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Experimental Methods in Biology Course Engages Students in Authentic Research on Phage SuperInfection Immunity Testing

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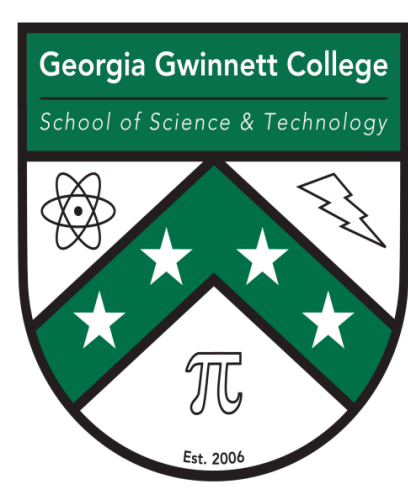
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EXPERIMENTAL METHODS IN BIOLOGY COURSE ENGAGES STUDENTS IN AUTHENTIC RESEARCH ON PHAGE SUPERINFECTION IMMUNITY TESTING

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STUDENT RESEARCH PROJECT

Georgia Gwinnett College along with almost 100 college and universities across the world is a member of the Howard Hughes Medical Institute Science Education Alliance's PHAGES Program under the mentorship of Graham Hatfull, PhD, Eberly Family Professor of Biotechnology and HHMI Professor in the Department of Biology at the University of Pittsburgh. As part of this program, GGC students have isolated and partially characterized 94 phages since 2011 (<http://phagesdb.org/institutions/GGWC/>; 1). Dr. Hatfull and his research team of organized the over 5,500 student-isolated phages that are able to infect the *Mycobacterium smegmatis* mc²155 strain into clusters (A through U) and sub-clusters based on sequence similarity.

The cost of complete, finished sequencing of a phage genome is about \$3,000 which means that here at GGC we must use other methods to classify the 87 phages that have not been sequenced. One option is to examine the ability of phages to infect a host that already contains a stably integrated phage genome – a lysogen. In a study of K cluster phages, the Hatfull group has demonstrated that phages in one of the K sub-clusters were unable to infect lysogens containing an integrated phage from that same sub-cluster (2, 3).

During the fall 2014 semester, 5 students (a) prepared high titer lysates of a panel of phages, (b) attempted to generate lysogens of these phages and (c) tested the immunity of the lysogens to superinfection by a panel of 7 phages as part of our Experimental Methods in Biology (BIOL4570) coursework (4-6).

The work accomplished during the semester was presented as a poster presentation during an on campus symposium, an oral project presentation and a written final scientific report. The project presentations (oral and written) were initially submitted in draft format and were reviewed/critiqued by their classmates and the instructor. Bi-weekly lab meetings were held in which a verbal report for each student was presented for comment and discussion.

BIOL4570 COURSE LEARNING OUTCOME GOALS

- Gather accurate information about a possible career path
- Effectively use the steps of the scientific method
- Communicate in-depth scientific information effectively in oral and written form using appropriate terminology and charts/graphs
- Collect and analyze data and present results in appropriate formats including charts, graphs and oral/written form

ASSESSMENT

Students were evaluated individually on the following items:

- Research Prospectus
- Data Presentation and Analysis
- Laboratory Notebook
- Final Scientific Report
- Project Presentation
- Class Participation

Overall course assessment was via the CURE Survey (7), the standard GGC course assessment and a laboratory and math skills assessment.

REFERENCES

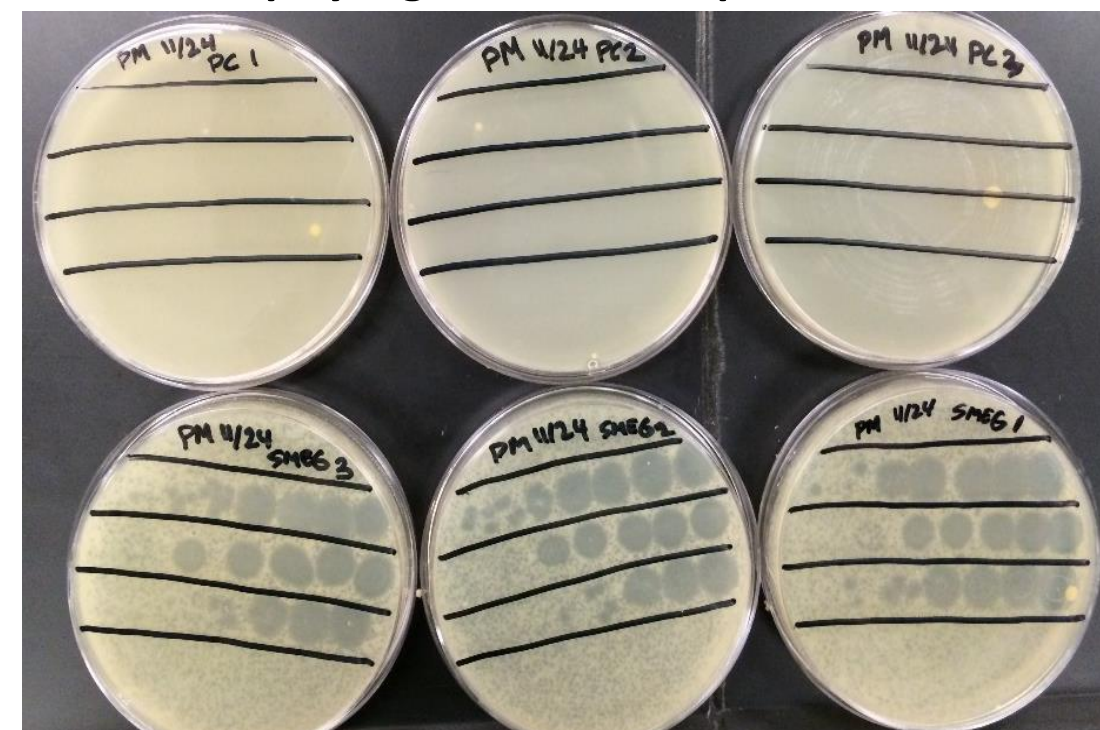
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2. WH Pope, CM Ferreria, D Jacobs-Sera, RC Benjamin, AJ Davis, et al, "Cluster K Mycobacteriophages: Insights into the Evolutionary Origins of Mycobacteriophage TM4," *PLOS One*, 6(10): e26750.
3. MK Donnelly-Wu, WR Jacobs, GF Hatfull, "Superinfection Immunity of Mycobacteriophage L5: Applications for Genetic Transformation of Mycobacteria," *Molecular Microbiology*, 7:407-417, 1993.
4. Science Education Alliance - Phage Hunting Advancing Genomic Research and Evolutionary Science (SEA-PHAGES) Resource Guide and Student Lab Manual <http://www.hhmi.org/seawiki/display/WIKI:NAV/In+Situ+Resources>
5. D Jacobs-Sera, Lysogeny Testing Protocol, last updated March 19, 2013 http://phagesdb.org/media/workflow/protocols/pdfs/LysogenyProtocol_3.19.13.pdf
6. D Dorsett and L Hammonds-Odie, A Theme Based Experimental Methods Course Provides Research Experience for Biology Majors, *Tested Studies for Laboratory Teaching*, 35, 110-114, 2014
7. LA Denofrio, B Russell, D Lopatto, Y Lu, Linking student interests to science curricula. *Science*, 318, 1872-1873, 2007.
8. Image of phage for title section: Bacteriophage T7 Model from <http://www.shapeways.com/model/452247/bacteriophage-t7-model.html?materialId=6>

STUDENT RESEARCH DATA

Students enrolled in the course:

- Prepared high titer lysates for 20 phages known to infect *M. smegmatis* mc²155.
- Each selected at least one of these phages and was able to generate candidate lysogens for the phages: Jomaryosh, Kratio, Mannie, Mufasa, Pork Chop and Quagmire
- The Kratio, Mufasa, Pork Chop and Quagmire *M. smegmatis* mc²155 lysogens demonstrated hetero-immunity to superinfection with 6 of the 7 phages in the panel.
- The Jomaryosh and Mannie *M. smegmatis* mc²155 lysogens did not demonstrate hetero-immunity to superinfection to any of the other phages in the panel.
- Mufasa phage lysates formed plaques on all 6 of the candidate lysogen lawns including to the Mufasa lysogen. Furthermore, the plaques contained a "mesa."
- All of the other lysogens demonstrated the anticipated homo-immunity to superinfection

Pork Chop Lysogen Lawn – Triplicate Trials

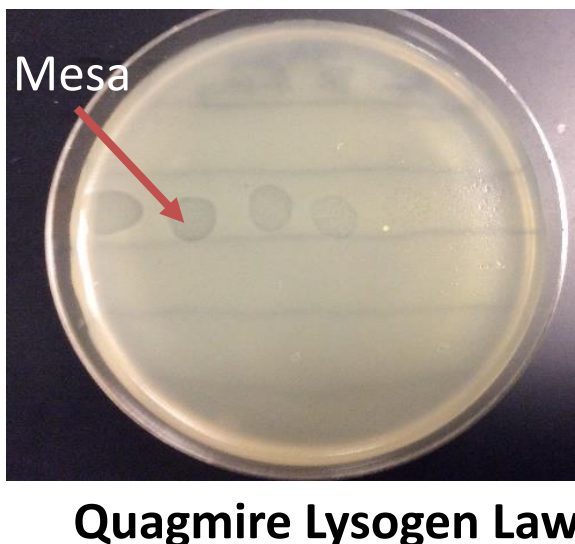


WT *M. Smegmatis* Lawn – Triplicate Trials

Adephagia
Jomaryosh
Kratio



Kratio Lysogen Lawn – Triplicate Trials



Quagmire Lysogen Lawn

Mannie
Mufasa
Pork Chop
Quagmire

Lysogen in <i>M. smegmatis</i> mc ² 155							
	Jomaryosh	Kratio	Mannie	Mufasa	Pork Chop	Quagmire	
Adephagia K1	Plaques no immunity	No Plaques Hetero-immunity	Plaques no immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity
Jomaryosh	No Plaques Homo-immunity	No Plaques Hetero-immunity	Plaques no immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity
Kratio K5	Plaques no immunity	No Plaques Homo-immunity	Plaques no immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity
Mannie	No Plaques Hetero-immunity	No Plaques Hetero-immunity	No Plaques Homo-immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity
Mufasa K2	Plaques no immunity	Plaques no immunity	?	Plaques no Homo-immunity	Plaques no immunity	Plaques no immunity	Plaques no immunity
Pork Chop	Plaques no immunity	No Plaques Hetero-immunity	Plaques no immunity	No Plaques Hetero-immunity	No Plaques Homo-immunity	No Plaques Hetero-immunity	No Plaques Hetero-immunity
Quagmire	Plaques no immunity	No Plaques Hetero-immunity	?	No Plaques Hetero-immunity	No Plaques Hetero-immunity	No Plaques Homo-immunity	No Plaques Homo-immunity

CHALLENGES

- The first quarter of the semesters was consumed by the students learning the required procedures to able to work the phages. Then, we spent the bulk of the semester generating the high titer lysates for the immunity testing. Prior experience working with phages and having lysates in hand at beginning of course would have allowed more time for students to work on their individual research projects.
- Students were very used to "cookbook" laboratory exercises where they will obtain "appropriate" results from their work. Stepping back and letting the students work out their own protocols was challenging as an instructor.
- Another challenge was keeping students focused and on-track, particularly around mid-semester, making steady progress on their research project and completing the necessary literature research and writing/editing.

STUDENT PERFORMANCE DATA

In fall 2014 semester, the BIOL4570 course had 10 students enrolled. Half of the students (5) chose the phage superinfection immunity testing project. The final lab report was scored using the GGC Biology Discipline Rubric. The range of the scores was 14.5 to 29.0 on a 30 point scale for the 5 students who completed the immunity testing project. The other graded assessment items were comparable for all 5 students. The 5 question laboratory and math skills assessment was given pre- and post-course and included questions about solution preparation. With each question scored as correct worth one point, the average for all students enrolled on the pre-assessment was 2.02 and post-assessment, 3.18 for a difference of 1.16 demonstrating an increased ability to detail how to prepare a solution.

CURE SURVEY DATA

At the start of the course: (n=8)

- 3 students planned on entering a doctoral program
- 1 student planned on entering a masters program
- 3 students planned on entering a health professional program
- 1 had no plans

By the end of the course: (n=6)

- 3 students planned on entering a masters program
- 1 student planned on entering a doctoral program
- 1 student planned on entering a masters program in a non-science field
- 1 student planned on pursuing a law, architectural or other degree

During the course, we discussed career plans and preparation for the next phase. The 5 students were asked to investigate the demographics of the last entering class at their top choice institution for their next step.

	Gain in Student Attitude and Experience with Course Elements (selected items from CURE Survey Results)	
	BIOL4570 fa14 (n=6)	National Results
Maintaining a Lab Notebook	4.0	3.58
Present Results Orally	3.88	3.61
Collect Data	4.13	3.88
Analyze Data	4.25	4.02
Write a Research Proposal	3.88	3.45
Work Individually	4.25	3.37
Read Primary Literature	4.13	3.55
Present Posters	4.00	3.26

	Gain in Student Attitude and Experience about Course Benefits (selected items from CURE Survey Results)	
	BIOL4570 fa14 (n=6)	National Results (n=4,807)
Clarification of Career Path	3.88	2.96
Tolerance for Obstacles	3.75	3.49
Readiness for More Demanding Research	3.88	3.41
Understanding Research Process	4.00	3.46
Learning Laboratory Technique	4.13	3.7
Skill in Science Writing	3.88	3.31
Self-Confidence	3.88	3.19
Learning to Work Independently	3.75	3.32

	Overall Course Assessment (from CURE Survey Results)	
	BIOL4570 fa14 (n=6)	National Results (n=4,807)
Course was good way to learn about the subject	4.50	3.88
Course was good way to learn about process of scientific research	4.63	4.00
Course had a positive effect on my interest in science	4.50	3.66
I was able to ask questions in class and get helpful responses	4.38	4.06

ACKNOWLEDGEMENTS

- Thank you to the students enrolled in the Fall 2014 BIOL 4570 course for their hard work and willingness to engage in a class-based research experience, twice a week for 2.5-3 hours.
- The GGC phages were isolated by the GGC students who were enrolled in Introduction to Biological Sciences 1101K in fall 2011 and fall 2012 and in Experimental Methods in Biology in spring 2013 under the guidance of Alessandra Barrera, PhD and Latanya Hammonds-Odie, PhD
- The other phages were part of the Mycobacteriophage Panel Spring 2013
- The assistance of Ms. Cindy Valenzuela, Mrs. Jessica Thompson, Ms. Amanda Sutton and Mr. Justin Madden during the course was invaluable.
- The course proposal for Experimental Methods was developed initially in collaboration with Dr. Allison D'Costa.
- The for supplies and reagents for the BIOL4570-01 fall 2014 course were supported by the USG-STEM II Initiative Grant to GGC SST for funding as a CER migrant.