Towards a New (Ph)age

Abigail Haddock, Alyona Kostyal, Bethany Serio, Bria Hocking, Caitlin Fernandez, Cara Romaniello, Colleen LaMagna, Daniel Shannon, Destiny McClain, Donovan Catchings, Gabriella DiToma, Hailey Rapisardi, Haley Toczko, Hannah Corcoran, Hayley Schultz, Jacqueline Knash, Javaughn Johnson, Jenny Patel, Joseph Chesek, Kaitlyn Kimes, Karamvir Singh, Kristen Rossignol, **Lilli Foote**, Matthew Leen, Megan Hedgelon, Mikayla Matthews, Nicholas Heiden, Paul Matarazzo, Prabh Kaur, Sabrina Benbader, Sarah Berube, Sean Oliver, Stephen Liuzzo, Zachary Lloyd

According to the World Health Organization (2015), each year over 480,000 people are infected with multi-drug resistant Tb. Of those people, only about 10% recover. To help combat this problem, Freshman Marywood University undergraduate students dug up bacteriophages, viruses that infect bacteria, from the soil in Northeastern Pennsylvania. Of these 50 soil samples, 17 bacteriophages were isolated. All of these bacteriophages infect Mycobacterium smegmatis, a bacteria related to Mycobacterium tuberculosis. Using Mycobacterium smegmatis as a host bacteria, the bacteria and soil extract was plated on a Luria agar plate, where the phages produced clearings called plaques. Morty007 and Marley1013 were then sent to have their genomes sequenced at the University of Pittsburgh. According to the data provided by the University of Pittsburgh, the phages were both found to be part of the lytic B3 subcluster and siphoviridae family. The number of genes present were also predicted using genome sequencing. Morty007 originally had 106 predicted genes, while Marley1013 had 101 predicted genes. However, after further analysis using various databases provided by the SEA-PHAGE program, which included PhagesDB, NCBI, HHPred and DNA Master, students deleted 5 of Morty007's genes and 2 of Marley1013's genes. Furthermore, these databases also provided information about the possible functions of different genes, their common GC content of 67.5%, and only an 11 base pair difference. Through this research, Morty007 and Marley1013 were added to the databases in order to be further analyzed for possible use in phage therapy in the future.

10th Annual SEA-PHAGES Symposium Abstract

Poster #034

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Joanne Turnbull

Isolation and Characterisation of unique Mycobacterium smegmatis bacteriophages native to New Zealand

Joanne Turnbull, Natasha Elliston-Boyes, Steen Grundy , Anezka Hoskin, Leani Oosthuizen, Emily Palmer, James Scott, Jenny Ann Sweatman, Nikki Freed, Heather Hendrickson

Research into the mechanisms of bacteriophages specific to *Mycobacterium smegmatis* has the potential to introduce a new method to treat or prevent disease caused by pathogenic Mycobacterial organisms. Prior to 2017, only two Mycobacteriophages native to New Zealand had been added to PhagesDB.org's Actinobacteriophage Database. The 2017 class of Massey University's Bacteriophage Discovery and Genomics course have since contributed information for an additional eight bacteriophages. DNA sequencing has been completed for five of these submissions, more than tripling the number of known DNA sequences for Mycobacteriophages found in New Zealand.

During the course of Massey University's 2017 Phage Hunt, eight students each isolated a unique bacteriophage specific to *M. smegmatis*. These phages were all found in soil or compost samples from the Auckland region of New Zealand, and discovered using direct isolation techniques as per the SEA-PHAGES (Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science) program guidelines. Each phage sample was purified using serial dilutions, and amplified to generate a high titer lysate of at least 109 pfu per mL. Each bacteriophage was viewed under transmission electron microscopy with the assistance of Dr. Adrian Turner at the University of Auckland. Upon measurement, all phages were found to be within the range of 55 to 75 nm in capsid width and 136 to 248 nm in tail length, with tail length showing much greater deviation from the mean value than capsid width.

10th Annual SEA-PHAGES Symposium Abstract

Poster #064

Merrimack College
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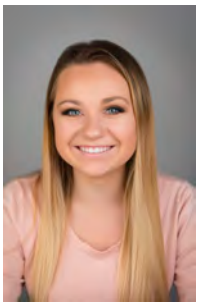

Micaela Caron

Genome Annotation of Four New and Novel M. smegmatis Mycobacteriophage: Emmina, Halena, Noella, MadMarie

Micaela Caron, JohnPaul Haley-Reed, Constantina Stavrou, Aaron Cochran, Ashley Figucia, Lisa Nguyenhoang, Katelyn Rae, Samantha Ridgewell, Charlotte Berkes, Mark Birnbaum, Christina MacLaren, Jennifer Pancoast, Janine LeBlanc-Straceski

Four *Mycobacterium smegmatis* bacteriophage, Emmina, Halena, Noella, and MadMarie, were discovered in soil samples collected at various locations across the Merrimack College campus in North Andover, MA during fall 2017. All four are temperate phage, each possessing either a serine or tyrosine integrase, and yielding turbid plaques that vary in size. All phage particles display siphoviridae morphology. Emmina, a cluster E phage, was discovered near the Mendel Pond and has the largest genome of the four phage, with a length of 75,299 bp. It is closely related to Upie, 244, and Hopey, which was also discovered on the Merrimack College campus. Halena is assigned to cluster L1 and has a genome length of 73,871 bp. Its closest relatives are Acquire49, Wyatt2, Tyson and Silverleaf. Noella and MadMarie are both A3 cluster phage. Noella has a 50079 bp genome and produces 3-4 mm turbid plaques with a clear center. MadMarie has a genome length of 50,849 and produces similar sized, but more turbid plaques. Although the genomes of the A3 phage are 92% identical throughout, they diverge in the central portion that contains the integrase gene, and other genes with no assigned function, as well as at both ends of their genomes. These A3 phage are related to Texage, another Merrimack discovery. The two major classes of integrases are represented among the four phage. Emmina, Halena and MadMarie all have tyrosine integrases, but from three different phams. Noella and the previously described Texage both contain a serine integrase from the same pham. Sequence comparisons in the integrase region between genomes in the A3 cluster reveals that the nucleotide sequence conservation that is found within the integrase genes is also maintained in the non-coding upstream region, which may contain putative attP sites.

10th Annual SEA-PHAGES Symposium Abstract

Poster #076

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Jacob W Schlichter

Magnar, a typical subcluster A1 phage with an unusual gene

Jacob W Schlichter, Brendan N Chetty, Susanna D'Silva, Ian G Kausch, Spencer N Kluth, Madelyn K Myers, Garrett M Schilling, Allison M Biedenharn, Amanda C Burgess, Elizabeth Lucas, Mariah S Squire, Kelly Z Abshire, Luis A Actis, Mitchell F Balish

The Miami University Bacteriophage Biology (MBI 223) class of Fall 2017 isolated 17 bacteriophages infecting *Mycobacterium smegmatis* from the soil at various sites in and around Oxford, Ohio. Among these phages, one, designated Magnar, which had been recovered from a trail in a cattle pasture, formed large plaques with distinctively large halos, had typical Siphoviridae virion morphology, and was selected by the class for genome sequencing. The remaining 16 phages were subjected to the DOGEMS protocol, and 12 complete or nearly complete sequences were obtained. Sequencing was performed at the Pittsburgh Bacteriophage Institute using Illumina technology. Of the 12 long sequences obtained by DOGEMS, 8 were cluster A members, and the rest were distributed among clusters B, E, F, and K. Because phages from these clusters are common, no effort was made to identify which genome was associated with which phage isolate. Magnar was revealed to be a member of subcluster A1. Annotation of Magnar by the Bacteriophage Genomics (MBI 224) class of Spring 2018 revealed 91 putative protein coding genes across its 51,428-bp genome, including one encoding an integrase, consistent with Magnar's lysogenic characteristics. Approximately 4% of the reads were from a different phage from subcluster A3. Magnar gene 45, encoding a 78-amino acid protein, was not assigned to a Pham by Phamerator despite being similar in size to genes in syntenic positions in other A1 subcluster phage genomes, immediately upstream of a gene for DNA polymerase I. The encoded protein's only BLAST hit, with an E-value of 1.90 using the default parameters within DNA Master, was to gp48 from A1 subcluster phage KBG, whose coding gene is syntenic with the Magnar gene. BLAST indicated that the predicted proteins share 59% similarity over almost their entire lengths. The KBG protein is a member of Pham 3953, which has 135 members in the Phamerator database as of this writing, none of which are associated with a known function, although the proximity to the DNA polymerase I gene suggests a potential role in DNA synthesis or its regulation. It is tempting to speculate that the unusual plaque morphology of Magnar resulted from contamination with the second phage or, given the absence of a halo in phage KBG plaques, the activity of the highly divergent gene 45.

10th Annual SEA-PHAGES Symposium Abstract

Poster #026

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Riley D Hellinger

Hannah E Sparks

Characterization and Genomic Analysis of Mycobacteriophage Wamburgrxpress, Including the Determination of the Essentiality of the Terminal 5 Kilobase Pairs for Plaque Formation

Riley D Hellinger, **Hannah E Sparks**, Marisa L Pedulla

Bacteriophages, viruses that specifically infect and kill bacterial cells, are the most numerous biological entities on Earth. Characterization of bacteriophages is necessary to understand their possible uses in medicine; for example, they could potentially be used as alternatives to antibiotics. Analysis of the genome of recently discovered bacteriophage, Wamburgrxpress, will provide new information about phage diversity by comparison to known phages. DNA sequencing revealed Wamburgrxpress contains a genome length of 74,392 bp, with 58.8% G/C content, belonging to phage cluster (L1) with a 10 bp 3' overhang. Genome annotation was performed to identify 126 putative protein coding genes of which 36 had predicted functions. The genome additionally contains 9 tRNA genes. Transmission electron microscopy revealed a siphoviridae morphology, similar to other known L1 cluster phages. Determination of whether lysogen *Mycobacterium smegmatis* bacteria containing Wamburgrxpress prophages can be isolated by standard protocols further characterized our bacteriophage relative to other L1 cluster phages that are known to be temperate. Due to the presence of a putative integrase gene within the genome, it was predicted that Wamburgrxpress is also temperate. A key goal of our research was to identify whether the last 5,373 base pairs of its DNA are essential for productive *in vitro* infection of *M. smegmatis*. It was hypothesized that the terminal 5 kbp at the 3' end of the genome are essential for infection (due to either the presence of essential genes or requirement of wildtype length to package its DNA correctly). Restriction digestions, agarose gel purification of the shortened phage genome, and electroporation into *M. smegmatis* of truncated DNA with and without a linker providing a cohesive end were performed. Plaques formed after plating with top agar were compared to those formed from electroporation of wildtype DNA.

10th Annual SEA-PHAGES Symposium Abstract

Poster #001

Montclair State University
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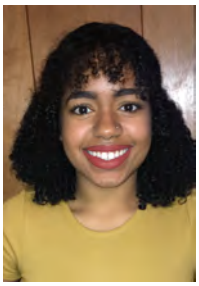
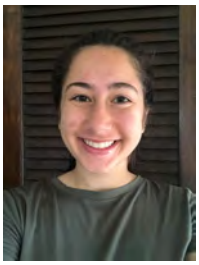


Ashly Reyes



Leen Khashashina

Isolation and Characterization of Novel Mycobacterium smegmatis and Arthrobacter sp. Bacteriophage from New Jersey Soil

Ashly Reyes, Leen Khashashina

The goal of this study was to isolate and characterize novel bacteriophages from New Jersey soil samples that infect Mycobacterium smegmatis and Arthrobacter sp. hosts. In 2017, 29 lytic phages that infect M. smegmatis and two that successfully infect Arthrobacter sp. were successfully isolated and further characterized through plaque morphology and scanning electron microscopy. Of the phages isolated in 2017, one M. smegmatis phage (BreSam8) and one Arthrobacter sp. phage (Tenno) were sequenced and annotated. BreSam8 is a subcluster A3 phage with a putative 90 genes (including three tRNA genes). Tenno is a subcluster AU1 phage with a putative 80 genes. Like other AU1 phages, all of Tenno's 80 genes are transcribed using the top strand of DNA, and none of Tenno's genes appear to be transcribed using the bottom strand.

10th Annual SEA-PHAGES Symposium Abstract

Poster #084

Morehouse College
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Charles P Smith

The Purpose of the Gene Functions in Mycobacteriophage Xavia

Larry E Ashby, Jared T Bailey, Nathaniel T Douglas IV, Jordahn M Edwards, Ashton M Farrar, Cameron Ford, Anthony Frazier Jr., Kyron M Freeman, Devin A Hawkins, DeAnte D Hoover, Malik S Jones, Tahvis J Magruder, Stuart A Phipps, Tiye' A Ridley, Stephon M Scott, Gregory Singleton II, **Charles P Smith**, Quandarius C White, Jibreel K Wright, Alexandra Peister, Lawrence S Blumer

Mycobacteriophage Xavia (GenBank MH230879) was isolated from a soil-water sample in Atlanta, Georgia, using *Mycobacterium smegmatis* as the host. Xavia is a Cluster P3 phage that is 49,808bp long. The purpose of our research was to finalize the draft annotation of this phage by manually confirming potential genes and identifying gene functions. Utilizing the annotation program, DNA Master, and guided by heuristic GeneMark output we determined the most likely open reading frames to identify each gene in this genome. Starterator reports were used to help identify the most conserved starts in each pham, and BLASTp searches and HHPred searches were used to identify the function of more than half of the genes in this genome. Comparisons between the Phamerator maps showed remarkable similarity in both nucleotide sequences and protein products early in each genome. Synteny in the Cluster P phages is very strong in the first half of each genome, but differences between the Cluster P phages is observed in the second half of the genomes and after ORF 28 in our phage. In our completed annotation, there are a total of 71 genes in Xavia and no tRNA or tmRNA sequences. The functions of 38 of the 71 genes were identified. A total of 19 genes have virion structure and assembly functions, one is a phage DNA replication gene, 3 are life cycle regulation genes, 3 are lysis genes, and 12 are other well-characterized genes. As seen in other members of Cluster P such as Bartholomew (P1) and Tortellini (P2), Xavia has a programmed translational frame shift in the tail assembly chaperone genes just upstream of the tape measure protein. The presence of integrase (Y-int), and immunity repressor genes suggest that Xavia is capable of lysogeny.

10th Annual SEA-PHAGES Symposium Abstract

Poster#110

Morehouse College
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Larry Blumer

Research Immersion Improves Outcomes for Underprepared Freshmen

Alexandra Peister, Lawrence S Blumer

Our implementation of the Howard Hughes Medical Institute, Science Education Alliance, Phage Hunters curriculum (www.seaphages.org) at Morehouse College differed from the implementations at other colleges and universities. We intentionally limited our enrollment to entering freshmen who were deemed underprepared to begin a biology major based on SAT scores. These students were not permitted to initially enroll in a traditional gateway survey-type biology course (BIO 111). Underprepared students were invited to apply for our Phage Hunters course to assess the effectiveness of this research immersion experience on their future success in BIO 111. Six cohorts (N=90) of Phage Hunters students have taken the gateway majors course permitting us to compare their academic performance to peers (N=45) who were similarly underprepared first-time freshmen but who did not participate in Phage Hunters, and to non-peers (N=182) all other students in the same gateway course. Phage Hunters students had a significantly greater pass rate (A,B,C grades) and a significantly lower withdrawal rate than did their peers. Compared to non-peers, Phage Hunters had a significantly lower withdrawal rate and no significant difference in pass rates. These findings indicate that an authentic research immersion experience can dramatically improve student outcomes for underprepared students and consequently improve freshmen student retention.

10th Annual SEA-PHAGES Symposium Abstract

Poster #054

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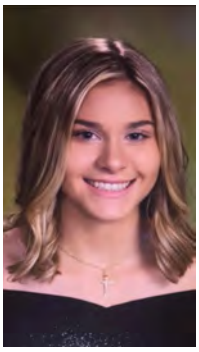
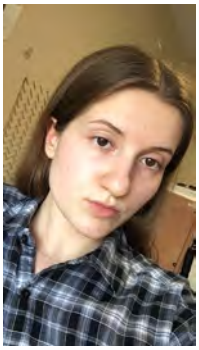


Jessica Musacchio



Sabina Zarod

The Molecular Journey: Annotation of Microbacteriophages Schnapsidee and Papafritta

Jessica Musacchio, **Sabina Zarod**, Kaitlyn Barber, Amanda Barnett, Destiny Bettica, Olivia Bracco, Erica Eack, Elizabeth Halpin, Camren Iorio, Nasayah Israel, Nicholas Mahoney, Chandlir Radcliffe, Andrew Velasquez, Suparna Bhalla, Evan Merkhofer

The Mount Saint Mary College Phage Hunters began isolating phages using *Mycobacterium smegmatis* mc^2155 in 2016-2017 and in 2017-2018 transitioned to the host *Microbacterium foliorum* to provide more insight into the diversity of genomes across the Actinobacteriophage phylum. This year, nine Microbacteriophages were isolated from soil samples gathered in Newburgh, NY. Phages *Papafritta* and *Schnapsidee* were obtained through enriched isolation, both yielding small, cloudy plaques after 48 hours incubation at 30 degrees. Sequencing of *Papafritta* (circularly permutated, 41.9 kB length, 63.4% GC content) and *Schnapsidee* (circularly permutated, 41.6 kB length, 63.4% GC content) identified them as members of the EA cluster and EA1 subcluster. Both phages were predicted to have a lytic life cycle based on the initial analysis of the genome sequences. BLAST analysis of *Papafritta* and *Schnapsidee* showed 85% nucleotide identity between these phages.

Bioinformatic programs including DNA Master, GeneMark, Phamerator and Starterator were used to successfully annotate these novel phages, and gene functions were assigned using the HHpred and NCBI BLAST programs. Upon annotation, these genomes were found to be very similar. There were however significant differences in the region of genes 28-33. Additional analysis is being performed on these phages to confirm the lytic life cycle. We are also investigating sensitivity of these phages to UV light. Finally, further bioinformatic analysis, including identification of possible regulatory elements and the presence of membrane proteins, is being performed to better understand the genome composition of these Microbacteriophages.

10th Annual SEA-PHAGES Symposium Abstract **Poster #069**

Nebraska Wesleyan University
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Jenna Whitmore

Isolation and Characterization of Bacteriophage Alleb

Jenna Whitmore, Mackenzie Batt, Caitlin Broekemeier, Claire Landgren, Annie Platt, MaKenzie Saltzman, Leah Treffer, Alison Wilson

During the Fall of 2017, students participated the Science Education Alliance Phage Hunters Genomics and Evolutionary Sciences (SEAPHAGE) program. This research-based program requires students to collect soil samples and isolate a bacteriophage, or virus that infects bacteria. The host used for our study was Microbacterium paraoxydans NWU1. In order to isolate the bacteriophage, basic microbiology techniques were utilized. Once isolated, the bacteriophage was purified and electron microscopy was utilized to visualize the bacteriophage. Finally, genomic DNA was isolated and the genomic DNA was sequenced. During the Spring 2018 semester, one bacteriophage (Alleb) was annotated using the computer software program DNA Master and other online tools. After annotation of the phage genome, it was determined that Alleb contained 113 putative genes. Each gene was investigated to determine its start and stop codons, similarity to other known genes and its function. Genes of interest found in Alleb and the role the genes plays in the bacteriophage life cycle will be discussed.

10th Annual SEA-PHAGES Symposium Abstract

Poster #104

North Carolina A&T State University
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Brianna Wiggins

Aggie Phamily Adopts Brylie

Brianna Wiggins, Ashley Addison, Myana Banks, Daja Bizzell, Ayana Byrd, Rixon Campbell, N'Dea Celious, Chyna Fisher, Adrinna Freeman, Arianna Grant, Najee' Green, Camille Harrison, Courtney Holt, Rebekah Jones, Nahndi Kirk-Bradley, Alexis Matias, Shayla McCray, Zipporah Melton, Adrianne Mitchell, Malek Mitchell, Nia Nickerson, Kaisi Peele, Tyra Penn, Chi Smalls, Vaniya Tisdale, Sydney Wheeler, Supriya Patwardhan, Anthony Postiglione, Robert Newman, Roy Coomans

SEA-PHAGES students at North Carolina A&T State University isolated 13 bacteriophages in the fall of 2017 using *Gordonia terrae* CAG3 as the host organism. Seven were isolated by direct plating, the other six were isolated from enrichment cultures. DNA extracted from three of the thirteen phages was sequenced at Pittsburgh Bacteriophage Institute. Two of the sequenced phages, Ali17 and Bizzy, are in Cluster DE; the third, Brylie, is one of eight sequenced Cluster DI phages in the Phagesdb databank. Brylie's genome is 49,870 base pairs and has a GC content of 67.3%. Annotation identified 73 genes. Brylie has a high degree of sequence homology with BetterKatz, the only Cluster DI phage previously annotated and entered in GenBank. Brylie and BetterKatz have a gene content similarity of 93.96% and share 70 phams. Phylogeny.fr was used to generate single gene phylogenetic trees of the eight Cluster DI phages. Trees for four different shared genes all grouped Brylie with Mulch, Nadeem, Parada, and WheatThin. Catenating the sequences for the four genes gave the same result. We also investigated protein structure prediction for selected gene products using I-TASSER.

10th Annual SEA-PHAGES Symposium Abstract

Poster #105

Northwest Indian College
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Destinee Hutchinson

Breanna Nguyen

Discovering SuperStar Phages at Northwest Indian College and Annotating Homura (Cluster K)

Destinee Hutchinson, **Breanna Nguyen**, John Rombold, Rachel Arnold

The Science Education Alliance – Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) was implemented at Northwest Indian College (NWIC) during the 2017–2018 academic year, making it the first program of its kind to be offered at NWIC. This program is a discovery-based research course that gave us the opportunity to dig through the soil to find new viruses in our local communities. We were able to gain a variety of microbiology techniques as the course progressed in the first quarter, including the aseptic technique, pipetting, culturing, and serial dilutions. In the first weeks of the program we had many issues with contamination and isolation of our samples. As a class, we all had attempted the first steps of the isolation process numerous times before we were able to successfully isolate and continue to the next steps. Both of us were able to complete the first components of phage discovery, and then one of us went on to take an optional Genetics and Evolution course. This course included working with a phage genome and doing complex genome annotation and bioinformatic analyses. For this portion, we were able to start annotating our adopted phage "Homura", a cluster K1 bacteriophage, using the programs DNAMaster, Phamerator, GeneMark, Starterator and the website PhagesDb. After our initial annotations, we proceeded to assign protein functions to each gene we found using the programs and rules of BlastP, HHpred, PECAAN, Synteny and PhagesDb.org. We gained an understanding of the inner workings of phages as we completed the annotation of Homura. This has also taught us the importance of affordable local research and how it can benefit the surrounding communities. The SEA-PHAGES program has motivated us to continue pursuing related fields of study and has expanded our knowledge with respect to our local environment.

10th Annual SEA-PHAGES Symposium Abstract

Poster #023

Northwestern College
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Chris Borchers

Bethany Muyskens

Sara S Tolsma

Production of Polyclonal Antibodies Against Structural Proteins of Viruses from Different Clusters Accompanied by Annotation of the Viral Genomes

Chris Borchers, **Bethany Muyskens**, Byron Noordewier, **Sara S Tolsma**, Jeanna Becker, Alexandra Boursiquot, Brittany Buren, Cole Cruse, Paxton De Haan, Kelsey Dorhout, Nathan Eide, Hans Epp, Amanda Everhart, Karsten Garwood, Emily Geraets, Colton Hage, Jacob Jenness, Hannah Jorgensen, Shay Kamstra, Joseph Kelly, Megan Kingsriter, Sidney Martin, Rachel Mercer, Sarah Meyer, Courtney Mithelman, Haley Muyskens, Lily Peschau, Hannah Powell, Renju Pun, Tanner Rensink, Zachary Rosson, David Rowley, Peace Preston, Sabrina Tarchione, Connor Tupper, Shelby Van Den Berg, Mitchell Van Kalsbeek, Michaela Van Riesen, Cole Verbrugge, Kristina Sevcik

Of 16 mycobacteriophages isolated in 2017, the genomes of three were sequenced and annotated: Sibs6, a member of the A1 cluster, Roots515, a C1 cluster phage, and CBorch11, a member of the rare H1 cluster. Sibs6 and CBorch11 are siphoviridae phages and Roots515 is a podoviridae phage. The 50,210 base-pair genome of Sibs6 has a 63.8 % GC content and includes genes that suggest this phage is lysogenic, consistent with its plaque morphology and other A1 phages. Its left arm contains 38 forward genes and its right arm contains 58 reverse genes. We assigned functions to 41 of its 96 genes. The genome of Roots515 is 156,288 base pairs in size and has a 64.7% GC content. Its 271 genes are mostly forward and include 33 tRNA genes. We assigned functions to 50 of the protein-coding genes in Roots515. CBorch11's genome is 68,508 base pairs in size and has a 57.6% GC content. CBorch11's 93 genes are all forward genes and with presumed functions identified for 22 of them. Concurrent with annotation, structural proteins of 64/65 kDa from each phage were gel purified and used to immunize Balb/c mice. The polyclonal anti-phage protein antibodies raised against the 64/65 kDa proteins cross-react with proteins of similar size produced by the other two phages even though the phages are from different clusters. We are in the process of raising monoclonal antibodies to CBorch11 high titer lysate. Our initial work suggests that, while some of the anti-CBorch11 antibodies in the mice from which we are isolating monoclonal antibodies recognize proteins of similar sizes in Sibs6 and Roots515, other antibodies are specific for CBorch11 proteins alone. We hope to use the antibodies to understand the production of these proteins in the context of phage life cycles and to examine biochemical similarities and differences in phages from different clusters. In addition to this annotation and antibody work, we isolated, purified, and characterized 20 more mycobacteriophages that infect M. smegmatis. These came from seven different geographical locations. Plaque morphologies of these phages overlapped with previous isolates (2017) but, in general, were smaller. In contrast to 2017, none of the 2018 isolates appear to be potential lysogens.

10th Annual SEA-PHAGES Symposium Abstract

Poster #030

Nyack College
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Sucely Ponce Reyes

Christal Rolling

Non-Mycobacterial Actinobacteriophages Expanding Our Knowledge of Phage Biology and Evolution

Sucely Ponce Reyes, **Christal Rolling**, Mariana Pereira Moraes, Maridalia Lillis, Maria I Paschalis, Grace K Anger, Marissa K Antonucci, Christina Dukehart, Rachel M Ewers, Kaelan Kanai, Franklin Mercado, Chelsea D Nichols, Ralph L Nicholson, Sofia M Osorio, Xylvie X Santiago, Djulie S Scaff, Kryscell N Stoner, Kathryn G Tamondong, Makayla Veracka, Daniel Kaluka, Peter J Park, Jacqueline M Washington

Compared to mycobacteriophages, much less is known about other actinobacteriophages. Consequently, isolation and characterization of non-mycobacterial phages will increase the diversity of bacteriophages and increase our knowledge about phage biology and evolution. During 2017-2018, Nyack College Phage Hunters isolated phages using several different hosts, including *Gordonia terrae* CAG3, *Gordonia terrae* 3612, *Rhodococcus erythropolis* NRRL B-1574 , and *Microbacterium foliorum* NRRL B-24224. Twenty phages were isolated, seven of which were sequenced. They include the *Gordonia* phages, EmsquaredA (CY1), TillyBobJoe (DC), Maridalia (CZ1), Easley (CZ4) and Nedarya (A15); *Rhodococcus* phage Shuman (CA) and *Microbacterium* phage Paschalis (EC). This diverse collection of sequenced phages range in size from 46,544-58,677bp.

As not much is known about many of these new non-mycobacterial actinobacteriophages, identification of gene functions can be a challenge. Gene order is mostly conserved in phages with the structural genes on the left end of the genomes. On this end, phages have both a small and large terminase gene used to package the genome in the empty capsid. Whereas, the large terminase subunit is more easily identified, the small terminase subunit is not. As it dimerizes, sequence analysis using the program COILS2 was used to try to identify the small terminase subunits. Bioinformatic analysis also revealed that Paschalis maybe atypical in regards to structural gene order. In most phages, the head to tail connector complex proteins are between the major capsid and major tail protein. In Paschalis, initial functional characterization places the head-to-tail connectors downstream the major tail protein. Paschalis also contains a unique gene, rifampin ADP-ribosyl transferase, which has been attributed to the resistance of Mycobacterium smegmatis to the antibiotic rifampin by glycosylation. To date it has not been identified in the genus Microbacterium.

Temperate phages usually have either a serine or tyrosine integrase. Interestingly, TillyBobJoe has both a serine and tyrosine integrase which appears to be typical for cluster DC as all 16 current members of the cluster have two integrases. The tyrosine recombinase integration site is predicted to be at tRNA Thr in the bacterial chromosome. Consequently, to further investigate the predicted tyrosine recombinase integration site we designed primers to probe the attL and attP sites in order to determine the frequency of integration using the tyrosine integrase as well as the stability of the lysogens.

All of our sequenced phages with the exception of Paschalis are predicted to be temperate and should form lysogens. Consequently, we have attempted to isolate lysogens from all our phages to perform immunity assays. Results show that some of these actinobacteriophages have a heterotypic defense mechanism similar to that found in mycobacteriophages.

10th Annual SEA-PHAGES Symposium Abstract

Poster #079

Ouachita Baptist University
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Cameron M Brownlee

RFLP-Guided DOGEMS and a Class Genome Announcement--Bioinformatics at OBU

Cameron M Brownlee, Hayden H Bowman, Haley K Davis, Emme M Edmondson, Savannah L Edwards, Jordan R Gills, Katlin R Jacobs, Gracie C Jones, Luke W Livingston, Morgan E Masengale, Madi Morrison, Austin M Mullins, Mariah D Pate, Beau T Pennington, Kenzie N Pickard, David R Rainwater, Allie C Studdard, Abby L Walker, Liam C Wooten, Nathan Reyna, Ruth Plymale

Students at Ouachita Baptist University isolated ten bacteriophage infecting Gordonia terrae 3612 from enrichment of seventy-five soil samples. Four bacteriophage were identified as candidates for DNA sequencing—DelRio, Ribeye, Ruthy, and SketchMex. In an effort to distinguish and prioritize these four phage for sequencing, the DNA of each phage was digested with the restriction enzymes BamH1, Cla1, EcoR1, HaeIII, and HindIII. The restriction fragment length polymorphism (RFLP) patterns were used simplify the DOGEMS (Deconvolution of Genomes after En Mass Sequencing) process. In traditional DOGEMS, multiple unknown phage are combined and unique phage are separated out as individuals during the sequencing assembly step. In our RFLP-guided DOGEMS, we combined two phage (Ruthy/Ribeye and DelRio/SketchMex) with dissimilar RFLP patterns into a tube, allowing effective sequencing and straightforward separation of multiple phage. Ruthy was found to be a singleton phage, DelRio to be in cluster DI, Ribeye in cluster DE, and SketchMex in cluster CT. All four phage genomes were annotated by the Bioinformatics class. DelRio was annotated as part of our Genome Hack-A-Thon, a regional high school outreach program where OBU students served as facilitators. After annotation, OBU student groups conducted additionally bioinformatic analyses. This data is currently being compiled and will be used to write a class genome announcement. Results will be presented.

10th Annual SEA-PHAGES Symposium Abstract

Poster #099

Purdue University
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Catherine Carroll

Comparative genomic analysis of mycobacteriophages and the quest for new protein folds

Jamie Arabshahi, Devon Amos, Karis Aucoin, Manvir Bains, Emmarie Ballard, Zach Berglund, Jillian Bouck, Morgan Carrithers, **Catherine Carroll**, Janice Chan, Jairus Chittenden, Maia Clare, Henry Cushing, Zachary Falender, Kevin Fitzgerald, Helen Flynn, Kartik Gannamaneni, Zihan Gao, Ethan Gaskin, Jenna Greene, Sanya Gupta, Jack Hamlin, Devany Harrell, Elena Haskins, Sarah Hermanek, Yunji Hong, Kaleigh Karageorge, Archana Kikla, Joey Krampen, Melody Ku, Violet Kuchta, Ellie Lang, Austin Larson, Chin Fang Lin, Derik Lovejoy, Alexis Macha, Michelle Mai, Kasey Martineau, Michael McCool, Sean McCormick, Cole Miller, Emma Misicko, Catherine Nagy, Ashley Otero, Anthony Park, Chandler Parrish, Chitra Ram, Vish Ravichandran, Vandana Reddy, Mika Reuhs, Jacob Riedel, Jayden Rosen, Julia Russo, Christina Sanchez, Evan Shank, Anqi Shao, Gabrielle Shapiro, Trevor Shoaf, Holly Spiritoso, Allie Stiffler, Rilee Taege, Jacob Washlock, Lindsey Wilson, Zhi Hui Yu, Zonghao Zhang, Emily Kerstiens, Gillian Smith, Yi Li, Ikenna Okekeogbu, Nicholas Noinaj, Kari Clase

Mycobacteriophages are ubiquitous viruses that infect mycobacteria. They have potential uses in the field of biotechnology and medical science with applications ranging from disease diagnosis, through phage typing, phage vaccine and phage therapy. Meanwhile, only a meager number of mycobacteriophages have been identified and characterized out of the multitudes present in the biosphere. In addition, a far majority of the bacteriophage genes that are discovered have no known function or structure. In this study, thirty novel mycobacteriophages that infect Mycobacterium smegmatis were isolated, characterized and fourteen were annotated per the most recent guidelines using both PECAAN and DNA Master. One of the characterized mycobacteriophages, Ochi17, has a genome size of 58kbp and GC content of 61% and was classified into cluster F, and sub-cluster F1. The second phage, VasuNzinga, was identified for sequencing by Dr. Hatfull's research group at University of Pittsburgh during a screen of archived lysate inventory using DOGEMS. It is only the eighth Cluster S phage ever found. Both genomes contained expected virion structural genes, such as the tape measure protein and other genes involved in tail assembly. Capsid related genes were also annotated including capsid maturation protease, scaffolding protein, major capsid protein. Both phages also contained integrase and putative proteins that mediate the lytic cycle such as lysins A and B, and holin. Ochi17 contained three tRNAs and several defense related genes such as the exo- and endo- nucleases, the immunity repressor, and the antirepressor. Notably, VasuNzinga contained multiple putative transferase enzymes for post-translational modification. Several genes were annotated with no known function (NKF), 59 genes in Ochi17 and 68 genes in VasuNzinga, respectively. In order to investigate the role of the genes with no known function and to confirm the functions of the predicted genes, the course research experience was expanded to include collaborations with a structural biology research group. The first step toward trying to discover new protein folds hidden within these uncharacterized genes from bacteriophages isolated through the SEA-PHAGES program, was piloted with the first phage discovered at Purdue in 2010, called 'MrGordo.' MrGordo contains 92 genes, 31 with homology to known proteins of function and/or structure; 61 have unknown function/structure. Twenty genes from Mr. Gordo were cloned, expressed using recombinant expression methods, and purified using metal affinity chromatography. Twelve of the proteins were soluble or could be refolded and structural characterization was performed using SEC, CD analysis, and/or SEC-SAXS. Current efforts are to determine the crystal structures of these twelve proteins. The goal of this project is to gain insight into the 3D fold of each of these mysterious novel phage genes in search for new protein folds.

10th Annual SEA-PHAGES Symposium Abstract

Poster #035

Queens University of Charlotte
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Oriana Jerez

Sami Tubolino

Queens University of Charlotte performed another "Nivrat" experiment along with "Burwell21"

Oriana Jerez, **Sami Tubolino**, Jennifer Easterwood, Joanna Katsanos

During Queens' second year in the SEA-PHAGES program, the discovery of two bacteriophages were made: "Burwell21" and "Nivrat." These phages were propagated in Mycobacterium smegmatis mc^2155 then isolated and characterized by students in the biology department. Using enriched isolation, both "Burwell21" and "Nivrat" were isolated from two different soil sample at Queens University of Charlotte. Bacteriophage "Burwell21" was extracted from a soil sample outside of Burwell Hall and bacteriophage "Nivrat" was extracted from a soil sample behind Wireman Building. The plaques from both phages appeared small and cloudy. Transmission Electron Microscopy revealed that "Burwell21" and "Nivrat" were both a part of the siphoviridae morphotype. After the isolation of the two bacteriophages, both "Burwell21" and "Nivrat" were sent to the Pittsburgh Bacteriophage Institute for further analysis and DNA sequencing. Both are a part of the F cluster and the F1 subcluster. By using the shock sequencing method of Illumina Sequencing, it was found that "Burwell21" contained 58,098 base pairs and a 61.5% Guanine-Cytosine (GC) content while "Nivrat" contained 58,009 base pairs and a 61.5% Guanine-Cytosine (GC) content. Annotations were conducted using a bioinformatics program, DNAMaster and 104 genes were predicted for both genomes.

10th Annual SEA-PHAGES Symposium Abstract

Poster #009

Queensborough Community College
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Diana Mata

Genomic Identification of Mycobacterium phage Hope4ever

Diana Mata, Memona Ali, Oscar Bermudes, Elle Casimir, Vanessa Chandie, Lara De Jesus, Katherine Diaz, Israel Escobar, Jahaira Garcia, Jasmine Gildon, Arlind Kacirani, Khushpreet Kaur, Yeeun Kim, Jin-Wei Darren Lee, Allisyn Lopez, Alexa Oltaci, Gabriel Palencia, Glenora Seecharan, Cheten Sherpa, Shanelle Smith, Sabrina Tolentino, Katelyn Urena, Xiaochen Yang, Celines Yauri, Urszula P Golebiewska

During the Spring 2018 semester, students at Queensborough Community College annotated and analyzed the genome of Mycobacterium phage Hope4ever. We were very grateful for the opportunity to adopt Hope4ever. The phage was found in 2012 by Brittany Sklenar, a student from Georgia Gwinnett College, in Lawrenceville, GA. Hope4ever was isolated from Mycobacterium smegmatis mc2155. It has a siphoviridae morphotype, characterized by a large head and a non-contractile tail. It is a temperate phage and forms plaques with bullseye pattern. Hope4ever belongs to the cluster A, subcluster A1. It has a genome with a length of 50455 bps with 86 predicted genes, all of them are protein coding sequences. Hope4ever is shorter and has fewer genes than an average A1 phage. The study of Hope4ever consisted of using bioinformatics programs such as DNA Master, GeneMarkS, HHpred, Blast, TMpred, TMHMM and other to determine homology, coding potential, gene function, open reading frames, length of the genes and possible protein structures. Cluster A is the largest cluster with 556 members, and A1 is its largest subcluster with 141 members. Thus it is not surprising that Hope4ever does not have unique genes and all of Hope4evergenes are homologous to other A1 phages except gp69 which is similar to cluster F. Unfortunately, we could not identify the function of gp69. The phage with highest protein similarity to Hope4ever is U2. On the nucleotide level Hope4ever's closest relatives are Myorolo and Jasper, both with 97% identity. Just like all other cluster A phages Hpe4ever has first half of the genome in forward direction and the second half reversed. In front of the tapemeasure protein there is a programmed frameshift that results in two different sizes of the tail assembly chaperones. Again a feature common in A phages. We identified functions for many of the genes. Hope4ever has an immunity repressor that is found in C1 phages and some A1 phages. It also has HNH endonuclease, integrase, LysinA, LysinB, terminase, portal, minor and major capsid and many structural proteins.

10th Annual SEA-PHAGES Symposium Abstract

Poster #047

Saint Joseph's University
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Emily E Costello

Investigating bacteriophage NatB6 and others members in the CR2 cluster that infect Gordonia terrae.

Emily E Costello, Natalie A Burkert, Nikhita C Pejavara, Giavonna M Picknally, David J Grana, Thi P Nguyen, Francesca C Samony, Iswarya V Vel, Nicole M Butch, Kayla M Gantz, Charles L Lawall, Natalie J Will, Skyler R Demis, Maura L Flynn, Thomas L Jenkins, Luis A Nunez, Elena M Christen, Joan C Reilly, Kaleigh C Williams, Corinne O Merlino, Michael P McCann, Julia Y Lee-Soety

Gordonia in the Actinomycetes genus consists of a diverse group of bacteria. While some species may cause disease in plants and animals, some are soil dwelling and are able to metabolize environmental pollutants. Studying bacteriophages that infect Gordonia may thus contribute to the pathogenesis of bacterial infections and soil contaminant remediations. We extracted bacteriophages from a soil sample collected from Audubon, PA and enriched specifically for *Gordonia terrae* phages. Bacteriophage NatB6 was purified and characterized; it is a lytic phage producing small clear plaques on bacterial lawns. NatB6 has is the siphoviridae morphology – icosahedral capsid with a long, flexible but non-contractile tail. The completely sequenced (Illumina) genome of NatB6 revealed that it is 67,081bp long, contains approximately 96 protein-coding genes, has 65.7% GC content, and ends with 3' sticky overhangs. Based on further sequence analysis, NatB6 is sorted in to CR2 cluster. Ten other sequenced phages are in this cluster, with average genome length of 67,311 bp (ranging from 64,124 – 68,626). NatB6 is most similar to Jifall16 (93.25%) and Foxboro (91.79%). We compared all 11 phages in the CR2 subcluster as well as aligned all 24 Gordonia phages in the CR cluster to make evolutionary inferences for phage diversity.

10th Annual SEA-PHAGES Symposium Abstract

Poster #101

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Savannah Houle

Geraldine Trahan

Bacteriophages

Savannah Houle, Geraldine Trahan

Introduction: Bacteriophages, also known simply as phages, are microscopic viruses in the soil which infect bacteria. Even though we have learned a tremendous amount about phages, there is still plenty left to learn.
Methods: In order to find the phages, we first had to collect soil. The next step was to enrich the soil so the bacteria can grow. The phage was then then spun in the centrifuge and the supernatant was collected, filtered, and M. smegmatis was added so the phage can amplify. The supernatant was then used for assay via spot test and purification. After purification, we named the phages, in our group we had Bones, Snazzy, Stulix, Walgreen, Coca, and Freyja. Several webbed plates were used to collect lysate in phage buffer to create a higher titer. When we had a high enough titer after flooding the plates and collecting the lysates, the phages were sent to the archive. With the leftover lysate, DNA from the phages was extracted, Bones and Snazzy were sent to be sequenced. When the DNA sequences came back, annotation was started.
Results: After performing multiple serial dilutions, and titers we finally received a high enough titer to be submitted to HHMI. We received the genomic sequence of Bones, and began the process of annotation. Bones has 88 genes and is in the A1 subcluster, it is in the siphoviridae family. By annotating each gene we were able to tell where each gene starts and stops, how they are related to similar phages, and what the function is if it had one, but many still have NKF (no known function) which makes the research challenging and exciting.
Conclusions: Throughout the process of isolating phages, we have become familiar with the procedures. We learned how to perform serial dilutions, plaque assays, titers, spot tests. We also learned about phage biology. Throughout the year we had 9 people working to collect a phage with a high enough titer, although only 6 of us succeeded. Of the 6 phages that were sent to HHMI, so far we have received the draft genome for Bones and Snazzy. The process of annotating genes was an entirely new and exciting process for us.

10th Annual SEA-PHAGES Symposium Abstract

Poster #098

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Faith Dent

Victoria Pickford

Musetta and Mulch: Novel Actinobacteriophages Isolated and Characterized at Seton Hill University

Faith Dent, **Victoria Pickford**, Kristen Butela

Microbacterium phage Musetta (ED2) and *Gordonia* phage Mulch (DI) were isolated from soil samples collected on the campus of Seton Hill University in Greensburg, PA and characterized by first-year undergraduate students participating in a one-semester combined phage discovery and bioinformatics SEA-PHAGES research course. Both phages were obtained through enrichment isolation at 25°C using the bacterial host *Microbacterium foliorum* NRRL B-24224 SEA (Musetta) and *Gordonia terrae* 3612 (Mulch), with Musetta producing clear plaques and Mulch producing turbid plaques after 48 h incubation at 30°C, indicating potential virulent and temperate properties, respectively. The genomes of Musetta (63.6 kb, 61.7% GC, defined linear ends) and Mulch (49.9 kb, 67.3% GC, 10 bp overhang) were annotated using PECAAN, DNA Master, HHPred, Phamerator, Starterator, tRNAScan-SE, Aragorn, and the Blast program suite. Musetta contains 122 putative protein-coding genes and 4 tRNAs, and Mulch contains 74 putative protein-coding genes and no tRNAs. Two orphams were identified in Musetta.

10th Annual SEA-PHAGES Symposium Abstract

Poster #061

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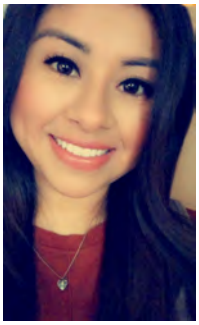
Ashley S Raymond



Maria F Lopez

Characterization of three uncommon bacteriophages, a global analysis of Actinobacteriophage predicted transmembrane domain-containing proteins, and a pan-genome analysis of Microbacteriophages.

Ashley S Raymond, **Maria F Lopez**, Timothy Abbot, Mallory Breban, Angela Galantini, Lucas Galster, Muhamed Khatib, Samantha Murphy, Megan Rebeschi, Dathan Stone, Nicholas P Edgington

So far approximately one hundred sequenced *Microbacteria foliorum* bacteriophages have been identified. We describe two new bacteriophages, 'Gilda' and 'Minima', that can infect this host, and belong to the clusters EF and EE, respectively. The EF cluster only has three members that are closely related to each other and are about 56.7kb in genome length, and the EE cluster has twelve members whose average genome size is only 17.4 kb. We present annotations and comparative genomics data on the Mycobacteriophage "S" cluster phage named 'Lilbit', which was isolated in 2014, but only recently sequenced last year by the Hatfull lab in a search for 'uncommon' mycobacteriophage lineages. Finally, we present a comprehensive examination of putative transmembrane domain-containing proteins in the Actinobacteriophages, and a pan-genome analysis of *Microbacterium* bacteriophages.

10th Annual SEA-PHAGES Symposium Abstract

Poster #082

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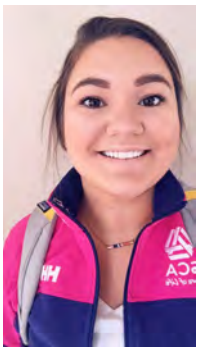

Isabelle I Russell

A Diversity of Marine Phage

Isabelle I Russell, Emily F Haggett, John F Onesti, Ornelly N Yanga, Megan Greenwood, Kathleen Pyburn

Marine phage have been associated with algal blooms either as a limiting factor on blooming species through infection or by infecting bacteria that in turn infect algal species. In 2018 we isolated bacterial hosts and phage from the water column and marine mud from the northwest Southern Maine Community College (SMCC) cove (43°38'48.74"N, 70°13'35.45"W). We isolated three hosts and four phage using enrichment techniques. Hosts were identified to genus level through barcoding of the 16S rRNA gene. One phage genome was sequenced, annotated and submitted to GenBank (NCBI Acc. # MG675557), this phage forms a putative lysogen with its host, Pseudoalteromonas. Electron Micrographs were taken of all phage, portions of their genomes cloned and sequenced and PCR primers were designed. We used the primers to look for the presence of phage in various parts of the marine ecosystem in Casco Bay. We hypothesize that as the waters warm in the spring, host populations will increase with a concomitant increase of phage.

10th Annual SEA-PHAGES Symposium Abstract

Poster #095

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Taylor Gray

Fredrick Wuliger

Isolation and Annotation of a Unique B1 Mycobacterium Phage - PinheadLarry

Taylor Gray, **Fredrick Wuliger**, Robert C Harms, Thomas J Peters

Mycobacteriophage are pathogens known to attack mycobacterium. Eradicating a pathogen with a pathogen seems poetically just. They could be the key to revealing the secrets behind the mechanisms of human pathogens like Mycobacterium tuberculosis and Mycobacterium leprae. Since phage can be grown in abundance in a laboratory setting, unlike M. tuberculosis, they can also be used as a potential model. Working backwards by understanding how phage "work," we can begin to understand the potential phage therapy of human pathogens like M. tuberculosis and M. leprae.

Since August of 2017, the students of St. Louis Community College have been working diligently to isolate, purify, amplify, and extract DNA from phage samples collected from soil found in numerous locations in the St. Louis area using Mycobacterium smegmatis as the phage's bacteria host. PinheadLarry was the phage that was successfully extracted; therefore, it was sent off to the University of Pittsburgh for genome sequencing.

At Pittsburgh, the DNA sample was cleaved into small, manageable fragments, hybridized to special adapters designed to anneal to oligonucleotides adhered to a channeled glass slide. The target DNA was amplified and sequenced during synthesis by observation of unique fluorescent signals emitted by the synthesized strand. By this method, many strands are sequenced simultaneously and indexed according to a specific, shorter sequence that is also catalogued. Pooled sequence libraries were divided according to index and subdivided according to similarity. Reverse and forward sequences were matched creating a whole contiguous genome sequence.

Gene annotation began after mapping with a computer program analyzing the genetic sequence, and defining all the open reading frames based on conditions determining their likelihood. However, the program is imperfect, particularly with a dearth of comparative data as is the case with phage, and thus the draft genome required a second, human annotation. During the 2018 Spring semester, eleven students worked together to annotate PinheadLarry using bioinformatic tools to guide their way to a fully annotated genome. DNA Master was the genome annotation program used along with coding potential algorithms, Glimmer and GeneMark, for gene start locations. NCBI Blast, PhagesDB, and HHPred were utilized for possible known functions for each gene, while the database Phamerator was examined for synteny among similar phage in the B1 cluster. After having to revise start locations, make ORF deletions, and adding new uncalled ORFs, PinheadLarry's genome sequence annotation was finalized. With the annotation complete, hopefully the genome will provide insight to how phage function, which in turn provide insight to how mycobacterium function.

10th Annual SEA-PHAGES Symposium Abstract

Poster #056

SUNY Old Westbury
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Characterization of Actinobacteriophages isolated at SUNY Old Westbury and annotation of Rahel, a C1 cluster Genome

Yara Abdelsalam, Jessica Ackloo, Navpreet Banger, Hason Chan, Tarana Chittineedi, Isabel Duplessy, Mark Dyce, Daesha Garrison, Ashley Gayle, Jaime Gonzalez, Sandra John, Imanjot Kahlon, Tania Kumar, **Adwoa Lewis**, **Karthik Madhira**, Rivka Mullokandova, Fernando Nieto, Christos Noutsos, Whitney Phair, Nellie Pirzadeh, Iman Raja, Kevin Ram, Ravi Ramdhari, Rita Reddy, Briana Saed, Phalan Smith, Stevee Sproul, Jane Thomas, Avia Yossefi

SUNY Old Westbury joined the 10th Cohort of HHMI SEA-PHAGES last fall 2017 semester. The Phage Discovery component was integrated into the General Biology I laboratory (23 students) in the Fall 2017 semester and the Bioinformatics component into the General Biology II laboratory in the Spring 2018 semester (25 students). During the Phage Discovery semester twenty-three viruses were isolated, and only two using direct isolation. All of them were isolated from soils in Nassau or Suffolk counties in an 18 miles radius using Mycobacterium smegmatis mc2155 as a host. Eleven of them were siphoviridae and twelve myoviridae. Rahel, a myoviridae Mycobateriophage was sent for sequencing to the University of Pittsburgh. Rahel forms plaques of approximately 1 mm in diameter and its titer was 3.07 x 1012 PFU/ml. During sequencing the genome was covered 471x. Rahel has 155,955 base pairs and 64.7% GC content C1 cluster of Mycobacterium smegmatis mc2155 phages. As reported by DNA master Rahel has 266 genes of which 9 are in the reverse strand, 155 in the forward strand and 32 are tRNAs. Its closest C1 cluster relative ArcherS7 has 269 genes 43 for which the function is known, 37 are tRNAs and 1 is tmRNA. Using comparative genomics we manually validated the starting codons of Rahel. Out of the 266 genes we changed the starting codon for 30 genes using Starterator and HHPred. Gene density in Rahel is 1.7 genes/kb indicating high gene density. ArcherS7 has 4 more genes and is around 1kb longer than Rahel indicating that genome expansion is due to gene content.

10th Annual SEA-PHAGES Symposium Abstract

Poster #057

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Megan Adams

Esperanza Sandoval

Discovery of Cluster A1 and O Bacteriophages, Arlo and Ryadel

Megan Adams, **Esperanza Sandoval**, Travis Miller, Brittany Stewart, Danielle Bachhofer, Ashleigh Cooper, Jessica Doty, Miranda Fuentes, Morgan Gaitan, Leeila Hanson, Josh Katuri, Brandi McElroy, Jonathon Musgrave, Aleksey Palumbo, Heidi Spann, Amanda Stone, Mario Tovar, Camille Trautman, Bianca Willis, Jesse Meik, James Pierce, Dustin Edwards

We isolated 16 bacteriophages that infect *M. smegmatis* mc2155 from soil samples in north Texas. DNA was extracted and whole genomes of mycobacterophages Arlo and Ryadel were sequenced at the Pittsburgh Bactriophage Institute. Arlo is a temperate cluster A1 bacteriophage with medium plaque size and Ryadel is a lytic cluster O bacteriophage. Transmission electron microscopy identified both Arlo and Ryadel to have siphoviridae morphology, however Ryadel has a prolate capsid which is characteristic of Cluster O bacteriophages. Arlo genome is 52,069 base pairs in size and contains a 10 base overlap of CGGATGGTAA, while Ryadel genome is 72,658 base pairs in size with a 4 base overlap of GTGT. Analysis of potential viral open reading frames indicate that Arlo could encode for a Cas4 protein, a component of CRISPR genome editing systems. These phages could provide further insights into bacterial immune functions and function of elongated capsid structures.

10th Annual SEA-PHAGES Symposium Abstract

Poster #087

The Ohio State University
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Natalie Cervelli

Dahlia Najjar

Genomic Analysis of Arthrobacteriophage MargaretKali, a singleton

Natalie Cervelli, **Dahlia Najjar**, Sarah Ball, Caroline Breitenberger, Charles Daniels

Students at The Ohio State University have been participating in SEA-PHAGES since 2011. During fall semester 2017, we isolated our first singleton, MargaretKali, using Arthrobacter sp. ATCC21022 as the host. The 39,448 base-pair MargaretKali genome has a 61.1 % GC content, which is slightly lower than that of the host's genome (63.4 %), and 69 predicted open reading frames, of which 29.5 % are orphams. A putative tyrosine-integrase was identified, suggesting MargaretKali may be a temperate phage. This is further supported by the fact that many predicted gene products had BLASTP hits corresponding to open reading frames in several different Actinobacteria genera, some of which could represent prophages.

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10th Annual SEA-PHAGES Symposium Abstract

Poster #055

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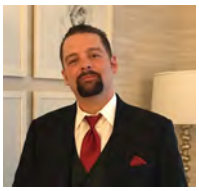

Frank H Robertson

Brendon C Ghiringhelli

Genomic Analysis and Biological Characterization of Novel Bacteriophages BrutonGaster and Schmidt

Frank H Robertson, **Brendon C Ghiringhelli**, Leonard Naegele, Meggan Rowley, Chandler Stucki, Sydney Garcia, Stassi Henry, Christine B Emmons, Tina Slowan-Pomeroy, Laura A Briggs

The genus *Gordonia* has piqued interest in recent years due to its diverse metabolic characteristics and increased frequency in clinical isolates. These bacteria tend to inhabit soil, but several members have been found in activated sludge, oil spills, and other toxic atmospheres, capable of rapidly degrading many pollutants. *Gordonia* spp. are frequently foam-forming bacteria that can be particularly problematic in wastewater treatment. Advancements in species identification via 16S rRNA sequence have led to proper classification of once misidentified pathogens to the taxon. For these reasons, interest has been building to establish better medical and environmental control tactics. Bacteriophages are re-emerging as alternatives to chemical control methods due to their highly selective nature and less destructive impact on fragile ecosystems. The purpose of this research was to study genetic characteristics and host specificity of two Gordonia phages isolated in fall of 2017; BrutonGaster and Schmidt were purified from soil in Northern Nevada and further categorized. BrutonGaster and Schmidt belong to the subclusters CQ2 and CU4, respectively. The genomes have been annotated and compared to closely related members of similar subclusters. BrutonGaster (91,510bp) has considerable genetic homology with OneUp, Toniann, and ClubL (90%, 81%, and 81%, respectively), while Schmidt (43,099bp) has modest genetic homology with Gsput1 and DinoDaryn (74% each). Preliminary data suggest the possibility of temperate lifecycles for BrutonGaster and Schmidt, putative integrase and immunity repressor genes have been identified for both. While Schmidt exhibits classic bullseye plaques indicative of a temperate phage, BrutonGaster results in predominately-lytic plaques when cultured with Gordonia terrae. While both phage genomes are characteristically modular, BrutonGaster had significantly higher synteny with compared species. Host range was examined using several Gordonia species with specific metabolic capabilities or medical significance. Future research will investigate the temperate nature of these two Gordonia phages.

10th Annual SEA-PHAGES Symposium Abstract

Poster #103

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Enrique A Guevara-Rivera

Cristian V Teran-Ochoa

Comparative genomics of recently annotated mycobacterium phage genome: Target.

Enrique A Guevara-Rivera, **Cristian V Teran-Ochoa**, Anaí Amaral-García, Samuel A Roa-Flores, Patricio A Zapata-Morín, Antonio Guzmán-Velasco, Elva T Aréchiga-Carvajal

The mycobacterium phage Target possesses a genome sequence of 49097 bp, GC% of 63.6, and the overhang sequence: CGGATGGTAA. It belongs to the subcluster A1 and it´s morphotype´s characteristic of the Siphoviridae group. 39 of its genes do not have an assigned function. The annotated sequence resulted in 85 genes annotation, some of which have been mentioned as necessary for the viral lifecycle, specifically the Mycobacterium spp. Some of the features in the genes include the characteristic scaffolding assembly protein that contains a frame shift, which follows the coding sequence of a fusion protein, and some others as the terminase, HNH endonuclease, lysine A and B, several minor tail proteins, etc. Comparative genomics methods were applied to the Target´s genome, including phylogenetical relations with other genomes of the same cluster and subcluster, so we could observe the Target´s divergence when compared to other viruses, taking the terminase sequence as the base for its construction and exploring the similarity of the genome nucleotide sequence against other mycobacteriophages. Also synteny was studied in other phylogenetic related phage´s genomes. After we could see all this characteristics and relations together we can infer that there must be some adaptive advantage conferred, that explains the conservation of this particular gene order.

10th Annual SEA-PHAGES Symposium Abstract

Poster #015

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Mckinley R Williams

Ote A Staton

Investigating Corynebacterium Phages: Similar Yet Very Unique

Mckinley R Williams, **Ote A Staton**, Adam R Aldaher, Katelin M Baird, Annisha Borah, Georgia E Haggard, Shriya Meesala, Sarah L Nealy, Aneesh Pathak, Raksha Ramdas, Manuel M Rocha, Sristi Das, Saakshi Thukral, Callie E Walls, Mohammad Waqas, Alexander K Winters, Katrina J Sahawneh, Denise L Monti

Over the past decade, the number of full-length phage genome sequences increased exponentially, in part, due to efforts of students in the HHMI SEA-PHAGES program. Although mycobacteriophages represent the largest group in the Actinomycetales database, several groups recently expanded efforts to isolate phages on alternate bacteria hosts, including Gordonia spp., Rhodococcus spp., Arthrobacter spp., and Microfloriorum spp., among others. For the past two years, students at the University of Alabama at Birmingham have studied phages infecting the host Corynebacterium. Here we report a comparison of Corynebacterium phages to those published in the Actinomycetales database and note unique characteristics of this group. Most *C. vitaeruminis* phages are lytic viruses and share a high degree of genetic similarity (>90% ANI). Moreover, most of these viruses share a common repeat region in the right arm not believed to be found in any previously isolated phage in the Actinomycetales database. Gradual degeneration of the consensus repeat sequence indicates this repeat may be a result of replication slippage. Unexpectedly, *C. vitaeruminis* phages do not all mediate cross-infection of the related bacterium *Corynebacterium pseudodiptheriticum* despite the high degree of genetic similarity among the *C. vitaeruminis* phages. To expand the pool of Corynebacterium viruses studied, we recently isolated five phages infecting the host *C. xerosis* and 3 additional *C. vitaeruminis* phages. In contrast to the *C. vitaeruminis* phages, all C. xerosis phages are likely temperate phages. Of the two viruses with complete full-genome sequences (Juicebox and SamW), both are singletons and appear to share a similar tyrosine integration system. Closer examination of the GC content across the genome revealed a significant drop in GC content particularly in the region of the integration complex for both Juicebox and SamW. Despite possibly sharing a similar integration complex, immunity studies show that C. xerosis phages are homoimmune. Future studies will expand isolation of Corynebacterium phages to further explore commonalities and differences of phages infecting this genus of bacteria. We are particularly interested in expanding phage isolation and sequencing efforts to the host Corynebacterium glutamicum, an important industrial microbe.

10th Annual SEA-PHAGES Symposium Abstract

Poster #046

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Elizabeth Vanderwall

Investigation of B1 and F1 Subcluster Mycobacteriophages' Life Cycle, Genomes, and Potential Applications for Detection of Pathogenic Mycobacteria

Kalin Patel

Elizabeth Vanderwall, **Kalin Patel**, Kevin Ha, April Johnson, Courtney Lee, Annie Odelson, Megan Satyadi, Steve Yoon, Michelle Bina, Andrew Lund, Kris Reddi, Amanda C Freise, Jordan Moberg Parker

Detection of pathogenic mycobacteria is emerging as a potential application of mycobacteriophages. Characterizing phage life cycle, phage-host interactions, and the phage genome is crucial in order to determine whether a phage will be a suitable candidate for diagnostic application. We studied phages from subclusters B1 and F1 to investigate these characteristics.

Phages AltPhacts, DaWorst, and Alexphander were isolated from soil using *M. smegmatis* as a host. AltPhacts presented both clear and turbid plaques, suggesting the possibility that AltPhacts was temperate. However, whole genome sequencing and PHACTS analysis classified AltPhacts as a lytic B1 phage. Other B1 phages have also been observed to produce mixed plaque morphologies, prompting an investigation of genomic elements that might produce this result. Genome comparison and BLASTp analyses within the B1 subcluster suggested DNA helicase variants may have been horizontally transferred from different bacterial species and may affect plaque morphology. Additionally, Phyre2 structural analysis identified gp46 as a lambda-like repressor DNA binding protein with 97.6% confidence. Phylogenetic analysis and multiple sequence alignment of gp46 from B1 phages with either clear or mixed plaques revealed divergent sequences due to a conserved two amino acid variation. In summary, genetic variants within the B1 subcluster may explain variation in plaque morphologies, and although AltPhacts is lytic, it may still have potential application as a detector or even therapeutic agent of *M. tuberculosis*. In addition to M. tuberculosis, there are other serious pathogens of the Mycobacterium genus that require new diagnostic developments as well. To evaluate whether F1 subcluster phages like Alexphander and DaWorst could serve as diagnostic agents of pathogenic nontuberculous mycobacteria, phage replication time and potential infectivity of pathogenic M. abscessus were assessed. A one-step growth curve determined DaWorst's latent period to be 100 minutes, around three hours shorter than most mycobacteriophages. Phylogenetic analysis demonstrated Alexphander's Y-integrase gene to be similar to that of a M. abscessus subsp. bollettii prophage, suggesting that M. abscessus may be within Alexphander's host range. Similar GC content and patterns of codon usage bias between Alexphander, DaWorst, and M. abscessus further suggested potential infectivity, although no conclusive results were found. These two F1 subcluster mycobacteriophages' short latent period and potential infectivity of M. abscessus suggest they and other F1 mycobacteriophages may be suitable for use as reporter phage candidates for mycobacteria. For all three phages, further research to confirm host range and to explore the incorporation of reporter genes will determine whether they can contend as detection agents for pathogenic mycobacteria.

10th Annual SEA-PHAGES Symposium Abstract

Poster #068

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William W Bushnell

Isolation and characterization of Streptomyces and their Phages from soil samples in the San Diego County.

William W Bushnell, Noorhan Z Amani, Maya E Ambroziak, Andrea Biju, Cianna N Calia, Yi J Chen, Lindsay T Hill, Sarna Karpinska, Keeley C Martinez, James R Medwid, Caroline Nguyen, Aaron A Oliver, Jessica P Pham, Marina R Ramsey, Saisri Ravi, Jefferey R Sardina, Savanna L Senecal, Jeanna Sheen, Neil V Shende, Caroline Y Shi, Lauren C Stuart, Linda Vu, Lily Q Wang, Laine J West, Alexandra C Westgaard, Roland Liu, Emily Pierce, Swarna Mohan, Joe Pogliano

Students in the Genomics Research Initiative course at UCSD isolated 26 *Streptomyces platensis* strain MJ1A1 phages from soil samples from around San Diego County and 47 *Streptomyces* strains from the same soil samples. Genomes from two phages, Hank144 and Darolandstone, were sequenced and annotated. Phage Hank144 belongs to subcluster BD2 and phage Darolandstone is the second phage to be added to the subcluster BC2. We screened the isolated *Streptomyces* strains for antibiotic production and 41 out of the 47 isolated strains, were classified by 16s rRNA sequencing. Several of the isolated *Streptomyces* strains produced molecules capable of killing E. coli ΔtolC and Bacillus subtilis PY79. Additionally, we examined the host range of our two sequenced phages against a subset of the isolated *Streptomyces* strains. We used the 16s rRNA results and the sequenced phage genomes to explain any observed differences in the host ranges.

10th Annual SEA-PHAGES Symposium Abstract

Poster #088

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Isolation and characterization of a mycobacteriophage from Oklahoma Soil

Raistland K Valenzuela, Elaine E Sawyer, Christopher J Patton, Hari Kotturi

Bacteriophages are viruses that infect bacterial host cells, take control of the host's replication machinery, and replicate inside the host cell. Our goals in this project were to isolate, characterize, and sequence a mycobacteriophage from Oklahoma soil. The mycobacteriophage Silverleaf was isolated from water runoff soil located in a backyard. The area was boggy with dead foliage covering the topsoil, which is an ideal environment for mycobacteriophages. We used soil enrichment method and agar overlay method for isolating the phage. We did three plaque purifications to obtain a pure phage. We used webbed plates for obtaining a high titer phage. Genomic DNA was extracted using the PCI method and was sequenced using Illumina sequencing technology. We were able to isolate, characterize and sequence a mycobacteriophage Oklahoma soil successfully. As of now, Silverleaf is the largest mycobacteriophage isolated and sequenced from Oklahoma soil. Annotation of Silverleaf's genome was completed using bioinformatics tools. Silverleaf has a genome that is 73,210 bp and belongs to Cluster L and subcluster L1. Silverleaf had 124 genes in total 10 of which code for tRNA. Gene number 114 is an orphan meaning it has no sequences in common with any other gene.

10th Annual SEA-PHAGES Symposium Abstract

Poster #093

University of Colorado at Boulder
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Jade Ponder

Q Custer Mycobacteriophage, Gancho, has recombination genes RecE/RecT adjacent to genes 57-63 which share homology with Mycobacterium vulneris, implying a mechanism for lateral gene transfer

Jade Ponder, Sophie Bai, Richard W Camp, Micklaus Garcia, Grace Hibshman, Katherine Howard, Hongyi Jiang, James Valenteen, Jianxuan Zhang

Bacteriophage are viruses that infect bacteria. These entities are highly diverse and can be grouped into clusters based on genome identity. Their genomes exhibit mosaicism, having acquired an array of genes through recombination and lateral gene transfer, that form distinct genome patterns. The Q cluster mycobacteriophage, Gancho, contains 54,250 base pairs with 82 genes, many with unknown functions. The RecE/RecT recombination system is shared among all Q cluster phages and is essential for the Q mycobacteriophage, Giles. The RecE/RecT recombination system has been implicated in generating mosaicism in phage genomes and recombination-dependent replication in bacteriophage T4. Genes 57-63, which are adjacent to the RecE/RecT genes in Gancho, share homology to genes found in *Mycobacterium vulneris*, a subset of the *Mycobacterium avium* complex, indicating possible lateral gene transfer.

10th Annual SEA-PHAGES Symposium Abstract

Poster #049

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Marcelio Shammami

Analysis of the Annotation of Salz (A11) and Darionha (G1) Bacteriophage

Amber Abram, Zahraa Alhabib, Ivan Barraza-Duran, Karen Crile, Victoria Krajcz, Yamiya Lloyd, DeAvion Logan, Tulsi Patel, Yashodhara Patil, Sabriyyah Ricketts, Leonardo Romero-Barajas, Marisa Rowland, Laila Sareini, **Marcelio Shammami**, Erin Sheardy, Nikol Shllaku, Racha Tiba, Jonathan Finkel, Jacob Kagey, Stephanie Conant

Two Mycobacterium smegmatis specific phage were extracted from soil and were then purified. DNA was extracted and sent to the University of Pittsburg for sequencing, followed by annotation by the 2017/2018 UDM SeaPhage class. The two phages annotated were Salz and Darionha, which belong to cluster A11 and G1 respectively. Cluster A has 19 sub clusters and 556 members, members of cluster A on average have a length 51,604 base pairs, 90.5 genes, 1.2 t-RNA, and are usually siphoviridae. Salz is a siphoviridae phage in cluster A sub cluster 11, it has 50,146 base pairs, and 95 predicted genes, genes 1-34 read forward, and genes 35-95 read reverse. Salz also has one t-RNA, one confirmed deletion, and seven confirmed changed starts. Cluster G has 4 sub-clusters and 46 members. On average cluster G phage have 42,274 base pairs, 62 genes, 0 t-RNA, and are specific to Mycobacterium. Darionha is a siphoviridae phage cluster G sub cluster 1 phage, it has 41,451 base pairs, and 62 predicted genes, there were three confirmed reverse genes, 32, 33, and 61, and the rest were all forward. Darionha has three confirmed added genes, one confirmed deletion and eight confirmed changed starts.

10th Annual SEA-PHAGES Symposium Abstract

Poster #072

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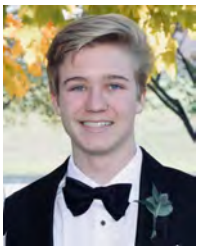

Ian Alberts

Corinne Croslyn

The discovery of Megamind, an isolation and annotation that required a lot of brain power

Ian Alberts, **Corinne Croslyn**, Evan Barnett, Tessa Hemmerlein, Jeremy Schmidt, Kristen Sportiello, Margaret Wolf, Ryan Wood, Joyce Stamm, Ann Powell

As part of our participation in the HHMI SEA-PHAGES program, we analyzed the genome of phage Megamind. Megamind produces small, clear plaques about 1 mm in diameter and has the Myoviridae morphotype. Megamind is a member of subcluster C1 and has 99% identity to the mycobacteriophage Ava3. Megamind was auto-annotated with DNA Master using GeneMark and Glimmer predictions. Auto-annotation showed that the Megamind genome was 154780 bp long with 225 genes. We then refined the genome annotation by using BLAST to compare the nucleotide sequence of all auto-annotated genes to genes in the PhagesDB and NCBI databases. We also examined Phamerator maps and Starterator to help determine the correct start of genes and utilized NCBI BLAST, HHPred, and Synteny to assign function to genes. Our analysis showed that Megamind contains 230 structural genes, 5 more than predicted by auto-annotation. Only 46 of these genes have known functions. We also developed PCR primers to determine phage identity for two complete-genome contigs resulting from a DOGEMS sequencing run for a mixture of genomic DNA from three phages (Primrose, Shelob, Tambudzayi). We plan to annotate the genomes for these two phages and compare them to the genome of Megamind.

10th Annual SEA-PHAGES Symposium Abstract

Poster #048

University of Kansas
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Studies on immunity and phage integration site of Gordonia bacteriophage Pollux

Bailey Edmonds, **Felicity Tso**, Emma Ashley, Andrew Bartell, Donnell Blue, Andres Cordova, Silvian Iordan, Lizzie Karras, Bryn OMeara, Chloe Ortbals, Sharyn Serbet, Joe Wimmergren, Erica Keffeler, Clinton Rice, Robert Ward

In 2017, the University of Kansas SEA-PHAGE class isolated and characterized Pollux, a temperate bacteriophage that infects *Gordonia terrae*. Sequence analysis revealed that it is a member of cluster CY1. BLAST analysis of the Pollux genome revealed strong sequence similarity to other CY phages in the left arm of the genome, and blocks of sequence similarity to clusters CV, CZ, DC, DH and DQ in the right arm of the genome. Repressor-based immunity and other prophage defense mechanisms have been extensively studied in mycobacteriophages, whereas similar functions in Gordonia bacteriophages are just being characterized. To investigate immunity of this phage, we generated a Pollux lysogen and infected it with 8 different Gordonia phages. The Pollux lysogen is immune from infection by CY phages and one CZ phage (Ebert), a DH and a DQ phage. Interestingly, one gene that is shared in 5 of the 6 genomes is pham 5013, which encodes a putative immunity repressor, confirming our annotation of that gene and suggesting that all of these immune responses are repressor mediated. Chidebere (a DQ phage) does not share the repressor gene, and has no obvious gene similarities. We would like to test additional phages related to Chidebere in order to identify candidate genes that may mediate this immunity. A second project in the lab sought to identify the integration site of Pollux in the Gordonia genome. BLAST analysis did not reveal a likely AttB site in Gordonia. Therefore, to identify the integration site experimentally, we isolated genomic DNA from the Pollux lysogen, digested it with 10 different restriction enzymes that cut near and on either side of the integrase gene. We diluted the digests, ligated them and performed polymerase chain reaction (PCR) with outward directed primers specific to the integrase gene in order to amplify the boundary between the prophage and bacterial genomes. Thus far we have not succeeded in generating a specific PCR product for sequencing. If we obtain a specific band we will sequence the product in order to identify the AttP and AttB sites in the phage and bacteria. In a third project, we investigated the prevalence of RNA splicing in mycobacteriophages. We queried the actinobacteriophage database for genomes having the same pham in two adjacent genes using SQL. We found over 1600 cases, but these represented only 152 unique instances. Many of these could be eliminated as programmed translational frameshifts. We obtained 8 mycobacteriophages having putative spliced genes, and designed primers spanning the potential intron. We then compared PCR fragment lengths from reactions using either genomic DNA or cDNA that we generated from RNA isolated from 2-hour phage infections of *M. smegmatis*. Thus far, we have not been able to generate PCR products from the cDNA and so do not have definitive results. However, the low incidence of putative splicing events suggests that splicing is a rare event in mycobacteriophage genes.

10th Annual SEA-PHAGES Symposium Abstract

Poster #025

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Seun D Bamisaye

Cluster A Mycobacteriophage Arcanine with a Shorter Tape Measure Protein

Seun D Bamisaye, Oluwatola E Femi-Olatunji, Ganiyu O Oyetibo, Imade Y Nsa, Matthew O Ilori

Cluster A is the largest group of Actinobacteriophages and has 141mycobacteriophage members in its A1 subcluster. Arcanine, a Subcluster A1 temperate phage was found by Jeff Bonin of Washington University, Saint Louis, MO in 2012 from an enriched soil sample. Its genome size is 5227 bp and has 96 ORFS with a GC content of 63.7%, few genes overlap. About 50% of the genes are in the reverse orientation and ~ 30% of the start sites begin with the codon GTG. The genome contains genes of unknown functions. Arcanine differs from the other A1 cluster phages in its possession of a hypothetical protein similar to that of Mycobacterium SargentShorty 9. The protein products of Genes 2, and 10 show weak similarity to hypothetical proteins from non-Mycobacterium host species. As expected, tail assembly chaperone (tac) is near tape measure and Arcanine has multiple copies (4) of minor tail proteins but the size of the minor tail protein is longer than tape measure. Genes 36 and 37 are copies of the same minor tail subunit, a pattern found in some Subcluster A1 phages. Unlike other members of the Cluster that have a long and short version of the tail chaperones. Arcanine has two tac genes that are about the same size. Its mosaic commonalities with other phages include structural, replication and regulation genes like. HNH Endonuclease domain proteins, tail protein, Lysin A, Lysin B, terminase, portal protein, capsid maturation protease, scaffolding protein, major capsid protein, head to tail connector protein complex. major tail subunit, tail assembly chaperone, tape measure, many copies of minor tail subunits, integrase, membrane proteins, DNA polymerase I, DNA B-like helicase, RecB like protein, immunity repressor, and DNA methylase No tRNA genes are present in this genome.

10th Annual SEA-PHAGES Symposium Abstract

Poster #013

University of Louisiana at Monroe
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Functional Annotation and Comparative Analysis of Three Gordonia terrae Phages – Sombrero (CS2 cluster), Catfish (singleton), and Dogfish (DT cluster)

Katherine E Feroben, Ivan A Alvarez, Misisipi Bhandari, Abby D Byford, Joe R Deselle, Quoc-Nghia N Duong, Ashlyn F Dupree, Minnisa E Garrison, Jeanelle L Higginbotham, Cade W Hunter, Beth A Knight, Jaden A Lee, Isabel C Lewis, Elizabeth L Long, Lauren Q Nguyen, Arohan Rimal, Connor C Roan, Salena Sinnasone, Jamuna Tandukar, Carl E Willis, Aaron V Nguyen, AnneMarie Hancock, Austin P Dicus, Grant E Gallien, Paul D Wiedemeier, Allison M Wiedemeier, Christopher R Gissendanner, Ann M Findley

We have successfully isolated twenty-four phages that infect the *Gordonia terrae* host, three of which have been sequenced. Gordonia phage Sombrero is similar to other CS2 cluster members, has 76,485 bp with a direct terminal repeat of 201 bases, 110 open reading frames, one tRNA, and a GC content of 59.0%. Dogfish constitutes one of two members of the DT cluster of Gordonia phages (with Nyceirae). It has 41,907 bp with a 3' sticky overhang of nine bases, 56 open reading frames, and a GC content of 67.5%. Catfish is presently classified as a Singleton and shares a limited degree of sequence homology with the CU1 subcluster of Gordonia terrae phages. Catfish has 46,888 bp with a 3' sticky overhang of eleven bases, 79 open reading frames, and a GC content of 65%. We provide functional annotations of these phage genomes and explore their relationship to other Gordonia phage clusters using the SplitsTree, Gepard DotPlot, and Phamerator visualization tools. Such analyses provide insight not only into the relationship between the *Gordonia terrae* phages but can point to extended comparisons between other Actinobacter phage group isolates.

10th Annual SEA-PHAGES Symposium Abstract

Poster#111

University of Louisiana at Monroe
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Potential of Mycobacteriophages as Candidates for Phage Therapy - Identification of Phage that Infect Nontuberculous Mycobacteria Pathogens

Katelyn M Setzer, Grant E Gallien, Lucy Darakjian, **Christopher R Gissendanner,** Ann M Findley

Nontuberculous mycobacterial (NTM) infections cause a wide range of debilitating diseases and are increasing in prevalence in the U.S and worldwide. With the increased resistance to antibiotics by NTM pathogens, phage therapy is gaining attention as an alternative for the treatment of such infections. Over 7,000 bacteriophages that infect the non-pathogenic species Mycobacterium smegmatis have been isolated. Host-range tests have identified specific subclusters of phage that can infect multiple M. smegmatis strains and a non-pathogenic strain of M. turberculosis, indicating that they may exhibit broad host range and can possibly infect NTM pathogens. Members of the A3 subcluster have multiple phages that are potentially broad host range. In addition, these phage share a specific variant of a gene (GP5) encoding a putative minor tail protein. We have tested the ability of a large series of A3 phage to infect six pathogenic Mycobacterium species (M. abscessus, M. chelonae, M. fortuitum, M. mageritense, M. porcinum, and M. septicum) to determine if A3 broad host range extended to NTM species and if the infectivity correlates with the gene variant. In addition, as part of the SEA-PHAGES broad host-range project, we have identified the cluster AB mycobacteriophage Muddy as a broad host-range candidate that infects all six of the NTM pathogens tested.

10th Annual SEA-PHAGES Symposium Abstract

Poster #042

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Abigail Moreau

Andrew Wilcox

The isolation and genome annotation of Cluster CV phage, Frokostdame

Abigail Moreau, **Andrew Wilcox**, Kesuma Laizer, Sitey Muktar, Kevin Smith, Jean Doty, Timothy Breton

Bacteriophages are useful for studying evolutionary questions and in medical applications, such as phage therapies. A novel bacteriophage capable of infecting the host Gordonia terrae was isolated from a wastewater treatment facility in Farmington, Maine, named Frokostdame, and its genome was sequenced, assembled and annotated. Frokostdame is a Cluster CV siphoviridae phage that produces small, clear plaques and consists of 52,531 bp and 84 genes. A translational frameshift was found in a tail assembly chaperone sequence, and gene with likely S-integrase and antirepressor functions were identified. No genes corresponding to an immunity repressor or excise, however, were found, and more information is needed to confirm Frokostdame's ability to produce lysogens. This adds to a growing database of actinobacteriophage research that may be useful in future research to understand gene evolution and combat antibiotic resistance.

10th Annual SEA-PHAGES Symposium Abstract

Poster #021

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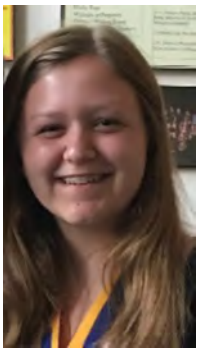

Dakota Archambault

Anna Schumann

Casting a wide net: exploring phage diversity using multiple hosts in a single course context.

Dakota Archambault, **Anna Schumann**, Melissa Maginnis, Keith Hutchison, Melody Neely, Sally Molloy

Bacteriophage, generally, and Actinobacteriophage, specifically, offer a wide array of genetic and biological diversity. Using three different bacterial hosts provided a means to isolate a wide array of phage that students could explore together. Isolated phage included both lytic and temperate phage. Temperate phage included examples of diversity in the immunity cassette, specifically phage that use integrase and phage that use the parABS system. In addition, one phage carries genes for two different repressors. Eden and Sansa are both strictly lytic phage isolated on the host Microbacterium foliorum. Eden is a cluster EB phage with a 40,833-bp genome that encodes 69 putative genes. Sansa belongs to subcluster EA2, has a 40,306-bp circularly permuted genome that encodes 62 putative genes. Two temperate phage, Neville and LastResort, were isolated using host Gordonia terrae. Neville is likely a singleton phage, sharing only 34% of it genes with singleton Octobien14, its closest relative. The Neville immunity cassette includes a leftward immunity repressor, and rightward Cro-like and excise genes located centrally in the genome. The tyrosine integrase is located on the very end of the left arm of the genome. LastResort is a cluster A15 phage that maintains lysogeny through a parABS system rather than with an integrase. The parABS system of LastResort consists of two genes that encode a highly conserved ParA ATPase and less conserved ParB DNA binding protein and eight parSL/R repeat sequences that flank the cassette. Cluster A12 mycobacteriophage, Steamy, isolated on host Mycobacterium smegmatis, also contains a ParABS system. Steamy's ParB sequence is shared with members of four other subclusters but no other member of the A12 cluster. Up to 8 parS-L repeats and 4 parS-R repeats were identified, 2 of which overlap with the 3´ end of parB. BLAST alignment indicates Steamy's parS is most closely related to that of subcluster A9, consistent with ParB pham assignments. Mycobacteriophage Aminay was also isolated on host M. smegmatis and is the only member in subcluster K7. Thirty percent of the 106 open reading frames are classified as orphams. Aminay encodes a tyrosine integrase that is most closely related to cluster K6 integrases, however the attP sequence shares the highest nucleotide identity with that of cluster F1 phage. Putative attB sequences were identified in M. tuberculosis, M. chelonae and M. smegmatis. Aminay encodes a cluster K4-like immunity repressor but also encodes a cluster A repressor. We would like to determine the immunity patterns of Aminay lysogens with cluster K and cluster A phage; however, we were unable to isolate stable lysogens of Aminay in M. smegmatis. We are currently determining the host range of Aminay and its ability to form lysogens in M. chelonae.

10th Annual SEA-PHAGES Symposium Abstract

Poster #065

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Joseph Angstman

Micaela Jones

Discovery of Poenanya: an F1 Bacteriophage from a River sample

Joseph Angstman, **Micaela Jones**, William Litchfield, Aidan Goblirsch, McKenna Frappier, Claire Schindler, Aaron LaBree, Justyce Duffield, Christine Fleischacker

A bacteriophage is a virus that infects bacterial cells, reproduces inside the host cells, and can lyse open the host cell in order to spread to other bacteria. At the University of Mary, the phage Poenanya was discovered and amplified in the Phage Discovery semester. This process began with the isolation and extraction of this phage from a river sample located near the University of Mary in Bismarck, ND. Using Mycobacterium smegmatis mc2 155 as the bacterial host cell, Poenanya was amplified to acquire a sufficient amount of the phage for DNA extraction and TEM imaging. In the Bioinformatics semester, genome sequencing of Poenanya assigned this phage to the F1 cluster of bacteriophages and the annotation revealed that the phage contains 109 genes total. Some of the genes include structural genes of the capsid, tail and minor tail proteins. Other genes include immunity repressor and anti-repressor as well as integrase genes for becoming a lysogen. Our results also show whether Poenanya had the ability to infect alternative Mycobacterium species host cells.

10th Annual SEA-PHAGES Symposium Abstract

Poster #002

University of Mary Washington
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Phage Hunting at the University of Mary Washington

Leland Burke, Samantha Everett, Tara Fitzgerald, Chase Forster, Faith Hodges, MacKenzie Johnson, **Macy Justice**, Francesca Maisano, Emily O'Lare, Natalie Padilla, Cindy Ramirez, Sarah Riddell, Lydia Samson, Jillian Stone, Nicole Taylor, Eleanor Tober, Theresa Vierow, M. Bradley Walker, Jenifer Grove, Lynn Lewis , Theresa Grana

UMW Phage Hunters classes have been isolating phages from *Bacillus* hosts since 2011. Our host this year was *B. thuringiensis* subsp. Kurstaki, which has been used as microbial insecticide for pest control and is used as a simulant for *Bacillus anthracis* in biowarfare/bioterrorism studies. Of the 18 phages isolated this year, one (Natp) was sequenced. Natp was isolated from an enriched culture, and was found in soil from Warrenton, VA. Natp has a genome length of 164,648 bp, which autoannotated with 294 features, a direct terminal repeat of 2,545 bp, and a GC content of 37.8%. Natp is most similar to BM5, Juglone, AvesoBmore, and Troll by BLAST. No tRNA genes were found when searched with tRNA Scan. Interesting features of the genome will be shared. During the spring semester, the class attempted a host range project and several phages were able to replicate on *Bacillus anthracis*, showing a potential phage therapy for anthrax. One new feature this year was that the course was taught by a new instructor, Theresa Grana, for the first time. Much guidance was provided by Lynn Lewis and other regional members of the SEA-phages network instructors.

10th Annual SEA-PHAGES Symposium Abstract

Poster #028

University of Maryland, Baltimore County
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Matthew Koert

Courtney L Mattson

Host Preference and Recombination in Phages Infecting the Phytopathogen Streptomyces scabiei

Zain U Abidin, Devin Blocker, Thomas D Burnett, Danielle N Frank, Nicole Gonzalez, Aloysius B Hora, Allie S Hutchison, Ajay Jani, Taha Jawed, **Matthew Koert**, Alyssa Lagasca, Anna Le, **Courtney L Mattson**, Mahla Nazarian, Harsh Patel, Priya J Patel, Rishit Patel, Hemanta Paudel, Olivia R Richter, Jacqueline Rivera, Sanjum Singh, Zachary Bustamante Tombo, Amina Touma, Matthew Vidmar, Ivan Erill, Steven M Caruso

Streptomyces species are well know for the production of bioactive compounds. Thirty bacteriophages were isolated using the phytopathogen *S. scabiei* RL-34 as as a host, and tested against eleven other *Strep*. species. Six phages were annotated and used for further comparative analysis against previously isolated Actinobacteriophages. Five BI phages were characterized, including three BI2s and two BI4s. Cluster BI phages are *Siphoviridae* with a low average nucleotide identity (ANI) at 0.76 ±SD 0.12, though the sub-cluster ANIs range from 0.88 - 0.98. The gene content similarity (GCS) of the BI cluster is 55.5% ±SD 21.6. One cluster BF *Podoviridae* was also investigated. When included, the cluster BF phages show an ANI and GCS of 0.86 ±SD 0.12 and 84.5% ±SD 12.9, respectively.

Host preference for *Strep*. phages was analyzed using named clusters and phylogenetic methods, using reported isolation host as a proxy for host preference. The analysis revealed that host preference is for the most part consistent within Strep. phage clusters, with one host typically dominating each cluster. This trend was confirmed in cluster BI through phylogenetic analysis of concatenated tail protein sequences and experimental host range data.

Gene content phylogenetic analysis of Strep. phages also revealed strong correlation between cluster membership and the presence of tRNA genes. This correlation was used to predict the presence of tRNA genes in cluster BK phages, which has not been annotated on PhagesDB, and was then verified with Aragorn. tRNA gene content was also shown to correlate strongly with phage lifestyle in Strep. phages. The eight annotated cluster BF phages encode 21 ±SD 1.8 tRNAs. Codon usage analysis of BF phages reveals poor adaptation to their host codon usage bias, suggesting a possible adaptive value for these tRNAs. However, multiple genome alignment of BF phages shows substantial variability within the tRNA gene segment, indicating the presence of a possible recombination hotspot.

Several Strep. phages encode either one or two HNH endonucleases on separate ends of their linearized genomes. Phylogenetic analysis using the protein sequences of these HNH endonucleases and their genetic environment reveals that they were most likely acquired independently from Firmicutes prophages. The upstream HNH endonuclease (R1) is present in several Arthrobacter phages and has bona fide homologs in Lactococcus phages. It is harbored in tandem with the downstream HNH endonuclease (R2) in multiple Strep. phages, and in two closely related Rhodococcus phages. R2 shows homology with Lactobacillus phage HNH endonucleases. Gene content phylogeny and BLASTN analysis reveal that, in contrast to the HNH endonucleases, the rest of these phages' genomes maps to related Actinobacteriophages. These data strongly support the notion that these HNH endonucleases where acquired through lateral gene transfer from Firmicutes prophages.

10th Annual SEA-PHAGES Symposium Abstract

Poster #113

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Steven Caruso

GOing forward: developing critical reading skills through an intercampus GO annotation competition

Steven M Caruso, Ivan Erill

The ability to read and critically assess published research is of import to the education of STEM students, but identifying effective methods to foster critical reading skills remains notoriously elusive. Inclusive Research Education Communities (iREC), like SEA-PHAGES, engage learners by facilitating their immersion in an authentic, yet orchestrated student-owned research experience. This approach has been shown to correlate positively with students' identification with and persistence in science. A main element of iRECs is that students from different institutions, and often with different backgrounds, address a specific scientific question, and that they do so with support from a centralized scientific and programmatic structure. In the SEA-PHAGES implementation, students and instructors are provided with detailed templates, protocols, software, databases and models that guarantee a sufficient degree of coherence and rep`roducibility among all the individual experiments carried out by undergraduates at different institutions.

The Gene Ontology (GO) is is a global community effort to define and systematize all the possible biological roles of a gene product. As such, it provides robust definitions for terms describing gene products and their logical relationships. Using GO, biocurators can formally describe the involvement of a given gene product in a particular biological process, its specific molecular function or its cellular location. This process, which maps gene products to particular ontology terms, is known as GO annotation. CACAO is an intercollegiate competition for GO annotation, in which students compete in teams to make GO annotations and critically assess and challenge those made by their peers. Much like in an iREC, the CACAO competition is operated through a centralized resource that provides training material and an intuitive wiki-based framework to assist students in the annotation process. Like in iRECs, students are free to decide what genes and aspects to annotate, find the necessary sources to do so, and open their work to critical assessment by their peers, but the overall structure of the GO and the CACAO competition provide enough constraints to make the critical reading exercise a collectively coherent experience.

Here we argue that the use of intra- or inter-campus CACAO competitions for GO annotation in the context of SEA-PHAGES complements and augments the iREC experience, providing a focused and well-defined layer of critical reading that is not directly addressed in the foundational iREC. After organizing five consecutive Phage Hunters CACAO competitions involving more than six colleges around the world, we report on a number of key elements that make the CACAO framework ideal for instilling and developing critical reading skills, and we put forward a CACAO unit implementation that maximizes the fostering of critical scientific reading and writing through structured dialog and iterative feedback.

10th Annual SEA-PHAGES Symposium Abstract

Poster #024

University of Nevada Las Vegas
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Chewbacca, Riparian and Reptar3000, three novel M. smegmatis phages from the Las Vegas area

Christopher Wallace, **Erica Torres**, Philippos Tsourkas, Alicia Salisbury, Kurt Regner, Christy Strong, Erin Cassin, Gustavo Reyes-Sanchez, Juvie Ines, Krystal Tran, Joseph Fersini, Heather Rhoden, Ramiro Cisneros, Jonathan Juste, Nicolas Barroga, Dyanne Macalinao, Spencer Muscelli, Rodney Tan, Gabriel Leyva, Tiffany Jeanite, Sophia Nhan, Georgette Uriarte-Valle, Simon Wong, Kevin Ayala-Pineda, Vanessa Cadiz

We present phages Chewbacca, Riparian, and Reptar3000, three novel Mycobacterium smegmatis phages from the Las Vegas area. These are the first phages of the SEA-PHAGES program from the Las Vegas area. Riparian was isolated from soil from a pond in Las Vegas Wetlands Park and belongs to cluster R, a relatively small cluster that consists of only 6 phages. Riparian has the largest genome of the three phages at 71199 bp, and we have identified 100 genes. Its GC content is 56% and it has circularly permuted genome ends. Chewbacca was isolated from soil in a residential area and belongs to cluster N. Its genome is 43575 bp and we have identified 74 genes. It has 3' sticky overhang genome ends and its GC content is 66.2%. Reptar3000 was isolated from soil in a residential area and belongs to cluster K, a relatively large cluster consisting of 114 phages. Its genome is 54601 bp long and we have identified 89 genes. Reptar3000 has 3' sticky overhands and GC content 67.6%. It has one tRNA gene. The three phages appear equidistant from each other in terms of nucleotide sequence identity (~40% nucleotide sequence identity to each other). The three phages we have isolated are not closely related and show there is diversity among M. smegmatis phages in the Las Vegas area. The Las Vegas area is distinguished from other SEA-PHAGES locations by its exceptionally long, hot and dry summers, with temperatures consistently above 100 degrees and humidity in the single digits for at least 4 months of the year. Consequently it is possible that phages from the Las Vegas area may have evolved unique adaptations to these conditions. Work is ongoing to identify unusual features in these phages.

10th Annual SEA-PHAGES Symposium Abstract

Poster #063

University of North Texas
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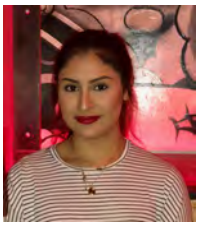

Jessica Barba

Brendan P Frederick

Annotation of Streptomyces Bacteriophages in the Newly Discovered Cluster BK1

Jessica Barba, **Brendan P Frederick**, Lee E Hughes

Bacteriophages which infect various bacterial hosts can typically be grouped into a variety of different phage types called clusters. These clusters are used to organize phages based on their morphology, gene content, and protein characteristics. Within the SEA-PHAGES program at the University of North Texas, we have helped to establish a new cluster, Cluster BK, with the discovery of bacteriophages found on the host genus *Streptomyces*. Currently, there are only two known subclusters within the growing BK cluster, BK1 and BK2. Of the five phages which have been discovered in the BK1 subcluster, 4 have been isolated on the bacterial host *Streptomyces griseus* at the University of North Texas. These phages include Annadreamy, Blueeyedbeauty, Comrade, and SparkleGoddess. Bacteriophages in subcluster BK1 typically have large genomes with an average of 128,659 bp and have terminal repeats between 700 and 800 bp in length. The G+C content found for the discovered phages average at 47.4%, which is among the lowest for phages found on the high G+C *Streptomyces* genus where the genomes are generally 68%-72% G+C. It can also be noted that these phages all appear to be lytic in their infection cycle due to the clear plaque morphology of each phage in the wet lab. Attempts to isolate lysogens have been unsuccessful. From what we know, the majority of these phages possess, on average, 38 tRNAs, which might be an adaptive strategy for expanding host range to more diverse host types. We are continuing to conduct bioinformatic analysis upon these phages and plan to utilize current and future research to further extend our knowledge of bacteriophages. Through our research conducted at the University of North Texas, we expanded the understanding of the diversity of *Streptomyces* phages as well as provided new insight into the properties of members of subcluster BK1.

10th Annual SEA-PHAGES Symposium Abstract

Poster #010

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Discovery and sequencing of Gordonia terrae phages reveals insights into prophage-mediated host defenses and phage evolution

Lindsay Ejoh, **Alec Kistler**, Pitt 2017-18 SEA-PHAGES students, PHIRE students, Marcie Warner, Matthew Montgomery, Meghan Bechman, Rebecca Bortz, Deborah Jacobs-Sera, Graham F Hatfull

Identification of a large collection of bacteriophages infecting a single bacterial host strain reveals insights into phage evolution and diversity. The collection of actinobacteriophages contains over 13,000 phage isolates of which more than 2,500 have been sequenced. Using *Gordonia terrae* as a host, we isolated 307 phages from environmental samples, contributing to the overall total of over 1,100 phages isolated using *Gordonia* strains; 49 of these phages were sequenced. To better characterize these phages, we explored the existence of prophage-mediated defense mechanisms in the *Gordonia* phages, as recently reported for the Cluster N mycobacteriophages. Preliminary studies show that lysogens of phage Wizard (Cluster DC) defend against infection of 11 phages, with plating efficiencies reduced by 10^{-4} to 10^{-8} relative to a non-lysogenic strain. One of the eleven phages (Kenna) has been sequenced and belongs to Cluster DN1, consistent with Wizard-mediated heterotypic defense rather than repressor-mediated superinfection immunity. In the Wizard genome there are only three genes between the lysis cassette and the repressor, two of which (44, 46) encode predicted integrases. Gene 45 has no predicted function but is a good candidate for conferring defense against Kenna infection. Future efforts will be directed at sequencing the remaining phages and investigating gene expression of the Wizard prophage and the phages it defends against. To further characterize the collection of newly acquired *Gordonia* phages isolated by Pitt students during academic year 17-18, the genomes of 49 samples were sequenced, constituting a substantial portion of the total of 297 sequenced *Gordonia* phages. These phage genomes span considerable diversity and are characteristically mosaic. The newly characterized phages can be assigned to 12 extant clusters (CS [6 phages], CT [1], CV [8], CY [1], CZ [10], DB [4], DC [3], DE [9], DJ [1], DL [1], DN [3], DP [1] and include one singleton (Gudmit). These phages differ in genome length ranging from 42,205 bp (Gudmit, singleton) to 90,490 bp (Daredevil, Cluster DL), and G+C% content, ranging from 50.2% (Volt, Cluster DP) to 68.5% (Leonard, Cluster DE2; *G. terrae* is 67.8%). Volt, along with two additional Cluster DP phages, Fryberger and Ronaldo, share very few genes with phages of other Actinobacterial hosts. Together with their relatively low G+C%, these observations suggest that the DP phages may have recently adapted to infect *Gordonia*, and evolved most recently from phages infecting hosts with lower G+C% than *Gordonia*.

10th Annual SEA-PHAGES Symposium Abstract

Poster #003

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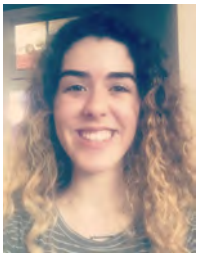
Agnes Cotto-Pereira



Natasha Gracia-Marquez

Genomic Characterization of Bacteriophages Isolated from Tropical Soils of Puerto Rico

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Genuine scientific discovery incorporated into the Biology curriculum engages freshman students in meaningful educational experiences piquing their interest and curiosity with the goals of preparing scientifically literate citizens and motivating them to persist in STEM fields. A positive impact on many of our students inspires a model for curriculum change. Over the past nine years, as participants in the SEA-PHAGES Program, approximately 300 undergraduate students at UPR-Cayey have isolated more than 200 bacteriophages, infecting *Actinobacterial* hosts, from soils of Puerto Rico, a tropical island with rich biodiversity in its varied ecosystems. Analyses of bacteriophage genomes, with variable and mosaic gene organization, identifies numerous novel genes and can result in potential therapeutic uses. We hypothesize finding unique bacteriophages containing genes with useful properties. Soil samples were collected throughout Puerto Rico and high titer phage lysates were prepared following enrichment 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. Bacteriophage genomic DNAs were purified, digested, and visualized using gel electrophoresis. Selected genomic sequences were determined and analyzed using bioinformatics tools. This year, nine novel virulent and temperate bacteriophages infecting Gordonia terrae 3612 were isolated followed by purification and partial characterization. These bacteriophages are predicted to encode many putative protein-coding genes similar to previously identified ones as well as novel proteins with unknown functions. Additional experiments are currently underway to further characterize these novel bacteriophages.

10th Annual SEA-PHAGES Symposium Abstract

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Additional experiments are currently underway to further characterize these novel bacteriophages. All characterized A subclusters are made up of mycobacteriophages except subcluster A15, a crossover cluster comprised of actinobacteriophages infecting Gordonia terrae. The eleven bacteriophages of this subcluster have an average genome size of 52,638 base-pairs with 62.0% GC content. Subcluster A15 bacteriophage Anon, isolated from Puerto Rico, contains twelve unique orphams. Subcluster A15 bacteriophages include nine phams contained in all members of this subcluster. One of these phams (38764), encoding a head-to-tail connector complex protein, is shared exclusively by all 556 sequencedCluster A bacteriophages. Some of these shared and unique phams may potentially contribute to bacteriophage broad host range that can result in mosaic genomes. At UPR-Cayey, seventeen bacteriophage genomes have been annotated and thirteen have been deposited in GenBank so far. The unique organization of these bacteriophage genomes provides a rich resource to elucidate their evolutionary origin and history through countless bacterial infections and continual geographic dispersal. SEA-PHAGES, HHMI, and UPR-Cayey supported this research.

10th Annual SEA-PHAGES Symposium Abstract

Poster #060

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Alexis Bailey

Marian Smallin

Analysis of Conserved Genes and Comparison of Protein Sequences Across Phages From F1 and C1 Subclasses

Alexis Bailey, **Marian Smallin**, Joshua Heuler, Montanna Jenkins, Ariana Saldoriga, Destiny Strickland, Taylor Reid, Meghan Weber, Hannah Sheppard, Hannah Grzech, Sydni Schlosser, Daniel Nunez, Caleigh Ertel, Shreya Patel, Sydney Bass, Oliva Sciandra, Logan Hedberg, Francesca Prieto, Jeannette Myrick, James Garey, Madison Davis, Richard Pollenz

Bacteriophages are the most abundant viruses on earth that infect and replicate their DNA within bacterial and archaeal cells. The genome that is contained within each bacteriophage expresses many of the proteins that are responsible for the replication and assembly of the virus. They are a model organism for understanding genomic information, and how organisms evolve over time. Although we worked in Microbacterium foliorum during the wet lab portion of the SEA PHAGES program to provide genetic diversity, we were unable to obtain DNA that could be sequenced. Thus, we adopted TinyTim and Girr, that are two actinobacteriphage that infect Mycobacterium smegmatis. TinyTim is a class C, subclass C1, myoviridae phage with a 1.53kb genome, and 262 predicted genes. Girr is a class F, subclass F1, siphoviridae phage with a 0.57kb genome, and 104 predicted genes. Therefore, this project endeavored to compare these genomes and identify similar and unique features. We hypothesize that genes encoding phage structural proteins will be conserved, while the majority of each genome will contain unique genes specific to the function of the phage. In addition, we hypothesize that proteins with the same function will have regions of high amino acid identity. The annotation of TinyTim revealed 223 verified coding sequences and 29 tRNAs. Girr had 101 verified coding regions and 1 tRNA. Alignment of these genomes showed minimal nucleotide minimal sequence homology. However, the annotation revealed that there are 34 genes in TinyTim that could be assigned a function compared to 32 in Girr. The common structural genes included portal, tapemeasure, major capsid, tail assembly chaperones and minor tail proteins. There also were several common enzymes including: carboxypeptidase, glycosyltransferase, helicase, and endonuclease. Alignment of the common structural proteins from TinyTim and Girr using three different programs revealed very small regions of identity and limited similarity across the entire sequence. However, secondary and tertiary structural analysis of the portal protein revealed high levels of protein structural conservation. Comparison of enzymes that had been ascribed similar functions revealed an overall higher level of sequence identity but most were still <40% conserved at the amino acid level. However, using protein alignment software, smaller regions within the proteins were identified with >60% similarity and these regions could be 3D-modeled into very similar structures even from very divergent phage classes. This research indicates the proteins with similar functions from phages in different clusters often do not have a high level of amino acid conservation and highlights the importance of rigorous analysis beyond simple amino acid comparisons when calling function. In addition, the process described in the project may assist in identifying key regions of unknown proteins for functional wet lab analysis.

10th Annual SEA-PHAGES Symposium Abstract

Poster #114

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Isolation of 29 Novel Actinobacteriophage that Infect Microbacterium foliorum: Lessons Learned from Running the SEA PHAGES Wet Lab for the First Time

Richard S Pollenz, Madison Davis

USF joined SEA PHAGES in cohort 10 and initiated two sections of the course in Fall 2017. Each section was capped at 16 students and both courses filled with a majority population of freshman biology majors. Each section met Mon/Wed for 2 hours with an optional 3 hour open lab on Fridays. The host bacteria utilized was Microbacterium foliorum. To validate the ability to utilize this host in Tampa and predict the number of samples that would need to be evaluated in the course, 30 soil isolates were screened prior to the start of the course. 5 of the samples were positive following direct isolation and an additional 7 samples produced plaques after the enrichment. Thus, 12 of 30 samples showed positive results (40%) suggesting that it was likely that all students in the course could isolate their own phage. In our first isolation, students were tasked with obtaining 4 soil samples each and in the direct screen 18 of the 131 samples (13.7%) showed positive results. Due to the hurricane, these samples were not processed using enrichment, thus, an additional 3 samples were collected and screened through direct and enriched procedures. 16 of 101 samples showed positive results after direct isolation (13.9%) with an additional 32 that showed positive results following the enrichment (31.2%). In total, 232 samples were processed with 34 phage isolated via direct isolation (14.6%) and a total of 66 samples were positive (28.4%). Thus, all but one of the students worked from a direct prep and only three had to adopt a phage that was not from one of their own soil samples. This data may be helpful for other groups working with foliorum. Procedurally, since each class session was only 2 hours, the instructor and TA added media, shook and centrifuged the soil samples prior to the class session. Students then received the freshly centrifuged samples and processed them from that point in the protocol. The 29 unique phage had varying plaque morphologies and growth characteristics. Generating high titer lysates for temperate phage or lytic phage with slow growth and very small plaque morphologies presented significant challenges. We did have some success in pooling centrifuged lysates or using the same lysate to flood multiple webbed plates in succession. DNA extraction proved to be the most challenging procedure and UPitt was unable to sequence the DNA that was isolated from four of the phage that all appeared to be podovirdea. Although the DNA appeared to pass QC, novel sequence was never obtained for these phage. Future work will assess if alternate DNA methods may be needed or if there were procedural issues with the DNA extraction techniques.

10th Annual SEA-PHAGES Symposium Abstract

Poster #036

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Paige Braddy

Evan Derrick

Functional analysis of conserved hypothetical genes from cluster K mycobacteriophages Hammy and Waterfoul

Paige Braddy, **Evan Derrick**, Sukriti Bhattarai, Chazden Blackmon, John Marshall Diffey, Chance Ford, Matthew Guillot, Brittney Johnson, Joshua Lange, Elizabeth Lawhead, Sanchirmaa Namjilsuren, Ronald Robinson, Kauri Runge, Robert Sanders, Dean Scafidi, Sarah Spence, Tevin Tillman, Donovan Weekley, Damayanti Chakravarty, Clint Pablo, Samuel Hendry, Dmitri Mavrodi

Bacteriophages that infect mycobacteria attract a lot of recent interest as therapeutic agents for treating infections caused by antibiotic-resistant *Mycobacterium tuberculosis*. To date, over 1500 phages that infect *M. smegmantis* and *M. tuberculosis* were discovered and characterized through genome sequencing and bioinformatics analysis. Most of these phages belong to the family Siphoviridae and are grouped into clusters based on genetic similarity. In addition to well-characterized genes that encode structural, regulatory, DNA metabolism, and lytic proteins mycobacteriophages carry numerous conserved hypothetical genes. Such genes are identified via cross-genome comparisons, but their function is currently unknown. In this study, we employed a combination of high-fidelity PCR and Gibson assembly to clone 70 hypothetical genes from the cluster K bacteriophage Hammy. The genes were cloned into the shuttle vector pSMEG and introduced into *M. smegmatis*, the natural host of Hammy. We then treated the plasmid-carrying clones with the inducer anhydrotetracycline and demonstrated that several hypothetical genes exhibit cytotoxicity and kill the bacterial host. Some of the newly-characterized cytotoxic Hammy genes have homologs in bacteriophage Waterfoul and are variably present in other clusters of mycobacteriophages. The ongoing work involves the identification of *M. smegmatis* targets of the cytotoxic proteins using a bacterial two-hybrid system. Results of this study will help to elucidate the role of poorly characterized viral genes in the biology of phages that infect M. smegmatis, M. tuberculosis, and closely related bacteria.

10th Annual SEA-PHAGES Symposium Abstract

Poster #067

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Elizabeth Boos

Cindy Hong

Phalling off of Clifton

Elizabeth Boos, **Cindy Hong**, Christian Fan, Radhika Govani, Kunal Kadakia, Vaishnavi Kundur, Reuben Sam, Luvpreet Singh, C.Nicole Sunnen

The SEA-PHAGES program is a full-year course that focuses on characterizing different phages and annotating their genomes to identify unique genes and their functions. The bacteriophage Clifton was isolated from a soil sample from Clifton, New Jersey, and infected the bacterium *Mycobacterium smegmatis*. Through serial dilutions, Clifton was isolated and found to form clear plaques. Clifton was found to be a siphoviridae and F1 cluster phage through electron microscopy and genomic sequencing. Afterwards, Clifton was annotated using bioinformatics tools, such as DNA Master, Phamerator, GeneMark, etc., to determine the location and function of each gene by comparing Clifton's genes to the genes of previously annotated phages. GP79 in Clifton was interesting because it had substantial coding potential and filled a large gap, but it was an orpham.

Therefore, there is not a lot of information about this gene and needs to be studied more to find its function. Similarly, we inserted a gene not called by Glimmer or Genemark, that aligns 100% with a current orpham in Pippy, GP47. Integrase and immunity repressor genes were indicative that Clifton was a temperate phage, despite its clear plaque morphology. Integrase allows Clifton to recognize specific sites in the host's genome to insert viral DNA and the immunity repressor allows Clifton to stay in the lysogenic cycle. The integrase and immunity repressor genes can also provide information about how phages remain undetected in their hosts and how these functions could be repressed. This information may be applicable for treating dormant viruses such as HIV or Herpes. Another interesting gene was WhiB family transcription factor because it allows Clifton to alter its host's cell wall to prevent other bacteriophages from infecting that same host. This can be useful in affecting the pathogenesis of bacteria, as seen in other bacteria such as Corynebacterium diphtheriae or Vibrio cholera. Studying Clifton's genome may be useful in medicine because it can provide information and preventative treatments for human pathogens.

10th Annual SEA-PHAGES Symposium Abstract

Poster #080

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Andrea Garcia

Lessons derived from three years of searching for Arthrobacteria phages in the Chihuahuan Desert

Andrea T Garcia, Alan Vaquera, Rafael Aleman, Jose Renteria, Angel Rivas, Jazmyne Arroyo, Andres Vasquez, Angel Avila, Jordan Francis, Mariana Gines, Miranda Luna, Erika Mogollon, Chelsea Porras, Nicholas Ruedas, Felipe Rodriguez, Germán Rosas-Acosta

This year, our Phage Hunters group continued attempting the isolation of Arthrobacter sp. phages from the Chihuahuan Desert. Out of 14 soil samples analyzed, 11 were collected in El Paso, TX, 2 in Cloudcroft, NM, and 1 in our border city of Ciudad Juarez, Mexico. Although Arthrobacteria are capable of growing under adverse conditions, our search for Arthrobacteria phages in our desert environment has continued to be largely unsuccessful: out of the 12 soil samples collected in the Chihuahuan desert, only 1 turned out positive. In sharp contrast, one of the two samples collected from Cloudcroft, NM, in the Lincoln National Forest, turned out positive. This finding, consistent with those of our previous Phage Hunters groups, indicates that Arthrobacteria phages are scarce in this geographical area. The possibility that Arthrobacter's abundance might be affected by the extreme heat that predominates during the season in which the samples are collected will be taken into consideration as an important variable for future iterations of this laboratory.

Due to issues related to the purification of DNA from the Arthrobacteria phages isolated by us, for the second part of the course, our group annotated the genomes of two Mycobacterium smegmatis bacteriophages isolated by students at Southern Maine Community College: Hegedechwinu and Anglerfish. Hegedechwinu, an F1-subcluster bacteriophage, has a 56,644 bp-long genome coding for 104 genes in the forward direction and 4 genes in the reverse direction; these reverse-direction genes were ignored by the automatic annotation performed using DNA Master and were found only during the manual annotation. Anglerfish, an A1-subcluster bacteriophage, has a 51,992 bp-long genome, coding for 38 genes in the forward direction and 56 genes in the reverse direction. Interestingly, neither of these phages contained tRNA genes. Considering that these phages were isolated in a very different environment from ours, we compared these phages with those previously isolated by UTEP students using Mycobacterium smegmatis in previous academic years. For Anglerfish we chose phages that belong to the A cluster, including Rebeuca, Airmid, Naca, and Yokurt. For Hegedechwinu we chose the only non-A cluster phages from UTEP, i.e., Leston (a K5 subcluster member), and Nicholasp3 (an L2 subcluster member). As expected, in spite of their different geographical origin, Anglerfish showed substantial similarities with the A-cluster phages isolated at UTEP. On the other hand, Hegedechwinu showed a similar functional gene arrangement as those observed in Leston and Nicholasp3 regardless of its different geographical origin and subcluster identity. Altogether, these findings emphasize the remarkable evolutionary relationships among bacteriophages in our biosphere.

10th Annual SEA-PHAGES Symposium Abstract

Poster #092

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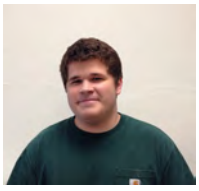
Nicholas C Pritchett



John D Larrimore

Ecological niche modeling and phylogenomic analysis of the C1 mycobacteriophage subcluster

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Cane17, a novel mycobacteriophage, was discovered in a soil sample collected from a farm in Emelle, Alabama. The phage was isolated using the host bacterium *Mycobacterium smegmatis* mc^2155. Cane17 (Myoviridae) forms small, lytic plaques and possesses a large head and short tail. It was chosen for genome sequencing because of its plaque morphology and high DNA concentration relative to other phages collected by the class. Cane17 was then assigned to the C1 subcluster and is 160,330 base pairs long. We annotated the genome using DNA Master and other supplemental programs. We discovered that Cane17 has 223 protein coding genes, 33 tRNAs, 1 tmRNA, a programmed translational frameshift, and a wraparound gene. There are 120 C1 subcluster phages, and we sought to understand the phylogenomic relationships within this subcluster. To accomplish this goal, we performed a Principle Coordinate Analysis and generated a Bayesian phylogenomic tree. Tonenili is the most basal taxon of the C1s and all other phages fall into one large cluster. Although all phages except Tonenili group together, there is high support making each phage unique. Given the atypical genome identity of Cane17 and its sisters, we wanted to see where else C1 phages are found and predict their distribution. We conducted ecological niche modeling using bioclimatic variables and present a map which indicates where C1 phages are likely to be found and where they should be scarce. Given the predicted distribution of C1 phages, we then performed a DOGEMS experiment to see if we had isolated any other C1 phages which weren't immediately chosen for genome sequencing. We found that our pool of phages did in fact include an additional C1 phage as well as A1, A7, B1, B4, B5, and F2 phages.

10th Annual SEA-PHAGES Symposium Abstract

Poster #032

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Samuel Alvarado

Rebecca Saults

Characterization of Gordonia phages with the help of DOGEMS

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Using Gordonia terrae as the bacterial host, our phage hunting started with soil collected from the UWF campus. Out of twenty soil samples collected independently, students have isolated and purified 10 phages: Griffith, YellowGate, Pogo, Semowler, Tetu, Fury, Spicyboi, Pleakley, HuiMin, and Forcado. Based on the whole genome sequence information, Fury and Pleakley are CR5 phages. The CR5 phages have lytic life cycle. To determine the cluster categories of the other eight phages isolated, we took the DOGEMS approach, sequencing the mixed DNAs of Griffith, YellowGate, Pogo, Spicyboi, HuiMin, and Forcado. The sequence information derived from the phage DNA mixture result indicated at least an additional CR5 phage was among these six, and maybe phages of clusters CR4, DI, DL, and DR. We were able to design 3 sets of cluster CR5-specific primers to identify phages Griffith, YellowGate, Pogo, Spicyboi, and HuiMin are CR5 phages as well. This result is consistent with the observation that these 7 CR5 phages all produce medium to large clear plaques. These 7 CR5 phages have their phage tail length at the range of 260-465 nm. We also designed primers based on other small contigs matched to clusters DI, DL, and DR for identifying the other three phages Semowler, Tetu, and Forcado. The results of these three phages' identification were not as conclusive as the CR5 phages; however, the results so far suggested these three phages might belong to cluster DL. These three phages Semowler, Tetu, and Forcado, all produced very small plaques and have their phage tails longer than 650 nm. Further exploration of phage Tetu will be carried out during the coming summer. While carrying out the DOGEMS project, we noticed a few supposed to be DI- or DL cluster specific primers also gave rise to PCR products out of CR5 phages. These PCR products appeared to have a larger size than original prediction. By observing the patterns of these PCR products derived from the CR5 phages, one could not help but to roughly profile these phages, that Griffith, YellowGate, and HuiMin are more similar to each other; the next kin would be phage Pogo, and then followed by Spicyboi. It might be worthy of exploring the application of the DOGEMS approach, that by using a more elaborated experimental design based on a series of PCR and sequencing experiments, one may be able to profile phages without the whole genome sequence information.

10th Annual SEA-PHAGES Symposium Abstract

Poster #038

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Serena K Jacob

Evan P Ruesch

Adventures in phage hunting on new Actinobacteria hosts: Risks and Rewards

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UWRF phage hunters have used several alternate hosts during our participation in the SEA-PHAGES program. While the success rate of students finding phages may be lower with these hosts, the phages isolated are often very interesting and yield new information about phage genomics. To increase the chances of students finding a phage, we have tested each soil sample on three different hosts. For 2017-18, we used *Arthrobacter sp*. 21022 and *Microbacterium foliorum*, as well as *Kocuria kristinae* NRRL B-14383, a new host not previously used for phage hunting. We selected *K. kristinae* because it grows well and we found phage in a pilot experiment. It is also related to Arthrobacter and Microbacterium, and we hypothesized that phages isolated on these hosts might exhibit interesting genomic relationships. We isolated five phages on *K. kristinae*, 17 on *M. foliorum* and 23 on Arthrobacter. Of the five *M. foliorum* phages sequenced, two (Fork and Lyell) formed subcluster ED2 with Musetta. The ED2 phages share 82-86% gene content similarity with each other and 37-40% with subcluster ED1 phages. Phages Quaker, KayPaulus, and VitulaEligans are all in cluster EE. These phages have small genomes (17,450-17,534 bp), consisting mostly of structural genes, but share no nucleotide or protein similarity with phages in other clusters with similar sized genomes. After four Kocuria phages were sequenced, it was determined that this host is actually Microbacterium paraoxydans. However, these phages are quite interesting. Jacko is in subcluster ED1, with phages infecting M. Paraoxydans strain NWU1, and Efeko is in cluster EE. Efeko and Jacko do not infect M. foliorum, and our cluster ED and EE phages isolated on M. foliorum do not infect this M. paraoxydans host. ValentiniPuff is a singleton distantly related to Arthrobacter cluster AL phages, sharing 3% gene content similarity. Burro is a unique singleton, with little similarity to any other phage; 48 of its 49 orfs are orphams. Burro appears to be a podovirus, and its genome contains the longest gene found in Actinobacteriophages, 13,476 bp. We hypothesize that this gene consists of fused structural genes, and we are carrying out protein analyses to better identify Burro's gene products. In preliminary host range testing, each of our M. paraoxydans phages exhibited a different pattern of infection on other strains of M. paraoxydans and M. aerolatum, and may yield new information about host range mechanisms. The one Arthrobacter phage sequenced, Tatanka, is in subcluster AU1. Cluster AU appears to be part of a supercluster that includes clusters AM, AW, BI, CC, DJ, and singleton Camille. These phages have 22-40% gene content similarity and share other genome features such as many membrane proteins (21-24), low GC content (51-59%) and repeats in intergenic regions. These new phages exhibit a range of genomic relationships and will enhance our understanding of phage biology and evolution.

10th Annual SEA-PHAGES Symposium Abstract

Poster #043

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Phighting bacteria entails: Comparative analysis of phage genomes and proteins

Ryan Butler, **Kahle Mercer**, Allison Johnson

Due to the alarming rates of bacterial antibiotic resistance, the field of phage therapy has recently garnered increased attention. The applications of this therapy are numerous and varied from dentistry all the way to agriculture. Bacteria and their phages have coevolved for so long that they respond quickly to adaptations, giving phage therapy a huge advantage over traditional "fixed" antibiotics. We used comparative genomics to better understand a collection of phage genomes and the whole genome as well as individual protein level.

As part of the 2018 VCU Phage lab, students annotated the genomes of four bacteriophages, Kamfam, ALPS, OmnioDeoPrimus (ODP) and Kioshi. The phages were found using Bacillus thuringiensis bacteria. These phages belong to the family myoviridae, which are characterized by double-stranded DNA and a long contractile tail. The genomes of three of the phages were very similar in length, averaging around 161800 bp with terminal repeats between 2000 and 3000 bp. ODP and ALPS have 294 and 291 open reading frames, respectively, while Kamfam has 284 along with 7 tRNAs. In contrast, Kioshi is 165,676 bp long, has 260 open reading frames and 22 tRNAs. All four phages had a GC content of 38%. In the lab, we used bioinformatics tools to further analyze these phages and compare them to larger databases of known protein functions, such as Blastp, HHpred, and PDB. These databases, paired with Genemark predictions, allowed us to create a complete annotation of the phages in DNA Master. Subsequently, a more in depth analysis of the phages was performed using comparative genomics tools for phylogeny, protein content comparisons, and dot plot comparison. This poster will also highlight projects examining the conservation of capsid protein and horizontal gene transfer of a family of intron encoded endonucleases that helped us understand more about inheritance of genes. Ultimately, these approaches were employed to gain a better understanding of the evolutionary similarities and differences between phages that infect Bacillus.

10th Annual SEA-PHAGES Symposium Abstract

Poster #070

Virginia Tech
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Novel mycobacteriophages Crespo and Dietrick: a comparison of genome length and structure

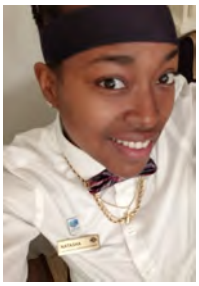
Jesse R Janoski, **Brenna V DeBellas**, Antonia C Broussard, Steven T Carter, Justice S Coggin, Alexandra I Crawford, Isabella A Crespo, Emma C Dodd, Christine M Ellis, Alexandra M Ellison, Marc O Hidalgo, Isabella Kim, Tessa A Kovats, Chanida Lerdritsomboon, Charles H Lynam, Caroline M McKenna, Catherine M Meyer, Benjamin R Nickerson, Quentin F Pitts, Soshiant A Raeesian, Sierra B Raney, Patrick J Reyes, Alyssa M Simon, Rachel M Smith, Corinne A Tran, Rebecca G Truban, Victor Van, Courtney L Walker, Megan E Walter, Nicole M Whalley

Two novel mycobacteriophages, Crespo and Dietrick, were isolated, characterized, and annotated by students in the Virginia Tech Phage Hunters laboratory during the 2017-2018 academic year. Crespo belongs to the G1 subcluster, a group of phage with relatively short genomes, while Dietrick is a C1 phage. Phages in the C cluster are known for having large genomes. Dietrick's genome size (153,582 bp) is over three times larger than that of Crespo (41,902 bp). Additionally, Dietrick is a lytic myoviridae phage, while Crespo displays the more common siphoviridae morphology and contains genes typically found in temperate phages. Crespo and Dietrick are excellent models to compare and contrast genetic elements related to both morphology and life cycles. The massive difference in genome size also offers insight into the minimal number of genes needed for a phage to function and highlights genes that likely perform other non-crucial roles.

10th Annual SEA-PHAGES Symposium Abstract

Poster #044

Virginia Western Community College
W. Roanoke VA
Corresponding Faculty Member: Heather Lindberg (hlindberg@virginiawestern.edu)



Natasha Whitaker

The discovery, characterization and annotation of DrFeelGood, an A1 cluster mycobacteriophage

Natasha Whitaker, Christopher Tice , Richard Juneau, Jacqueline Biscardi, Stacie Deaver, Heather Lindberg

At Virginia Western Community College, over the course of the 2017- 2018 academic year, we isolated, characterized and annotated the genome of a unique bacteriophage, DrFeelGood. DrFeelGood was isolated from soil collected from a compost pile comprised of potting soil mixed with clay in the Roanoke Valley using Mycobacterium smegmatis mc155 as the host bacterium. Isolation and purification of Dr.FeelGood occurred during the fall semester in Biology 101, while genome annotation took place in Cellular Biology during the spring semester. Sequencing done by the Univeristy of Pittsburgh revealed DrFeelGood to be an A1 cluster phage. For genome annotation, several databases were used to investigate the sequence of DrFeelGood's 83 genes and their correlative functions. The overall goal is the submission to GenBank with expectations of contributing to the comprehensive knowledge of phage genetics, biodiversity, and microbial ecology.

10th Annual SEA-PHAGES Symposium Abstract

Poster #089

Washington University in St. Louis
St. Louis MO
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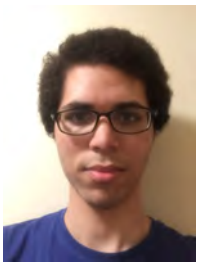


Muhammad Mousa

Characterizing the Novel Bacteriophages of the St. Louis Area

Muhammad Mousa, Aja Drain, Adeshola Fanegan, Katie Gemperli, Laura Goh, Lauren Kang, Benjamin Kelsky, Sofia Kling, Tuka Mousa, Jerry Sun, Jesse Yavner, Christopher Shaffer, Kathy Hafer

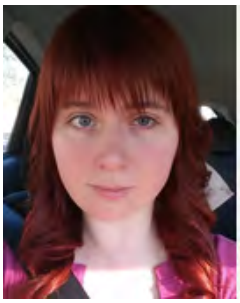
The students of Washington University in St. Louis, in conjunction with the SEA-PHAGES program, isolated and annotated 7 novel *Streptomyces griseofuscus* and *Streptomyces lividans* bacteriophages. Collectively, these discoveries can provide significant contributions to our understanding of the overall diversity of phages in the St. Louis Area. The 7 annotated phages all belong to the Siphoviridae morphotype; two are members of the BE2 cluster, one was assigned to each of the BM, BK1, and BK2 clusters, and two new phage are Singletons. Phages Kromp and Kradal are highlighted here as bioinformatically interesting targets for specific further investigation. Kromp is a novel lysogenic bacteriophage with no close neighbors. It has a 58,268 bp genome with a 9-base 3' overhang. Kromp is almost entirely genetically novel, with BLASTn showing only 0.40% genome alignment to its closest related phage (pZL12). Only 27 of its 95 protein-coding genes were placed in phams, most of which only contain 2 members. Kromp's gene cluster 65-67 contains an unusual pattern of coding potential that is indicative of active coding in multiple frames. This could be the result of a very large overlap between genes, or alternatively, the presence of an undetermined slippery sequence. Kradal is the third phage to be added to the prolate-head BM Cluster, which also contains phages Satis and JustBecause (both isolated at Washington University in St. Louis in 2016). Its 186,383 bp genome is very similar to that of Satis, save for a ~300bp deletion. In addition to their prolate head shape, these phages share a lytic life cycle and extremely large genomes – JustBecause possesses the smallest genome in the cluster, yet is it is still 184,281 bp. With just three phages in the BM cluster, there is already distinct diversity among them: JustBecause is only about 34% similar to Kradal and Satis by BLASTn comparison, suggesting that they have had significant time to diverge. Future discoveries of St. Louis-area prolate-headed phages should provide a clearer picture of the diversity and relative age of the cluster

10th Annual SEA-PHAGES Symposium Abstract

Poster #091

Webster University
Webster Groves MO
Corresponding Faculty Member: Stephanie Schroeder (schroeds@webster.edu)

Kelsey Durban



Meredith Harrison

Shiloh Bradley

An ORFan Operon in Actinobacteriophage Bobby

Kelsey Durban, Meredith Harrison, Shiloh Bradley, Weijia Xie, Deloren Thomas, Eric Davis, The Ha Lai, Yalda Mohamed, Stephanie Schroeder, Victoria Brown-Kennerly, Mary Preuss

Through the SEA-PHAGES program, our undergraduate research students are isolating and characterizing novel bacteriophages. Phage Bobby is a J-Cluster Actinobacteriophage collected on the campus of Webster University, that infects Mycobacterium smegmatis. Upon sequencing its genome, we noted striking similarity to Kalah2, another J cluster phage isolated nearby. A comparative analysis to this and other J cluster phages reveals Bobby has undergone a genome rearrangement and harbors a novel stretch 3,490 bp of orphan PHAMS showing protein coding potential, yet yielding no significant homology to any known genes in NCBI databases. These 'ORPHAMs' correspond to ab initio ORFs 98-103 in Phamerator with genome coordinates 59900-63389. Blastn of the NCBI databases with this region returned no significant similarity, whereas blastx found modest homology in ORF 101 to a hypothetical protein of Mesorhizobium muleiense, a bacterium isolated from chickpea Cicer arietinum in Xinjiang, China. HHpred (MPI) analysis of ORF 101 showed weak structural homology to Conotoxin TVIIA of the sea snail Conus tulipa (1EYO, RCSB Protein Data Bank). ORF 102 shows very weak homology to an ATP-binding protein in Porphyromonas canoris, a bacterium associated with dogs, and by HHpred was found to have significant structural homology to a DNA mismatch repair protein domain highly conserved from humans to bacteria. Of the 232 ORFs originally predicted in Bobby, we concluded 220 are likely real and twelve should be deleted: ORFs 1, 30, 33, 38, 40, 70, 72, 84, 108, 148, 181, 196. Ongoing analysis seeks to determine the function of the putative ORPHAM region, that appears to be an orphan operon of unknown origin.

10th Annual SEA-PHAGES Symposium Abstract

Poster #096

Western Carolina University
Cullowhee NC
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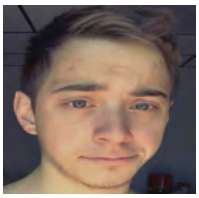

Brandon Stamey

Ethan Holcomb

Puzzling Virion Morphology and DNA Characterization Results from Microbacteriophages Discovered at Western Carolina University

Brandon Stamey, **Ethan Holcomb**, Megan Eckardt, Jamie Wallen, Maria Gainey

Western Carolina University (WCU) has been a member of the SEA-PHAGES program for three years, and during this time WCU students have isolated and archived 62 Actinobacteriophages. In previous years students used the host *Mycobacterium smegmatis* for bacteriophage isolation. This year two new hosts, *Microbacterium foliorum* and *Gordonia terrae*, were used. In the past only bacteriophages belonging to the family *Siphoviridae* have been isolated at WCU using *Mycobacterium smegmatis*. However, this year our electron microscopy (EM) results revealed that we had potentially isolated 1 *Myoviridae* and 3 *Podoviridae* bacteriophages using the host *Microbacterium foliorum*. The isolation of 3 potential *Podoviridae* bacteriophages was especially surprising given that only 1 *Podoviridae* bacteriophage has been reported to have been isolated using an Actinobacterial host by the entire SEA-PHAGES program. The purified DNA of 3 Microbacteriophages potentially representing all 3 tailed bacteriophage families (Andromedas-*Siphoviridae*, Neferthena-*Myoviridae*, and ColaCorta-*Podoviridae*) was sent to the University of Pittsburgh for whole genome sequencing. In contrast to what we expected, our sequencing results revealed that all 3 bacteriophages likely belonged to the family *Siphoviridae*. We were even more surprised to learn that bacteriophages Andromedas and ColaCorta belonged to the same subcluster (EA2) and were almost genetically identical. Our restriction enzyme analysis results from ColaCorta and Andromedas were also puzzling. The DNA of Andromedas and ColaCorta were not cut even by restriction enzymes whose target sequences were abundantly present in these genomes. Current studies are focused on clarifying the discrepancies between our sequencing results and EM and restriction enzyme analyses.

10th Annual SEA-PHAGES Symposium Abstract

Poster #007

Western Kentucky University
Bowling Green KY
Corresponding Faculty Member: Claire Rinehart (claire.rinehart@wku.edu)

Wyatt R Ringo

Features for Six Mycobacteriophage: BeeZoo (K1), Childish (B1), Kahve (B1), Priya (A9), Sandalphon (F1), and Adnama (E)

Gillian D Brown, Marcus Q Brown, Alison M Cash, Miriam A Chinkers, Hannah K Congleton, Katherine E Crider, M'Kyia N Davis, Christian P Dillard, Madison J Ellis, Anas Gondal, Olive M Halmadine, Evan C Hendrickson, Mason B Herschberg, Georgia A Hoffman, Brooklyn N Kassinger, Samantha J Kitchen, Reinhard M Knerr, Emma A Lamb, Elliott J Law, Zavyore C Martinez, Noah L Middlebrook, Sabid S Mir, Erin E Moe, Jeffery K Moreman, Connor J Patterson, Elizabeth P Peden, **Wyatt R Ringo**, Sasha V Sairajeev, Hannah K Shapiro, Aashka N Sheth, Julia M Stekardis, Alexander A Stewart, Anna R Strunjas, Bailey N Thompson, Briley A Thompson, Isabella G Uhls, Emina Vranjkovina, Naomi S Rowland, Amanda K Staples, Bobby L Gaffney, Claire A Rinehart, Rodney A King

Five phages, BeeZoo, Childish, Kahve, Priya, and Sandalphon were isolated from soil samples taken in Bowling Green, KY. Adnama was isolated from soil which came from Louisville, KY. All phages were grown on Mycobacterium smegmatis mc2155.

BeeZoo is a typical cluster K1 phage that displays large 3.2 mm turbid plaques after 48 hrs of growth at 30°C. It codes for a tyrosine integrase. It has a genome that is 60,494 bp long with 98 genes. It is a siphoviridae morphotype that has a 50 nm diameter head and a 220 nm long tail. It codes for 1 tRNA.

Childish is a typical cluster B1 phage that displays large 3-4 mm plaques with large halos after 24-48 hrs incubation at 37°C. It has no integrase. Childish has a genome size of 69,044 bp with 100 genes. It is a siphoviridae morphotype and has a head diameter of 47 nm and a tail length of 327. It carries no tRNAs.

Kahve is also a typical cluster B1 phage that displays clear plaques after 24 hrs at 37°C. Kahve has a genome size of 69,031 bp with 102 genes. It has not integrase. It is a siphoviridae morphotypes and has a head diameter of 55 nm and a tail length of 297 nm. It carries no tRNAs.

Priya is a typical cluster A9 phage that displays 2 mm plaques. It has a genome size of 52,949 bp with 96 genes. It carries no integrase. It is a siphoviridae morphotype with a 35 nm diameter head and a 170 nm long tail. It codes for 2 tRNAs.

Sandalphon is a typical cluster F1 phage that displays 1 mm slightly turbid plaques after 24 hrs at 37°C but with a turbid halo after 48 hrs. It does not code for an integrase. It has a genome size of 59,540 bp with 103 genes. It is a siphoviridae morphotype with a 62 nm diameter head and a 230 nm long tail. It carries no tRNAs.
Adnama is a typical cluster E phage that displays 2 mm turbid plaques and codes for a tyrosine integrase. It has a genome size of 75,208 bp with 148 genes. It is a siphoviridae morphotype with a 35 nm diameter head and a 170 nm long tail. It codes for 2 tRNAs.

10th Annual SEA-PHAGES Symposium Abstract

Poster #115

Western Kentucky University
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Claire Rinehart

PECAAN, a Phage Evidence Collection And Annotation Network

Claire A Rinehart, Bobby L Gaffney, James D Wood, Jason R Smith

PECAAN was developed to facilitate the collection of gene evidence and to implement a consistent and complete presentation during the annotation and the quality review. This database driven web application runs on many web-enabled devices including laptops, tablets and phones with an html5 compliant browser. New phage entries into PECAAN use data derived from a FASTA file and GeneMark. The gene calls and the Glimmer / GeneMark notes are obtained from internal PECAAN execution of these programs. Pham information is pulled from Phagesdb.org and Starterator information for the phams comes from Washington University. The host-trained GeneMark output is submitted to PECAAN as a pdf file and when displayed in PECAAN it scrolls horizontally instead of vertically, thus allowing easy connection of each reading frame between pages. Whenever a new phage is entered and whenever a new start site is selected, PECAAN automatically pulls database matches for the gene's protein from drawn from Phagesdb, NCBI protein BLAST, the CDD, HHPred and TMHMM.
Annotation of a gene in PECAAN consists of five steps:

1- Choosing the start site from a table of all possible start sites after consulting the Glimmer, GeneMark, and Starterator suggestions, 2- Entry of a function or NKF, 3- Checking boxes next to the function evidence from Phagesdb BLAST, NCBI BLAST, CDD, HHPred, and TMHMM, 4- Entry of notes. 5- Clicking the "Save" button to enter the annotator's name, a time/date stamp, and the changes into a log and the database.

To identify tRNAs and tmRNAs, the DNA sequence is scanned with Aragorn and tRNA-Scan. The evidence from each is displayed and evidence boxes can be checked to select the correct models.
A checkbox at the top of each gene page allows a choice to include the gene or not to include the gene in the final annotation. A button also allows additional genes to be added including genes with multiple coding domains, such as the frameshift in the tail assembly chaperones.

PECAAN can also display live PhamMap results that lets you view your current genome along side one other genome from the same cluster. It is easy to change between phages in the comparison view allowing the user to compare several genomes to the genome that you are annotating. PECAAN also allows viewing of the genome along with the six reading frames which are highlighted with the called genes. All potential start (green) and stop (red) sites are highlighted making it easy to find frameshifts. PECAAN can export several files to be used in preparation for genome submission. Two can be used to populate DNA Master through the "Documentation" "Parse" option. Gene notes and author files can be exported separately. If student annotations are to be graded, the change log can also be exported. PECAAN is written in JAVA and is available on GitHub. We are happy to accommodate additional users and phages from the SEA. We welcome suggestions for extensions to PECAAN.

10th Annual SEA-PHAGES Symposium Abstract

Poster #006

Winthrop University
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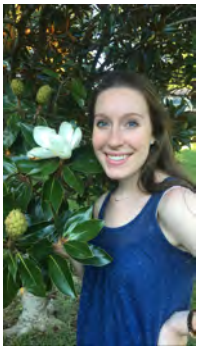
Corresponding Faculty Member: Kristi Westover (westoverk@winthrop.edu)

Joshua LeClerg



Allison Reed

Phage Isolation and Characterization at Winthrop University: Dueling Hosts- Mycobacterium smegmatis vs. Microbacterium foliorum

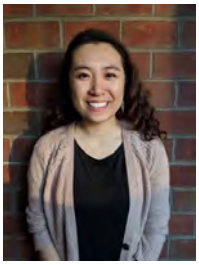
Brady Black, Daniel Croke, Baily Crolley, **Joshua LeClerg**, Allyssa Lewis, Samantha McNeil, Alyssa Paskowitz, **Allison Reed**, Charlene Simpson, Hallie Smith, Mikaela Way, Victoria J Frost, Kristi M Westover

This is Winthrop University's second year as part of the SEA-PHAGES program (Phage Hunters Advancing Genomics and Evolutionary Science) sponsored by the HHMI Science Education Alliance. During fall 2017, ten students used microbiological techniques to each isolate a unique bacteriophage that infected the bacterial host *Mycobacterium smegmatis*$^{mc^2\ 155}$. Individual phages were purified to obtain identical plaque morphologies and then amplified to collect high viral titer (HVT) lysates. Using transmission electron microscopy (TEM), the phages were visualized and shown to all belong to the Siphoviridae group of mycobacteriophages. Molecular techniques, including the comparison of restriction enzyme digest patterns of the viral DNA, enabled the students to decide on which phages should be further analyzed at the genomic level. Three, Rhynn, ExplosioNervosa, and Hamish, were sequenced at Pittsburg State University. Rhynn and ExplosioNervosa shared sequence homology with known members of the Cluster A mycobacteriophages. Rhynn belongs to sub-cluster A1 and ExplosioNervosa belongs to sub-cluster A9, each containing approximately 90 open reading frames. Hamish belongs to sub-cluster B1. ExplosioNervosa is 53,014 bp in length with 61.9% GC content, while Rhynn is 52,522bp in length with 62.0% GC content. Hamish is 68,585 bp in length with 66.5% GC content, and has approximately 100 putative genes. In addition to the mycobacteriophages, we used a relatively new program host organism, *Microbacterium foliorum*, as a pilot organism for next year. The Microbacterium foliorum strain NRRL B-24224 SEA was originally isolated from grass in Germany. Phages that infect this species have been classified according to their genome sequences and organized into clusters EA, EB, EC, ED, EE, EF, EG, and EH, with a small number of singletons. A novel soil phage, Scamander was subsequently characterized. Scamander, also belonging to the Siphoviridae, was identified following sequencing as a member of the EE sub-cluster. Only three EE phages have been annotated to date. Scamander is 17,452 bp in length with 27 putative open-reading frames and 68.7% GC content. The resulting annotation revealed 19 of the 27 genes were associated with known function. This is a relatively high percentage compared to other phages. In addition, we identified a -1 frameshift in the tail assembly chaperone at nucleotide position 7, 284. This research not only adds to the increasing characterization and knowledge of novel phages being discovered in this area of South Carolina but also continues to include Winthrop University and its students as members of a nationally renowned research program.

10th Annual SEA-PHAGES Symposium Abstract

Poster #039

Worcester Polytechnic Institute
Worcester MA
Corresponding Faculty Member: JoAnn Whitefleet-Smith (jaws@wpi.edu)



Ann V Le

A Story of A4 Bacteriophages: The Split DNA Primase with Dramatic Overlap in Two Different Frames

Ann V Le, Liam M Goodale, Megan T Hoppe, Alyssa A Nicolella, Kylie A Dickinson, Daniel C Millard, Nina A Murphy-Cook, Katherine A Nugai, Daniel M Champlin, Michael W Gauvin, Akshaye A Shah

At Worcester Polytechnic, four DNA samples were sent for sequencing after being isolated: AVLE17 (isolated from Charlton, MA compost), Tsukiryu, Houdini22 and NotAPhaseMom (all three isolated from different places in Worcester, MA). Sequencing results showed that Tsukiryu is exactly identical to Cocoaberry and that the other three novel phages are extremely similar to Cocoaberry. Cocoaberry is bacteriophage WPI students isolated from a compost sample in Charlton, MA in 2015 that has not been worked on since then. The EM microscopy results showed that the circular plaques of each of our three new bacteriophage also matched that of Cocoaberry, and all four are temperate siphoviridae. NCBI BLASTn was the first tool used to align and compare each sequence to Cocoaberry. While Houdini22 and NotAPhaseMom each shows a single-nucleotide polymorphism (SNP) at the 15,316th and 24,639th base pair respectively, AVLE17 shows several SNPs concentrated throughout the first 10,000 and last 5000 bps. Phamerator also confirmed the maximum homology between these four phages which are categorized in the A4 subcluster. Additionally, each sequence has many similarities to other bacteriophages such as Kampy (isolated from Virginia in 2012) and Peaches (isolated from Louisiana in 2008), both of which also belong to the A4 subcluster.

It was interesting to note the pattern of a split DNA primase gene with a dramatic overlap in two different frames within the four phages. Therefore, one of our major project goals was to understand the function and conservation of this overlap through a comparative genomic amino acid analysis using Phamerator, Protein NCBI BLASTp, T-Coffee, and HHPred. To study the conservation of the two primase sequences throughout the A4 subcluster, all non-draft phages, plus the three draft sequences from this study were chosen. Through closer inspection, the first primase was consistently a DNA primase catalytic domain while the second primase had a zinc-binding motif. Both primase sequences are highly conserved as seen in the extreme sequence similarity within the A4 family. To study the conservation of these two domains in the entire A family, the first non-draft phages in subclusters A1-A18 were analyzed. The two-piece split primase structure was found across the A family, particularly in subclusters A1-A12 and A14-A18. T-Coffee analysis further showed that there was significant sequence conservation within the catalytic domains and less in the zinc binding domains. Spud, a C1 phage, has a one-piece DNA primase that was compared to the split primases using BLASTp and T-Coffee, resulting in higher catalytic and lower zinc-binding conservation. The results from this project suggests a starting point for determining how the split primase region has evolved and potentially how they may function on a molecular scale.

10th Annual SEA-PHAGES Symposium Abstract

Poster #078

Xavier University of Louisiana
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Corresponding Faculty Member: Joe Ross (jross@xula.edu)

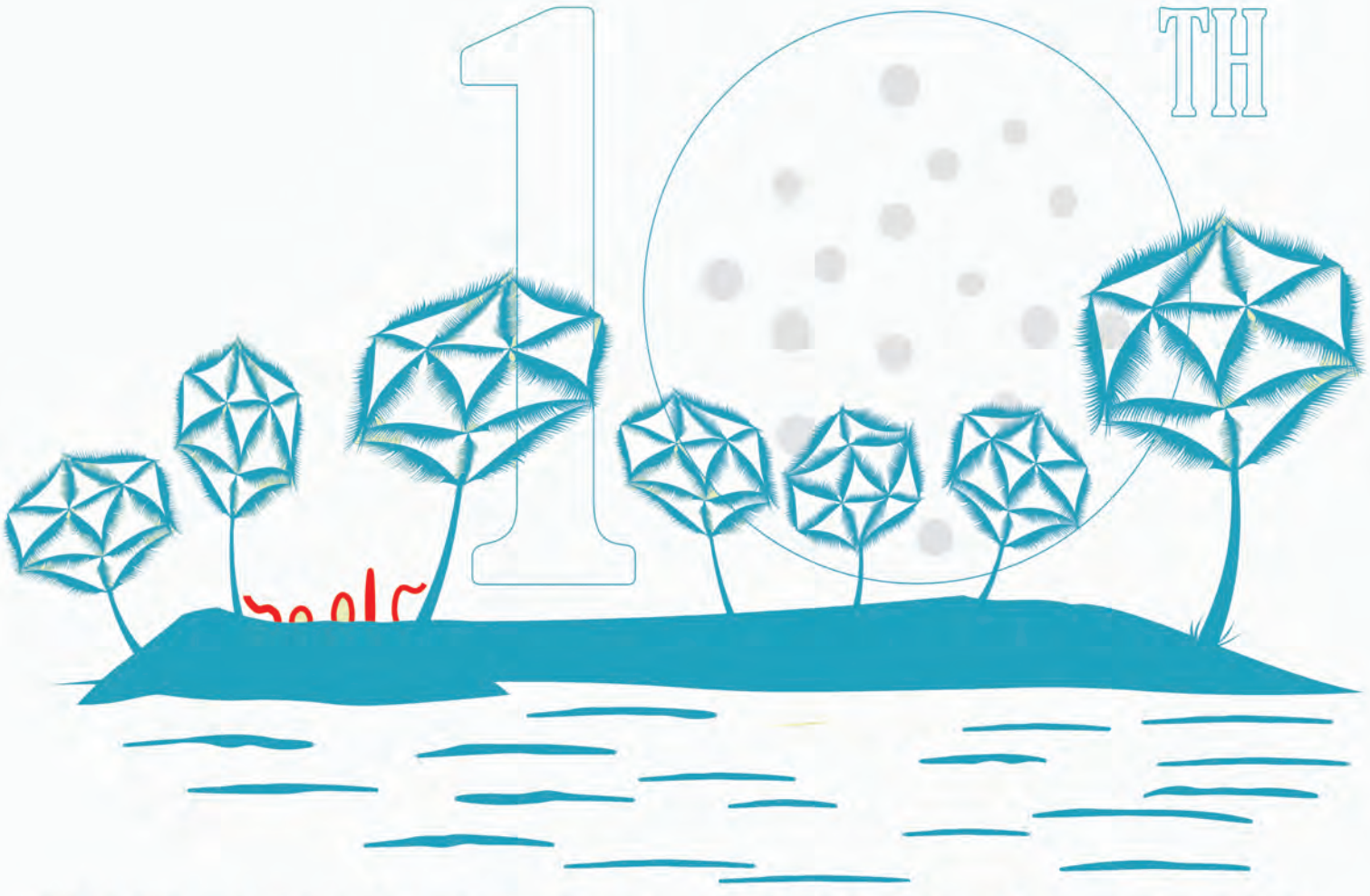
George E Olverson

Ugenie Coming Out of the Bottle—More Big Easy Phaging

Hanna Al-Moaswes, Hana Alkhafaf, Myles A Bartholomew, Cara P Ford, Tommy Giang, Lauren A Johnson, Karmen M Jones, Taylor M Landry, **George E Olverson**, Stacey N Umeozolu, Joseph F Ross

Phages were isolated from a variety of locales in and around the Greater New Orleans, Louisiana area using standard microbiological techniques. Genomic DNAs from two phages were sequenced and one genome, that of ugenie5, was chosen for analysis. The sequenced genome is 50,799bp in length, with cohesive ends showing a ten base pair overlap. BLASTn analysis reveals considerable nucleotide homology with the genomes of other known mycobacteriophages in cluster A, subcluster A9. Automated annotation employing Glimmer, GeneMark and Aragorn in the DNAMaster environment calls about 89 features including one putative tRNA gene. Analysis with Aragorn via the World Wide Web, external to the DNAMaster environment, substantiates the tRNA call. This feature is located in the left arm beginning at about 5900bp and putatively encodes a glutamate tRNA, with an anticodon in DNA form *ctc*. The external Aragorn prediction is corroborated by analysis with tRNAscan-SE. With the help of BLASTp analysis and similar tools, it is possible to make at least tentative proposals for the functions of possible gene products in the case of a significant minority of putative protein-encoding ORFs. The structure of the genome appears to be similar to that of many mycobacteriophages, with ORFs that at least potentially encode structural products at the left end, while the right end has a more complex and difficult to predict functional picture.

GENERAL INFORMATION



10th SEA Symposium

June 8 – 10, 2018

GENERAL INFORMATION

PROGRAM BOOK & AGENDA ACCESS

This year, we're excited to announce that the program book will be completely online. You can access the Program Book and Agenda using most web-capable devices, and instructions to do so are provided below.
A downloadable/printable agenda (PDF) is also available at the program website at https://seaphages.org/meetings/31/. Hard copies of the program book and agenda will **NOT** be available at the symposium.

1. You can access the online program book and agenda using the mobile app (recommended) or web browser.
2. For access via the mobile app,
    - follow the instructions provided in the email from CrowdCompass on June 04,
    - if you have not received the email, download and install the CrowdCompass AttendeeHub app from the **App Store** (iOS devices) or **Google Play Store** (android devices)
    - Once installed, search for "sea-phages", then login using your name and email address.
3. For access via a web browser, visit https://crowd.cc/2018seasym and login using your name and email address.

HASHTAG

#SEAsymp2018

ATTIRE

Attire for the entire SEA Symposium is business casual.

PRESENTERS & MODERATORS | TALKS

Those selected to give talks must upload their presentations to the "SEA Symposium 2018 Talks" folder in Dropbox by Thursday June 7th, 2018. Presenters will receive a link to this Dropbox folder. Please name your files as indicated in the document "Symp_Filename", which is included in the Dropbox folder. You can continue to update your talk until 2 hours prior to your talk, working off of the file you uploaded to Dropbox.
Slides for Session Moderators should similarly be place in the Dropbox Folder, using the filename as indicated.
As some fraction of attendees will be seated in an overflow room, we recommended that you avoid the use of laser pointers during your talk, as it cannot be seen in the overflow room.

PRESENTERS | POSTERS

Every school is required to present one student poster at the symposium. The maximum height and width for each poster cannot exceed 48"x 48". There are two poster sessions, one for odd-numbered posters and another for even-numbered posters. Poster assignments can be found in the program book.

LOCATION

HHMI Janelia Research Campus
19700 Helix Drive
Ashburn, VA 20147
http://www.hhmi.org/janelia

MEETING ROOMS & SEATING ASSIGNMENTS

All talks will be presented in the Auditorium. Talks will also be projected in the Seminar Room, which are also equipped with microphones and video capabilities.

- All students are assigned to the Auditorium for talks throughout the symposium.
- All faculty all assigned to the Seminar Room for talks throughout the symposium. A rotating subset of faculty will be assigned to the Auditorium. Faculty should review their seating assignments on the name badges before each session.

MEALS

All meals will be provided at Janelia Research Campus. Those observing Ramadan should inform SEA Staff during check-in, and boxed meals will be provided. Dining tables will be reserved for Cohort 11 faculty and Phage Discovery Workshop facilitators for lunch on Saturday.

LODGING INFORMATION

Lodging assignments were provided to participants when travel plans were made. Participants will be lodged at one of the hotels below:

- Janelia Research Campus (Janelia) –19700 Helix Dr, Ashburn, VA, 20147 – 571-209-4000
- DoubleTree by Hilton Hotel Sterling-Dulles Airport (Doubletree) – 21611 Atlantic Boulevard, Sterling, Virginia, 20166 – 703-230-0077
- Holiday Inn Dulles International (Holiday Inn) - 45425 Holiday Drive, Dulles, Virginia, 20166 – 703-230-0077

TRAVEL

If you have not received your travel and housing confirmations from HHMI Travel Services, you will need to contact hhmimeetings@hhmi.org to request a copy of your travel itinerary.

SHUTTLE BUS SCHEDULE

Shuttle buses will be provided for participants. All participants are required to use the shuttle buses during their published operating hours. The shuttle bus schedule can be found in the online program book, online at https://seaphages.org/meetings/31/, in the attached downloadable/printable agenda (PDF), and at the end of this message.

PARKING

Parking at Janelia Research Campus is available **ONLY** for overnight guests staying at Janelia, and for day guests. Overnight guests staying at the DoubleTree and Holiday Inn hotels must leave their cars at the hotels and use the shuttle busses provided from the hotels to Janelia.

REIMBURSEMENT OF EXPENSES

Participants are expected to cover the cost of incidentals (e.g. meals during travel, baggage fees, or lodging not required for the meeting). HHMI will NOT reimburse you for travel insurance, personal charges such as entertainment, or taxis to tour D.C. or visit friends.
If you believe special circumstances justify reimbursement, please contact Billy Biederman at sea@hhmi.org. In such cases, it is important that you keep all receipts and travel stubs.

10th SEA Symposium

SHUTTLE INFORMATION

Reston Limousine shuttle buses will operate from: Dulles to Janelia; Dulles to DoubleTree; Dulles to Holiday Inn.
Upon arrival at Dulles Airport, proceed to Baggage Claim Area, Carousel #3.
Look for a Reston Limousine representative holding a sign for HHMI.
The representative will direct you to either the bus going to Janelia, Holiday Inn, or the DoubleTree

ARRIVALS – Friday, June 8

SHUTTLES – Dulles to Janelia, Dulles to DoubleTree, and Dulles to Holiday Inn
Depart at:
12:00 PM
1:15 PM
2:30 PM
3:45 PM
Additional Shuttle – Dulles to Janelia
Depart at 5:00 PM

SHUTTLES – DoubleTree to Janelia, and Holiday Inn to Janelia
Depart at:
2:30 PM
3:30 PM
4:30 PM
Guests staying at the DoubleTree or the Holiday Inn can also use the non-HHMI hotel shuttle from Dulles to their assigned hotel.

EVENINGS – Friday and Saturday

SHUTTLES – Janelia to DoubleTree, and Janelia to Holiday Inn
Depart at:
9:00 PM
9:30 PM
10:00 PM
10:30 PM
11:00 PM

MORNINGS – Saturday and Sunday

SHUTTLES – DoubleTree to Janelia, and Holiday Inn to Janelia
Depart at:
6:45 AM
7:00 AM
7:15 AM
7:30 AM
7:45 AM
8:00 AM

DEPARTURES – Sunday, June 10

SHUTTLE – Janelia to Dulles, and Janelia to Reagan National Airport (DCA)
Depart at 1:00 PM

SHUTTLE – From Janelia to HHMI HQ (for SEA Faculty Meeting Guests Only).
Depart at 2:00 PM

SHUTTLE – From Janelia to Bethesda Marriott (for SEA Faculty Meeting Guests Only).
Depart at 2:00 PM

Additional transportation information will be posted on the departure list at the registration desk.

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SYMPOSIUM & HOTEL LOCATIONS

SYMPOSIUM

HHMI Janelia Research Campus
19700 Helix Drive
Ashburn, VA 20147
http://www.hhmi.org/janelia

HOTEL LOCATIONS

Janelia Research Campus (Janelia)
19700 Helix Dr,
Ashburn, VA, 20147
Tel. 571-209-4000

DoubleTree by Hilton Hotel Sterling-Dulles Airport (Doubletree)
21611 Atlantic Boulevard,
Sterling, Virginia, 20166
Tel. 703-230-0077

Holiday Inn Dulles International (Holiday Inn)
45425 Holiday Drive,
Dulles, Virginia, 20166
Tel. 703-230-0077