

Vincent Liu - ASP Logbook 2023 - 24

Daily Notes:

September

Sept. 1:

- Set expectations for class:
 - Calendar planned for classes and meetings for 2 months ahead of current, e.g. September and October planned out
 - Email professor once a week and initiate conversations as they are busy and could forget you
- Use NCBI PubMed for papers, “Free PMC Articles” are available to download without professor
- When you cite a line that the authors of the paper cited from somewhere else, always cite the source of the authors before the paper you are reading
- Write research proposals by mid of October

Sept. 6:

- Paperpile
 - Reference manager
 - Organize literature
 - Cite sources
- Individual meeting:
 - Biweekly Checks
 - 6 - 7 in the 1st term, out of 15 marks in “schedule, organization, and communication” which is 20% of total marks
 - Mentors Evaluation, out of 15

Sept. 8:

- Began reading “*Escherichia coli* ST131, an Intriguing Clonal Group”
 - See details in “Background Research”

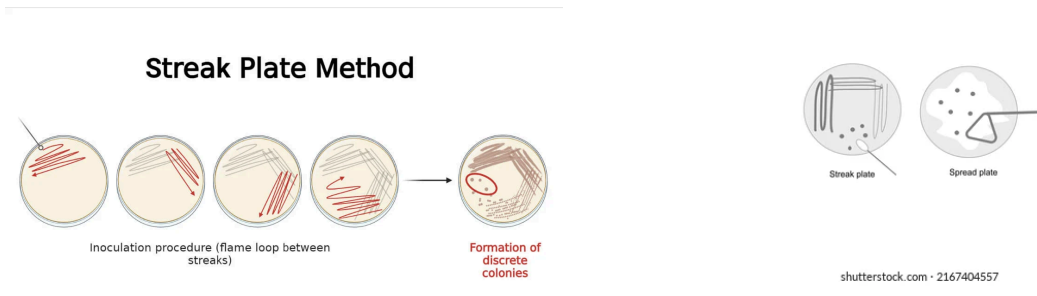
Sept 13.

- Went to lab to practice operating equipment with assistant researcher

- Learned and practiced operating single pipette (2-20 μm , 20 - 100 μm , 100 - 300 μm , and 100 - 1000 μm)
- Learned and practiced multipipette (8 nozzle and 12 nozzle)
- Discussed schedule for Sept. 14
 - Will practice more techniques with a different lab technician

Sept 14.

- Went to lab to practice operating equipment with assistant researcher
 - Learned and practiced procedure of dilution using multi and single pipettes
 - Learned bacterial colony growing technique
 - Streaking
 - Spreading



Sept 18.

- Guidelines for Research Proposal
 - Introduction
 - Broader topic \rightarrow Narrower topic
 - Need for the research, innovation
 - Convince reader that there is a significant need for said research
 - Questions
 - **GO RIGHT AFTER THE INTRODUCTION*****
 - “Goal of the study is to answer the question _____”
 - Objectives
 - Short term and long term
 - Variables and Hypothesis
 - **DISCUSS WITH MENTOR*****
 - Variables written with the methodology
 - Methodology
 - Variables at the start
 - Significance
 - How it will impact the field of studies
 - Contributions to society, the field, or to individuals

- Answer the question “What are we getting out of that?”
- References
 - Paperpile
- Continued reading “*Escherichia coli* ST131, an Intriguing Clonal Group”
 - See details in “Background Research”
- Created title page of the Research Proposal

Sept. 22

- Practiced Techniques at the Lab
 - Practiced serial dilutions of tryptic soy broth phage samples using 1000 um multichannel pipette
 - Practiced serial dilutions of test strain bacteria then plated using cell spreader method



Sept. 28

- Research proposal introduction draft:
 1. Identify problems: E.g. Urinary Tract Infection or Gastrointestinal infections
 2. Lead into the cause: These infections are caused by multidrug resistant ST131 E.coli...
 3. Possible solution: Bacteriophage therapy could be used to target MDR pathogens...
 4. Lead into research question
 5. Goals and future plans

October

Oct. 2

- Research question determined

- Are bacteriophages isolated from municipal wastewater effective against clinical isolates of Escherichia coli ST131 strain?
- Practiced making tryptic soy broth (TSB) and tryptic soy agar (TSA)
 - Broth: Mix 30.0g of TSB powder to water for 1L of TSB
 - Agar: Mix 40.0g of TSA powder to water for 1L of TSA

Oct. 4

- Add information in “Background Research” for previously read but not written articles
 - “Community-Acquired Urinary Tract Infection by Escherichia coli in the Era of Antibiotic Resistance”
 - “Decolonizing drug-resistant E. coli with phage and probiotics: breaking the frequency-dependent dominance of residents”
 - “Escherichia coli ST131, an intriguing clonal group”

Oct. 6

- Read and annotated “Community-Acquired Urinary Tract Infection by Escherichia coli in the Era of Antibiotic Resistance” see “Background Research”

Oct. 7

- Read and annotated “Decolonizing drug-resistant E. coli with phage and probiotics: breaking the frequency-dependent dominance of residents” see “Background Research”

Oct. 13

- Continued annotating “Escherichia coli ST131, an intriguing clonal group” see “Background Research”

Oct. 17

- Read and annotated “Morphological, Host Range, and Genetic Characterization of Two Coliphages” see “Background Research”

Oct. 19

- Complete course for WHMIS 2015 and start Hazard Assessment Training on my.ucalgary.ca

Oct. 23

- Finish Hazard Assessment Training
- Presentation format:
 - Punch Line
 - Background (2-3)
 - Research Question/ Aims
 - Methods
 - Variables
 - Significance ****CONNECT TO PUNCH LINE****
- Include all parts of the Research Proposal in the Presentation

Oct. 25

- Finish Occupational Health and Safety Orientation
- Finish Spill Response Training

Oct. 27


- Complete Introduction of Research Proposal

Oct. 31

- Participated in presentations:
 - Jessica:
 - Design and Construction of CRISPR/Cas9-Mediated Knockout Plasmids for Autism-Risk Genes
 - Autism is Highly heritable
 - Gene knockout as a study method to determine more therapeutic methods to combat Autism
 - Using CRISPR/Cas9 as a knockout tool
 - Plasmids can replicate independently
 - Design gRNA Sequences
 - gRNA for 10 ASD-risk genes will be designed using CRISPOR
 - Improved methodology for future studies
 - Cooper:
 - Effect of concussions on the level of proteins used for a vital brain development process in adolescent mice
 - Neurodegenerative Diseases such as alzheimer's
 - Synaptic Pruning
 - Increased and Decreased
 - Microglia is a immune response
 - How do repeated mild traumatic brain injuries affect C3 and C1q complement proteins expression in the motor cortex of adolescent mice

November

Nov. 2

- Completed introduction, questions, hypothesis, and objectives of the research proposal.
- See detail in document for research proposal
 -  ASP Research Proposal

Nov. 4

- Finished the research proposal entirely.
 - Please see document on Google Classroom for specific informations
 - [ASP Research Proposal](#)
 - Asked mentor to review said proposal according to the given rubric from the course
- Due to mentor canceling meeting on Nov. 3, calendar needs to be shifted
 - Please see calendar section for updated dates

Nov. 5

- Finalized and handed in research proposal on turnitin.com and Google Classroom, please see documents there
 - Applied the feedback from mentor and made corrections and new citations to the research proposal
 - Added new information and citations from websites recommended by mentor
 - [ASP Research Proposal](#)

Nov 6.

- Attend lab meeting on zoom and in mentor's lab
 - Viewed examples of research paper presentations
 - Prophage research:
 - Why are prophages important?
 - Phage prediction software
 - Use phages with no genes of prophages as a negative control, analyzed using whole genome sequencing
 - Spontaneously induced prophages to *Lactobacillus gasseri* contribute to horizontal gene transfer

Nov 8.

- Participated in presentations:
 - Owen:
 - Electrocardiogram 5 waves (P, Q, R, S, T)
 - Detect Acute Coronary Syndrome
 - ST-Segment Elevation
 - Non-ST-Segment Elevation
 - Angina
- Began working on oral presentation of research proposal
 - Finished introductions, questions, and Objectives sections (Please see Google Slides titled "Research Proposal Oral Presentation - Vincent Liu" on Google Classroom)

-  Research Proposal Oral Presentation - Vincent Liu

Nov. 9

- Worked during spare to finish oral presentation of research proposal
 - See finished copy in Google Classroom


Nov. 14

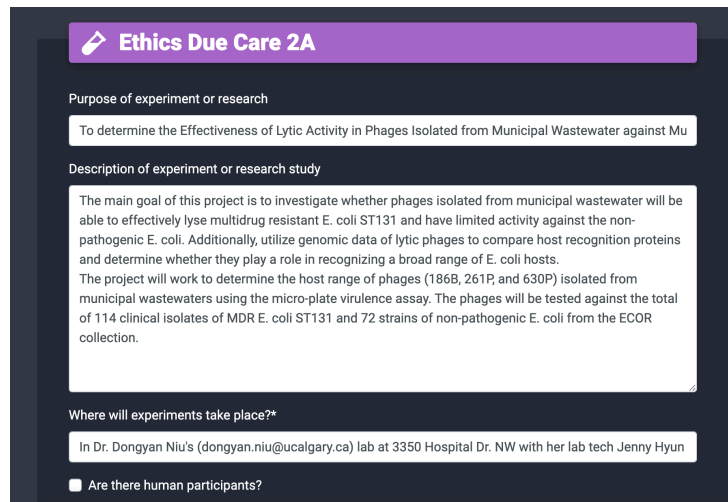
- Performed Microplate Virulence Assay in lab with mentor
 - See “Experimental Procedures”

Nov. 17

- Oral presentation presented

Nov. 21

- Individual meeting
 - Intro section for final paper include goals and research questions
 - Make tentative dates for December calendar
 - Rewrite methodology, see edits
 -  ASP Research Proposal
 - Ask about form 2B
- Filled out basic info on CYSF Portal
 - Ethics approval



Ethics Due Care 2A

Purpose of experiment or research

To determine the Effectiveness of Lytic Activity in Phages Isolated from Municipal Wastewater against Mu

Description of experiment or research study

The main goal of this project is to investigate whether phages isolated from municipal wastewater will be able to effectively lyse multidrug resistant E. coli ST131 and have limited activity against the non-pathogenic E. coli. Additionally, utilize genomic data of lytic phages to compare host recognition proteins and determine whether they play a role in recognizing a broad range of E. coli hosts. The project will work to determine the host range of phages (186B, 261P, and 630P) isolated from municipal wastewaters using the micro-plate virulence assay. The phages will be tested against the total of 114 clinical isolates of MDR E. coli ST131 and 72 strains of non-pathogenic E. coli from the ECOL collection.

Where will experiments take place?*

In Dr. Dongyan Niu's (dongyan.niu@ucalgary.ca) lab at 3350 Hospital Dr. NW with her lab tech Jenny Hyun

Are there human participants?

Nov. 23

- Performed Microplate Assay with Ecor 4 - 7
 - See “Experimental Procedures”

Nov 27.

- December
 - Deepen understanding of theoretical aspects of the experiments
 - MOST IMPORTANT: Data collection AND increase familiarity and understanding of the methodology
 - Methodologies need to answer the question directly

- Science Fair on March 4, 2024
- Filled out Hypothesis, Variables, and Procedures section on the CYSF portal

Hypothesis

Content*

Format | B I U S x₂ x² A- | I_x | ¶ | ☰ | ☲ | ☱ | ☴ | ☵ | ☶ | ☷ | ☸ | ☹ | ☺ | ☻ | ☼ | ☽ | ☾ | ☿ | ♀ | ♂ | ♁ | ♂ | ♃ | ♄ | ♅ | ♆ | ♇ | ♈ | ♉ | ♊ | ♋ | ♌ | ♍ | ♎ | ♏ | ♐ | ♑ | ♒ | ♓ | ☾ | ☽ | ☿ | ♀ | ♂ | ♁ | ♂ | ♃ | ♄ | ♅ | ♆ | ♇ | ♈ | ♉ | ♊ | ♋ | ♌ | ♍ | ♎ | ♏ | ♐ | ♑ | ♒ | ♓

Phages isolated from municipal wastewater will have lytic activity against *E. coli* ST131 and not be effective against non-pathogenic *E. coli*. 2. Phages with lytic activity against both pathogenic and non-pathogenic *E. coli* may carry a specific host recognition protein that allows them to be effective against both.

SAVE

Variables

Content*

Format | B I U S x₂ x² A- | I_x | ¶ | ☰ | ☲ | ☱ | ☴ | ☵ | ☶ | ☷ | ☸ | ☹ | ☺ | ☻ | ☼ | ☽ | ☾ | ☿ | ♀ | ♂ | ♁ | ♂ | ♃ | ♄ | ♅ | ♆ | ♇ | ♈ | ♉ | ♊ | ♋ | ♌ | ♍ | ♎ | ♏ | ♐ | ♑ | ♒ | ♓

Independent Variables:

- Phages used to test on *E. coli* ST131 and ECOR collection
- Concentration of phages used to test on *E. coli* ST131 and ECOR collection
- The location of municipal wastewater the phages were extracted from

Dependent Variable:

- Whether the bacteriophages were able to lyse the clinical isolates of *E. coli* ST131 and ECOR collection

SAVE

Procedure

Content*

Format | B I U S x₂ x² A- | I_x | ¶ | ☰ | ☲ | ☱ | ☴ | ☵ | ☶ | ☷ | ☸ | ☹ | ☺ | ☻ | ☼ | ☽ | ☾ | ☿ | ♀ | ♂ | ♁ | ♂ | ♃ | ♄ | ♅ | ♆ | ♇ | ♈ | ♉ | ♊ | ♋ | ♌ | ♍ | ♎ | ♏ | ♐ | ♑ | ♒ | ♓

1/10 Serial Dilution:

In this study, phage and bacteria stocks will be diluted using 1/10 serial dilution. 1/10 serial dilutions were prepared in 96-well plates. Undiluted samples of phages or bacteria will be placed in the first row of the 96-well plates. All other rows will be filled with 9/10 of the capacity with tryptic soy broth. Single pipettes will be used to take 1/10 of the Phages and bacteria stocks from the first row and mixed into the tryptic soy broth in the second row. Repeated until satisfied dilution (-6 and -7) was achieved.

Tryptic Soy Agar (TSA):

Tryptic soy agar will be used in growth of bacterial colonies to kindle the growth of bacteria during incubation. 40.0g of TSA

SAVE

Nov 29.

- Made correction to introduction of research proposal
 - See specific document on google classroom
 - [Vincent Liu - Introduction Section Paper](#)

- Submit draft to Dr. Garcia for feedback
- Give yourself enough time to prepare a poster

December

Dec 1.

- Performed Microplate Assay with Ecor 8 - 11
 - See “Experimental Procedures”

Dec 5.

- Individual Meeting
 - Record details into logbook
 - Bio Render website for diagrams of biology related procedures and objects
 - Add diagrams to make procedures clearer
 - February 28 Oral Presentation
 - Poster by February 26
 - Plan with mentor about getting data for science fair of pathogenic and non pathogenic strain to show that this project has potential
- Add to the logbook based off of previous comments made by Dr. Garcia
 - Insert screenshots of CYSF Portal of filled out sections to previous dates (Nov. 21 and Nov 27) in “Daily Notes”
 - Linked “Research Proposal”, “Oral Presentation”, and “Introduction of Final Paper” into respective dates and granted access to people with the link
 - Made reports more detailed for Assays previously performed on Nov. 14 and Nov. 23
- Read and annotated pages 1-5 of “Meta-analysis of pandemic Escherichia coli ST131 Plasmidome proves Restricted plasmid-clade Associations” and added to Paperpile
 - See “Background Research”

Dec 7.

- Homework: Create a plan for a science fair with dates and present it to Dr. Garcia by the next individual meeting.
- Introduction Section Paper
 - Made changes proposed by Dr. Garcia and handed in
 - [Vincent Liu - Introduction Section Paper](#)

Dec 11.

- Discussed plan with mentor
 - January 2nd to January 5th from 9:00 - 3:30 during winter break to screen 8 phages a day
 - Jan 2 Ecor 20 - 27
 - Jan 3 Ecor 28 - 35

- Jan 4 Ecor 36 - 43
- Jan 5 Ecor 44 - 51
- Discuss lab time during exam break when exam break schedule comes out
- February start screening pathogenic *E. coli* ST131
- Digital recording of microplate assay steps
 - Microplate Assay Result..xlsx
 - 1. Go into results format
 - 2. Go under Microplate Ecor Strains
 - 3. Go under Positive Host Control
 - 4. Go under Negative Host Control
 - 5. Skip "Blank Control"
 - 6. Go under Host Counting CFU and MOI
- Performed Microplate Assay with Ecor 12 - 15
 - See "Experimental Procedures"

Dec. 13

- Recorded Data from previous dates after mentor has taught how to record data
 - For the dates of Nov. 30 and Dec. 11
 - See "Data Collection and Results"
- Finished reading and annotating "Meta-analysis of pandemic Escherichia coli ST131 Plasmidome proves Restricted plasmid-clade Associations"
 - See "Background Research"
- Individual meeting:
 - Think about how to compare data from different phages and non pathogenic vs pathogenic strains of *E. coli*
 - Google slides for individual presentations but bring the poster to Dr. Garcia for practice
 - Push for doing pathogenic *E. coli* sooner
 - Ask Jenny of how data is presented

Dec. 15

- Change logbook according to feedback from Dr. Garcia
 - Added "Take Home Message" to all annotated papers
 - See previous documents in "Background Research"

Dec. 18

- Prepared Overnight Culture
 - Inoculated into 5 ml of TSB and incubated at 35 degrees Celsius overnight

Dec. 19

- Performed Microplate Assay with Ecor 16 - 19
 - See "Experimental Procedures"
- Added "January" to calendar section

- Discussed with mentor
 - Finish screening by mid February
 - Work on poster after screening
 - Get printed at end of February
 - Mentor will send layout template for poster

January

Jan 2.

- Performed Microplate Assay with Ecor 20 - 27
 - See “Experimental Procedures”
- Collected results from previous experiment
 - See “Data Collection and Results”

Jan 3.

- Performed Microplate Assay with Ecor 28 - 35
 - See “Experimental Procedures”
- Collected results from previous experiment
 - See “Data Collection and Results”

Jan 4.

- Performed Microplate Assay with Ecor 36 - 43
 - See “Experimental Procedures”
- Collected results from previous experiment
 - See “Data Collection and Results”

Jan 5.

- Performed Microplate Assay with Ecor 44 - 51
 - See “Experimental Procedures”
- Collected results from previous experiment
 - See “Data Collection and Results”
- Meeting with Mentor
 - Start screening ST131 after midterms
 - Get poster printed before last week of February
 - Send phages for transmission electron microscopy to get images of phages
 - Confirm with Dr. Garcia for date to wrap up lab work

Jan 24.

- Email mentor to confirm meeting and preparing overnight culture on Jan. 29
- Updated dates:
 - Jan 31 Dec/Jan logbook due
 - Feb 13 Procedures section due
 - March 4 marks lock for term 2

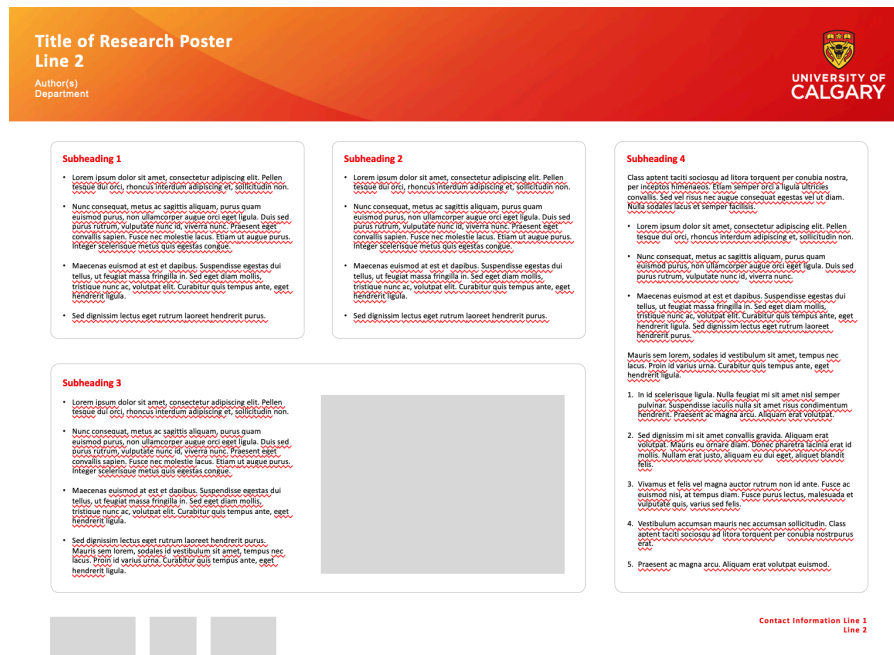
- Procedures section
 - “What was done” or “How it was done”
 - Past perfect tense
 - Citations: Any data that was not performed by you and based off experiments done in past papers
 - Name of Manufacturer
 - ([Name of company], [City], [Country])
- Made changes to the Procedure section of the research proposal
 - Vincent Liu - Procedures Section

Jan 26.

- Individual meeting
 - Cite results for science fair for results that was not done by me
 - Start working on graphs and visuals while collecting data
 - BioRender for visuals
 - Read more literature to see who is doing the same work as me
- Worked on procedure section of final paper
 - Microplate Assay
 - CFU/mL
 - Overnight culture
 - Vincent Liu - Procedures Section

Jan 30.

- Performed Microplate Assay with *E. coli* C1 Subclade (Resistant to fluoroquinolones)
 - See “Experimental Procedures”
- Mentor sent poster template and will send pictures and data



February

Feb 1.

- Meeting with Dr. Garcia
- Science Fair
 - Monday March 4th
 - Gone all morning so email teacher
 - 5 Judges
- Work on poster/oral presentation

Feb 5.

- Poster format:
 - Background
 - Research question, goals, hypothesis
 - Methods
 - Results
 - Analysis/Conclusion
 - Future Directions, “What next?”
- Added Research question, goals, and hypothesis to poster

Vincent Liu
Webber Academy, Calgary, AB

Background

- Urinary tract infections (UTIs) are one of the most common types of infections to occur in the human body. Specifically, urinary tract infections caused by *Escherichia coli* (*E. coli*) accounts for up to 80% of community-acquired uncomplicated UTIs.¹ Furthermore, antibiotics, the only treatment for bacterial infections, were failing as bacterial developed multi-drug resistance (MDR).
- The MDR *E. coli* serotype 131 (ST131) strain can cause UTIs and bacteremia (bloodstream infection), potentially leading to the death of the host if fatal.²
- Bacteriophages (phages) are viruses that infect specific bacteria, replicate inside them, and then lyse the bacteria, ultimately leading to the death of the bacteria.³ The phages use host receptor recognition proteins to infect a specific host.⁴
- Many researchers claim that bacteriophages can become the solution for the crisis of antimicrobial resistance in many bacteria strains due to their ability to adapt to better combat the defense mechanisms of the bacteria as the bacteria is also evolving.⁵

Research Question

Can phages isolated from municipal wastewater effectively and specifically lyse clinical isolates of *E. coli* ST131 and have limited activity against the non-pathogenic *E. coli*?

Objectives

The first short term goal is to determine the host range of phages (186B, 261P, and 630P) isolated from the municipal wastewater using the microplate virulence assay. The phages will be tested against the total of 114 clinical isolates of MDR *E. coli* ST131 and 72 strains of non-pathogenic *E. coli* from the ECOR collection.

Hypothesis

Phages isolated from municipal wastewater will have lytic activity against *E. coli* ST131 and not be effective against non-pathogenic *E. coli*.

Feb. 7

- Performed Microplate Assay with *E. coli* C2 Subclade
 - See “Experimental Procedures”
- Collected results from previous experiment
 - See “Data Collection and Results”

Feb. 9

- Finished edits of Procedures Section and handed into turnitin.com
- Vincent Liu - Procedures Section

Feb. 13

- Compiled data sent by mentor into graphs

Host Range of Isolated Phages

Table 1 Summary of phage genome size, host range, and classification.

Phages	Classification		Genome Size (bp)	ECOR Collection (n = 72) (%)	C1* (n = 28) (%)	C2** (n = 52) (%)	C1-M27* (n = 8) (%)	No. of Susceptible Strains (%)
	Family	Genus						
186B	Straboviridae	Mosivirus	169,185	20/72 (28%)				
261P	Autographiviridae	Vectrevirus	44,505	15/72 (21%)				
630 P	Autographiviridae	Vectrevirus	44,631	10/72 (14%)				

* Most C1 and C1-M27 strains are resistant against ciprofloxacin (fluoroquinolone), trimethoprim/sulfamethoxazole (folate pathway inhibitor), ceftriaxone (cephalosporin).

** Most C2 strains are resistant against ciprofloxacin, ceftriaxone, tobramycin (aminoglycoside)

Feb 15.

- Performed Microplate Assay with *E. coli* C1-M27 Subclade
 - See “Experimental Procedures”
- Collected results from previous experiment
 - See “Data Collection and Results”

Feb 20.

- Individual meeting
 - Print posters
 - Use slides for oral presentation
- Finished poster and sent to mentor

Determining the Effectiveness of Lytic Activity in Phages Isolated from Municipal Wastewater against Multidrug-Resistant *E. coli* ST131 and non-pathogenic *E. coli*.



UNIVERSITY OF CALGARY

Vincent Liu
Webber Academy, Calgary, AB

Background

- Urinary tract infections (UTIs) are one of the most common types of infections to occur in the human body. Specifically, urinary tract infections caused by *Escherichia coli* (*E. coli*) accounts for up to 80% of community-acquired uncomplicated UTIs.¹ Furthermore, antibiotics, the only treatment for bacterial infections, were failing as bacterial developed multi-drug resistance (MDR).²
- The MDR *E. coli* serotype 131 (ST131) strain can cause UTIs and bacteremia (bloodstream infection), potentially leading to the death of the host if fatal.³
- Bacteriophages (phages) are viruses that infect specific bacteria, replicate inside them, and then lyse the bacteria, ultimately leading to the death of the bacteria.⁴ The phages use host receptor proteins to infect a specific host.⁴
- Many researchers claim that bacteriophages can become the solution for the crisis of antimicrobial resistance in many bacteria strains due to their ability to adapt to better combat the defense mechanisms of the bacteria as the bacteria is also evolving.⁵

Research Question

Can phages isolated from municipal wastewater effectively and specifically lyse clinical isolates of *E. coli* ST131 and have limited activity against the non-pathogenic *E. coli*?

Objectives

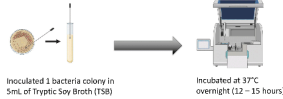
The first short term goal is to determine the host range of phages (1868, 261P, and 630P) isolated from the municipal wastewater using the microplate virulence assay. The phages will be tested against the total of 114 clinical isolates of MDR *E. coli* ST131 and 72 strains of non-pathogenic *E. coli* from the ECOR collection.

Hypothesis

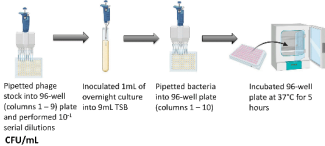
Phages isolated from municipal wastewater will have lytic activity against *E. coli* ST131 and not be effective against non-pathogenic *E. coli*.

Methods

Overnight Bacterial Culture



Microplate Phage Lytic Activity Assay



CFU/mL



Results

TEM Morphologies

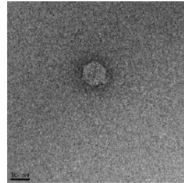


Figure 1. Autographiviridae (head diameter: 59nm, tail length: 12.5nm)

Phage Microplate Assay

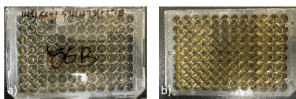


Figure 2. (a) Labeled microplate including the strain of phage, bacterial hosts, and controls. (b) Microplate displaying turbidity (no lysis) in columns 1-4 and 10 as well as clear solution (lysis) in columns 5-9.

Host Range of Isolated Phages

Table 1 Summary of phage genome size, host range, and classification.

Phages	Classification				ECOR Collection (No.) (%)	C1* (n=4) (%)	C2** (n=12) (%)	C1.6227* (n=12) (%)	No. of Susceptible Strains (n)
	Family	Genus	Genome Size (bp)	GC Content (%)					
1868	Saubeviridae	Mutapervus	169,185	30.71 (28%)	20/40 (75%)	3/165 (57%)	7/8 (88%)	99/185 (53%)	
261P	Autographiviridae	Vectrovirus	44,500	10.72 (21%)	17/40 (43%)	36/65 (55%)	0/8 (75%)	73/185 (39%)	
630P	Autographiviridae	Vectrovirus	44,631	10.72 (14%)	17/40 (43%)	20/65 (31%)	1/8 (13%)	53/185 (29%)	

* Most C1 and C1.6227 strains are resistant against ciprofloxacin (Fluoroquinolone), trimethoprim/sulfamethoxazole (folate pathway inhibitor), ceftriaxone (cephalosporin).

** Most C2 strains are resistant against ciprofloxacin, ceftriaxone, tobramycin (aminoglycoside)

Conclusions

- Bacteriophages effective against *Escherichia coli* ST131 clade C were isolated from municipal wastewater
- Different family and genus of phages displayed different host range within the ECOR Collection strains and *E. coli* ST131 clade C
- Different concentrations of phages showed different effectiveness of lytic activity against non-pathogenic ECOR Collection *E. coli* and
- Phages isolated from Municipal wastewater showed limited lytic activity against non-pathogenic ECOR Collection

Future Directions

- Analyze the genomic data of isolated phages (1868, 261P, and 630P) to examine its role in host recognition, both *E. coli* ST131 and non-pathogenic *E. coli*.
- Find combinations of phages that work together in a phage cocktail to reduce the chance of bacteria developing phage resistant strains.
- Perform host range assay to determine the activity of phages isolated from municipal wastewaters on other MDR bacteria.
- Evaluate phage capabilities for medicinal and therapeutic use in treating MDR bacterial infections.

References

1. Lee DS, Lee SJ, Chee HS. Community-Acquired Urinary Tract Infection by *Escherichia coli* in the Era of Antibiotic Resistance. *Biomed Res Int.* 2018;2018:7656752. doi:10.1155/2018/7656752.
2. Nicolas-Chateleine MH, Bertrand X, Midec JY. *Escherichia coli* ST131, an intriguing clonal group. *Clin Microbiol Rev.* 2014;27(3):543-574. doi:10.1128/CMR.00125-13
3. Goodridge L, Galicchio A, Griffiths MW. Morphological, host range, and genetic characterization of two coliphages. *Appl Environ Microbiol.* 2003;69(9):5364-5371. doi:10.1128/AEM.69.9.5364-5371.2003
4. Bertozzi Silva J, Storms Z, Sausagete D. Host receptors for bacteriophage adsorption. *FEMS Microbiol Lett.* 2016;363(4). doi:10.1093/femsli/fmw002
5. Forsyth JH, Barron NU, Scott L, et al. Decolonizing drug-resistant *E. coli* with phage and probiotics: breaking the frequency-dependent dominance of residents. *Microbiology.* 2023;169(7). doi:10.1099/mic.0.001352

Images: Created with BioRender.com

Acknowledgements

Jenny Hyun (ACWA (Advancing Canadian Wastewater Assets) Microbiology Laboratory)
Dr. Dongyan Niu (University of Calgary)
Mawra Gohar (ACWA (Advancing Canadian Wastewater Assets) Microbiology Laboratory)
Dr. Beatriz Garcia-Diaz (Webber Academy)

Feb 22.

- Finalized Poster with feedback from mentor
- Prepare for Oral Presentation by on google slides
 - Science Fair Prep Oral Presentation - Vincent Liu
- Always answer research question in the conclusion

Feb. 26

- Participated in presentation and asked question
 - Brynn:
 - Environmental stress causes plants to become endangered
 - Synthetic biology
 - Engineer for biodiversity preservation
 - Environmental stressors
 - Cooper:
 - Asked question about injuring different parts of the mice head
- Finished Oral Presentation
 - Science Fair Prep Oral Presentation - Vincent Liu

Feb. 28

- Presented oral presentation
- Participated in presentation and asked question
 - Tiffany:
 - Therapy, set goals for recovery
 - Eye movement desensitization reprocessing
 - Stigma around first responders feeling mentally unwell
 - Education in the workfield or everywhere?
 - Did you find the amount of mentally ill first responders from their websites?
 - Lack of general education of these topics
 - Mariska
 - HSF diets linked to obesity
 - When fats get into the tissue it can cause damage
 - Previous only male rats
 - Why do female and male fibrosis differ?

March

Mar. 7

- Results section
 - Full of figures and tables
 - Explain all about the figure before adding the figure
 - DESCRIPTION of the figure not and explanation
 - Past tense
- Reflection on Science Fair
 - More images and also discuss sources of error
 - Add a venn diagram (?) of the phage overlap in lytic activity instead of just verbal mention
 - More confidence in presentation
 - Emphasis on problem, social costs, make it sound more significant

Mar. 11

- Logbook:
 - Reflections on judges comments
 - Heavy on science fair reflections
- Send email to mentor about science fair advice from judges
- Individual meeting:
 -

Experimental Procedures:

November

Nov. 14

- Performed Microplate Virulence Assay in lab with mentor
 - Used phage stocks of 630P at dilutions of 0 (undiluted phage stock + bacteria) to -8 (-8 dilution phage stock + bacteria) in a single 96-well plate
 - Columns 1-2 is Ecor 1, 3-4 is Ecor 2, 5-6 is Ecor 3
 - Column 7 is the positive control (Phage + Host Strain)
 - Column 8 is the negative control (Host Strain only)
 - Column 9 is the blank control (TSB only)
 - Incubated 96-well plate at 37 degrees Celsius for 5 hours

Nov. 23

- Performed Microplate Assay with Ecor 4 - 7
 - Mentor will send data and images from Assay
 - Used phage stocks of 186B, 261P, and 630P at dilutions of 0 (undiluted phage stock + bacteria) to -8 (-8 dilution phage stock + bacteria) in 3 separate 96 well plates (1 for each strain of phage)
 - Columns 1-2 is Ecor 4, 3-4 is Ecor 5, 5-6 is Ecor 6, and 7-8 is Ecor 7 and repeated for all phage strains
 - Column 9 is the positive control (Phage + Host Strain, for example phage 630P would have a host strain of E. coli 630)
 - Column 10 is the negative control (Host Strain only)
 - Column 11 is the blank control (TSB only)
 - Incubated all 3 96-well plates at 37 degrees Celsius for 5 hours

December

Dec. 1

- Performed Microplate Assay with Ecor 8 - 11
 - Mentor will send data and images from Assay
 - For “Experimental Procedures” and “Data Collection and Results” mentor will teach format and how to record the next meeting on Dec. 11

- Used phage stocks of 186B, 261P, and 630P at dilutions of 0 (undiluted phage stock + bacteria) to -8 (-8 dilution phage stock + bacteria) in 3 separate 96 well plates (1 for each strain of phage)
- Columns 1-2 is Ecor 8, 3-4 is Ecor 9, 5-6 is Ecor 10, and 7-8 is Ecor 11 and repeated for all phage strains
- Column 9 is the positive control (Phage + Host Strain)
- Column 10 is the negative control (Host Strain only)
- Column 11 is the blank control (TSB only)
- Incubated all 3 96-well plates at 37 degrees Celsius for 5 hours

Dec. 11

- Performed Microplate Assay with Ecor 12 - 15
 - Mentor will send data and images form Assay
 - Used phage stocks of 186B, 261P, and 630P at dilutions of 0 (undiluted phage stock + bacteria) to -8 (-8 dilution phage stock + bacteria) in 3 separate 96 well plates (1 for each strain of phage)
 - Columns 1-2 is Ecor 12, 3-4 is Ecor 13, 5-6 is Ecor 14, and 7-8 is Ecor 15 and repeated for all phage strains
 - Column 9 is the positive control (Phage + Host Strain)
 - Column 10 is the negative control (Host Strain only)
 - Column 11 is the blank control (TSB only)
 - Incubated all 3 96-well plates at 37 degrees Celsius for 5 hours

Dec. 19

- Performed Microplate Assay with Ecor 16 - 19
 - Mentor will send data and images form Assay
 - Used phage stocks of 186B, 261P, and 630P at dilutions of 0 (undiluted phage stock + bacteria) to -8 (-8 dilution phage stock + bacteria) in 3 separate 96 well plates (1 for each strain of phage)
 - Columns 1-2 is Ecor 16, 3-4 is Ecor 17, 5-6 is Ecor 18, and 7-8 is Ecor 19 and repeated for all phage strains
 - Column 9 is the positive control (Phage + Host Strain)
 - Column 10 is the negative control (Host Strain only)
 - Column 11 is the blank control (TSB only)
 - Incubated all 3 96-well plates at 37 degrees Celsius for 5 hours

January

Jan 2.

- Performed Microplate Assay with Ecor 20 - 27

- Used phage stocks of 186B, 261P, and 630P at dilutions of 0 (undiluted phage stock + bacteria) to -8 (-8 dilution phage stock + bacteria) in 6 separate 96 well plates (1 for each strain of phage and 4 Ecor strains per well plate)
- 1st set of plates: Columns 1-2 is Ecor 20, 3-4 is Ecor 21, 5-6 is Ecor 22, and 7-8 is Ecor 23 and repeated for all phage strains
- 2nd set of plates: Columns 1-2 is Ecor 24, 3-4 is Ecor 25, 5-6 is Ecor 26, and 7-8 is Ecor 27 and repeated for all phage strains
- Column 9 is the positive control (Phage + Host Strain)
- Column 10 is the negative control (Host Strain only)
- Column 11 is the blank control (TSB only)
- Incubated all 3 96-well plates at 37 degrees Celsius for 5 hours

Jan 3.

- Performed Microplate Assay with Ecor 28 - 35
 - Used phage stocks of 186B, 261P, and 630P at dilutions of 0 (undiluted phage stock + bacteria) to -8 (-8 dilution phage stock + bacteria) in 6 separate 96 well plates (1 for each strain of phage and 4 Ecor strains per well plate)
 - 1st set of plates: Columns 1-2 is Ecor 28, 3-4 is Ecor 29, 5-6 is Ecor 30, and 7-8 is Ecor 31 and repeated for all phage strains
 - 2nd set of plates: Columns 1-2 is Ecor 32, 3-4 is Ecor 33, 5-6 is Ecor 34, and 7-8 is Ecor 35 and repeated for all phage strains
 - Column 9 is the positive control (Phage + Host Strain)
 - Column 10 is the negative control (Host Strain only)
 - Column 11 is the blank control (TSB only)
 - Incubated all 3 96-well plates at 37 degrees Celsius for 5 hours

Jan 4.

- Performed Microplate Assay with Ecor 36 - 43
 - Used phage stocks of 186B, 261P, and 630P at dilutions of 0 (undiluted phage stock + bacteria) to -8 (-8 dilution phage stock + bacteria) in 6 separate 96 well plates (1 for each strain of phage and 4 Ecor strains per well plate)
 - 1st set of plates: Columns 1-2 is Ecor 36, 3-4 is Ecor 37, 5-6 is Ecor 38, and 7-8 is Ecor 39 and repeated for all phage strains
 - 2nd set of plates: Columns 1-2 is Ecor 40, 3-4 is Ecor 41, 5-6 is Ecor 42, and 7-8 is Ecor 43 and repeated for all phage strains
 - Column 9 is the positive control (Phage + Host Strain)
 - Column 10 is the negative control (Host Strain only)
 - Column 11 is the blank control (TSB only)
 - Incubated all 3 96-well plates at 37 degrees Celsius for 5 hours

Jan 5.

- Performed Microplate Assay with Ecor 44 - 51

- Used phage stocks of 186B, 261P, and 630P at dilutions of 0 (undiluted phage stock + bacteria) to -8 (-8 dilution phage stock + bacteria) in 6 separate 96 well plates (1 for each strain of phage and 4 Ecor strains per well plate)
- 1st set of plates: Columns 1-2 is Ecor 44, 3-4 is Ecor 45, 5-6 is Ecor 46, and 7-8 is Ecor 47 and repeated for all phage strains
- 2nd set of plates: Columns 1-2 is Ecor 48, 3-4 is Ecor 49, 5-6 is Ecor 50, and 7-8 is Ecor 51 and repeated for all phage strains
- Column 9 is the positive control (Phage + Host Strain)
- Column 10 is the negative control (Host Strain only)
- Column 11 is the blank control (TSB only)
- Incubated all 3 96-well plates at 37 degrees Celsius for 5 hours

Jan 30.

- Performed Microplate Assay with *E. coli* ST131 C1 Subclade (Resistant to fluoroquinolones)
 - Used phage stocks of 186B, 261P, and 630P at dilutions of 0 (undiluted phage stock + bacteria) to -8 (-8 dilution phage stock + bacteria) in 6 separate 96 well plates (1 for each strain of phage and 4 Ecor strains per well plate)
 - Columns 1-2 is 171, 3-4 is 256, 5-6 is 492, and 7-8 is 578 and repeated for all phage strains
 - Column 9 is the positive control (Phage + Host Strain)
 - Column 10 is the negative control (Host Strain only)
 - Column 11 is the blank control (TSB only)
 - Incubated all 3 96-well plates at 37 degrees Celsius for 5 hours

February

Feb. 7

- Performed Microplate Assay with *E. coli* ST131 C2 Subclade
 - Used phage stocks of 186B, 261P, and 630P at dilutions of 0 (undiluted phage stock + bacteria) to -8 (-8 dilution phage stock + bacteria) in 6 separate 96 well plates (1 for each strain of phage and 4 Ecor strains per well plate)
 - Columns 1-2 is 526, 3-4 is 599, 5-6 is 622, and 7-8 is 633 and repeated for all phage strains
 - Column 9 is the positive control (Phage + Host Strain)
 - Column 10 is the negative control (Host Strain only)
 - Column 11 is the blank control (TSB only)
 - Incubated all 3 96-well plates at 37 degrees Celsius for 5 hours

Feb. 15

- Performed Microplate Assay with *E. coli* ST131 C1-M27 Subclade

- Used phage stocks of 186B, 261P, and 630P at dilutions of 0 (undiluted phage stock + bacteria) to -8 (-8 dilution phage stock + bacteria) in 6 separate 96 well plates (1 for each strain of phage and 4 Ecor strains per well plate)
- Columns 1-2 is 149, 3-4 is 564, 5-6 is 572, and 7-8 is 473 and repeated for all phage strains
- Column 9 is the positive control (Phage + Host Strain)
- Column 10 is the negative control (Host Strain only)
- Column 11 is the blank control (TSB only)
- Incubated all 3 96-well plates at 37 degrees Celsius for 5 hours

Background Research:

Sept 8.

- Read summary and introduction of “*Escherichia coli* ST131, an Intriguing Clonal Group”
- Summary: In 2008 *Escherichia coli* ST131 was discovered
 - Is the predominant *E. coli* lineage among extraintestinal pathogenic *E. coli*
 - Is found to have strong resistance to fluoroquinolones (a type of antibiotics to treat bacterial infection)
 - This paper highlights what is currently known about *E. coli* ST131 (as of 2014) to help public health authorities to combat this pathogen
- Introduction:
 - *Escherichia coli* is a common bacteria with many different strains that infect and reside in the gastrointestinal system of humans and animals

Sept 18.

- Introduction:
 - Causes diarrheal diseases and leading cause of urinary tract infections
 - Need for research because ST131 is a harmful pathogen that is multidrug resistant to all forms of antibiotics
 - Extended-spectrum β -lactamases (ESBL) by *E. coli* isolates have increased steadily over the last 20 years and this allows them to be resistant to fluoroquinolones.
 - Goal is to “provide readers with a practical overview, a documented summary of the most important microbiological and epidemiological data published to date, and an indication of what remains to be discovered”

Oct. 6

- See annotated document
 -  Lee et al. 2018 - Community-Acquired Urinary Tract Infection by *Escherichia coli* ST131

Oct. 7

- See annotated document
 - Forsyth et al. 2023 - Decolonizing drug-resistant E. coli with phage and pro...

Oct. 17

- See annotated document
 - Goodridge et al. 2003 - Morphological, host range, and genetic characteriza...

Dec. 5

- See annotated document
 - Kondratyeva et al. 2020 - Meta-analysis of Pandemic Escherichia coli ST1...

Dec. 13

- See annotated document
 - Kondratyeva et al. 2020 - Meta-analysis of Pandemic Escherichia coli ST1...

Data Collection and Results:

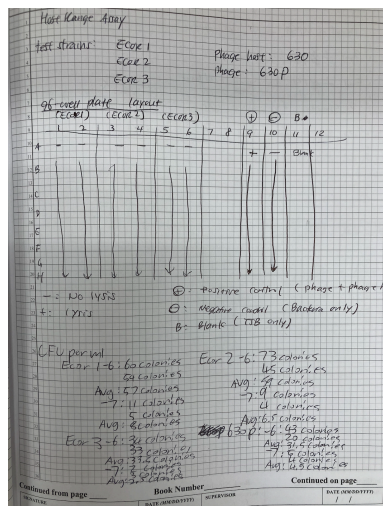
For All Data in Excel Format:

- Turbidity was determined by eye to see if the result was clear, partially clear, or turbid.
 - +++ = Phage activity/successfully lysed (clear solution)
 - ± = Partial phage activity/not fully clear but not fully turbid
 - - = No phage activity/completely turbid

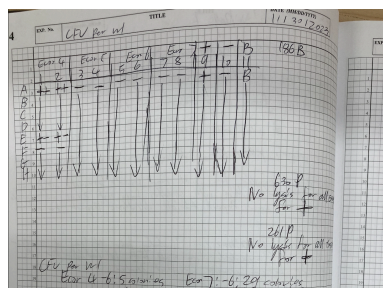
November

Nov. 22

- See under date of November 22, 2023
 - Microplate Assay Result.....
 - Raw Data Sheet:



Nov. 30

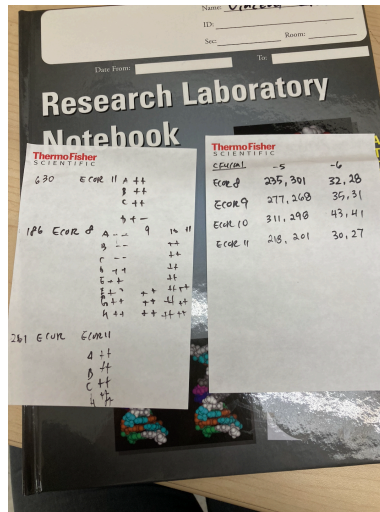


- See under date of November 30, 2023
 - Microplate Assay Result..xlsx
 - Raw Data Sheet:

December

Dec. 11

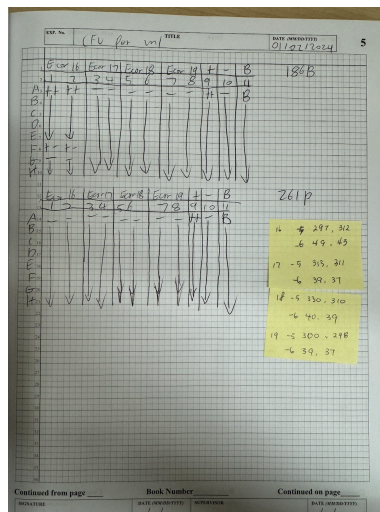
- See under date of December 11, 2023
 - Microplate Assay Result.....
 - Raw Data Sheet:



January

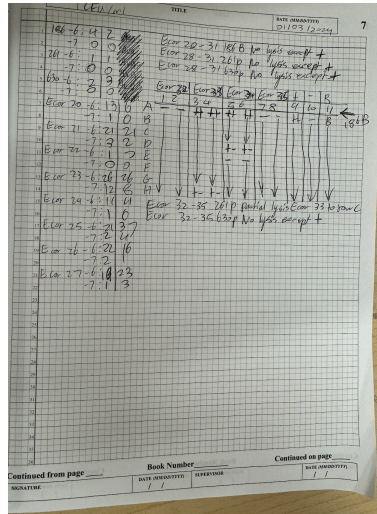
Jan 2.

- See under date of January 2, 2024
 - Microplate Assay Result.....
 - Raw Data Sheet:



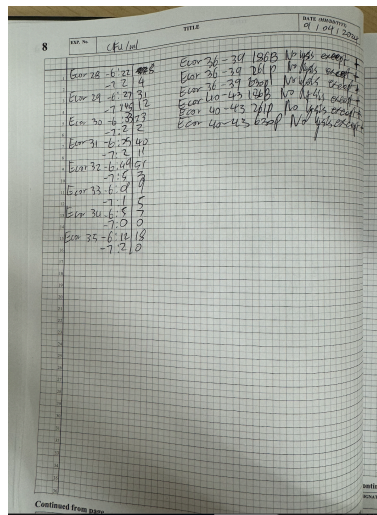
Jan 3.

- See under date of January 3, 2024
 - Microplate Assay Result.....
 - Raw Data Sheet:



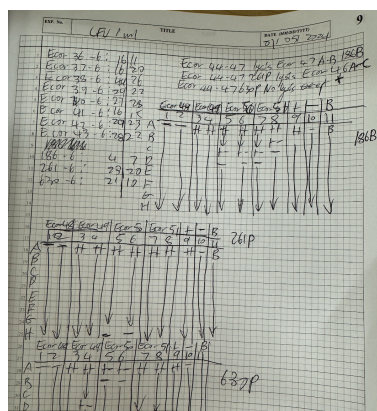
Jan 4.

- See under date of January 4, 2024
 - Microplate Assay Result.....
 - Raw Data Sheet:



Jan 5.

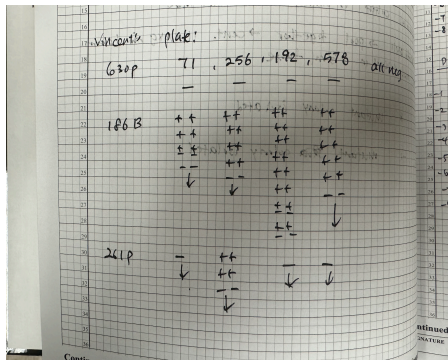
- See under date of January 5, 2024
 - Microplate Assay Result.....
 - Raw Data Sheet:



February

Feb. 7

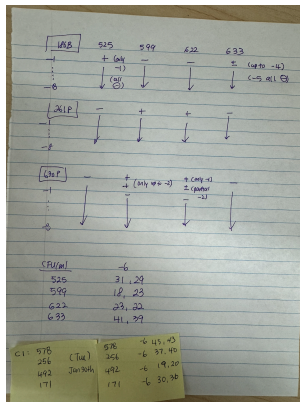
- C1 Subclade
- Raw Data Sheet:



- CFU/mL
 - 578 -6 Dilution: 45 and 43 colonies
 - 256 -6 Dilutions: 37 and 40 colonies
 - 492 -6 Dilutions: 19 and 20 colonies
 - 171 -6 Dilutions: 30 and 36 colonies

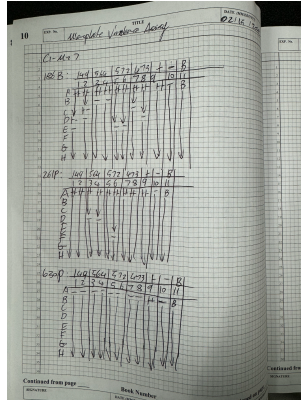
Feb. 15

- C2 Subclade
- Raw Data Sheet:



- CFU/mL
 - 525 -6 Dilution: 31 and 29 colonies

- 599 -6 Dilutions: 18 and 23 colonies
- 622 -6 Dilutions: 23 and 22 colonies
- 633 -6 Dilutions: 41 and 39 colonies
- C1-M27 Subclade
 - Raw Data Sheet:



Calendar:

September

SEPTEMBER 2023						
Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
27	28	29	30	31	1	2
3	4 Labor Day	5	6	7	8	9
10	11	12 Individual Meeting	13 Go to lab after school	14 Go to lab before school	15	16

17	18	19	20	21 Review materials sent by Jenny	22 Lab Before School	23
24	25	26 Individual Meeting	27 Sick but read article on MDR	28 Work on draft of the introduction	29 Meet in the lab maybe?	30

October

OCTOBER 2023						
Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1	2 Read articles on phage resistance + go to lab	3	4 Individual Meeting	5	6 Annotate "Community-Acquired Urinary Tract Infection by Escherichia coli in the Era of Antibiotic Resistance" Paper	7 Annotate "Decolonizing drug-resistant E. coli with phage and probiotics: breaking the frequency-dependent dominance of residents"
8 Annotate "Escherichia coli ST131, an intriguing clonal group"	9 Columbus Day	10	11 Meet with mentor (TBD) If no meeting then work on introduction and objectives in	12	13 Continued annotating "Escherichia coli ST131, an intriguing clonal group"	14

			research proposal			
15	16	17 Individual Meeting + work on annotating Morphological, Host Range, and Genetic Characterization of Two Coliphages	18	19 Complete WHMIS 2015 and begin Hazard Assessment training	20	21
22	23 Complete Hazard Assessment training and start lab safety training on my.ucalgary.ca	24	25 Finish lab safety training and all other training	26 Meet with mentor after school	27 Finish introduction of research proposal if not finished before + Annotate Standard Reference Strains of Escherichia coli from Natural Populations	28
29	30	31 Work on Variables and Hypothesis in the research proposal Halloween	1	2	3	4

November

NOVEMBER 2023						
Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday

29	30	31	1 Work on research proposal Methodology (provided by mentor) and significance	2	3 Finish research proposal Go to the lab and present my research proposal	4
5 Prepare Overnight Culture for Examination on Monday	6 Finalize and hand in a research proposal. Lab meeting postponed to this date, was supposed to be last Friday	7 Quick results check	8 Participate in Oral Presentations as well as begin the presentation aspect of the research proposal. Finish introductory slides if time permits	9	10	11 Veterans Day
12	13 No School	14 Perform microplate assay and check results after school	15 Oral Presentation	16	17 Oral Presentation Fill out information in CYSF Portal	18
19	20	21 Individual Meeting Prepare overnight culture and materials for the next day	22 Perform microplate assay and check results after school	23	24	25
26	27 Complete all sections that can be filled out now for CYSF portal (hypothesis and variables)	28	29 Make corrections to introduction of research proposal	30 Grow overnight cultures for tomorrow Create 20 Agar plates Prepare MTSB in 96-well plates	1	2

December

DECEMBER 2023						
Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
26	27	28	29	30	1 Perform Microplate Assay with Ecor 8 - 11	2
3	4	5 Work on literature review and log book Make changes to logbook according to feedback Individual meeting	6	7 Read papers that will be sent by mentor	8	9
10	11 Perform Microplate Assay with Ecor 12 - 15 Mentor will teach proper format to record data	12	13 Finish introduction of the research paper / Literature review Individual meeting Finish reading article (See Background Research”	14	15 Read literature that will be sent by professor **Introduction of final paper due	16
17	18 Prepare Overnight Cultures	19 Perform Microplate Assay with Ecor 16 - 19	20	21	22	23
24	25 Christmas	26	27	28	29	30

31	1	2	3	4	5	6
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January

JANUARY 2024						
Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
31	1 New Year's Day	2 Screen Ecor 20 - 27	3 Screen Ecor 28 - 35	4 Screen Ecor 36 - 43	5 Screen Ecor 44 - 51 Meeting with Dr. Niu regarding screening ST131	6
7	8 Begin work on Procedures section of final paper	9	10 Submit draft of Procedures section of final paper for feedback	11	12 Midterm Exam Break Starts	13
14	15	16	17	18	19	20
21	22 Midterm Exam Break Ends	23	24 Make changes to the procedures section of Research Proposal suggested by Dr. Garcia	25	26 Begin writing Procedures section of Final paper Individual meeting	27

28	29 Prepare overnight culture for ST131	30 Perform Assay for ST131	31 Dec/Jan Logbook due	1	2	3
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February

FEBRUARY 2024						
Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
28	29	30	31	1 Finish Draft of Procedures section and submit to Dr. Garcia	2	3
4	5 Make changes Procedures section if handed back, if not then next day If not handed back, prepare Oral Presentation Individual Meeting	6 Prepare overnight culture in the lab	7 Perform microplate assay using overnight culture	8	9 Make changes to the Procedures section if not handed back the last day. If it was handed back, Prepare Oral Presentation	10
11	12	13 Procedures section due. Hand in procedures section Compile data sent by mentor that I have not screened into graphs and put onto poster	14	15 Long weekend start Screen Subclade C1 - M27 and create poster with help of mentor	16	17

FEBRUARY 2024

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
28	29	30	31	1 Finish Draft of Procedures section and submit to Dr. Garcia	2	3
4	5 Make changes Procedures section if handed back, if not then next day If not handed back, prepare Oral Presentation Individual Meeting	6 Prepare overnight culture in the lab	7 Perform microplate assay using overnight culture	8	9 Make changes to the Procedures section if not handed back the last day. If it was handed back, Prepare Oral Presentation	10
18	19 Last day of long weekend	20 Completed poster and submit hand into mentor before printing	21	22 Use poster template sent by mentor and plan for arrangement and fitting	23	24
25	26 Prepare Oral Presentation	27 Schedule with Dr. Garcia to rehearse Oral Presentation	28 Oral Presentation	29	1	2

March

MARCH 2024

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
25	26	27	28	29	1	2
3	4 Science Fair	5 Taken up by assembly	6	7 Reflect on science fair feedback	8	9
10	11 Fill out CYSF portal except video which will be recorded outside of class Individual Meeting	12	13 Begin results section by adding in description of figure 1 graph	14	15 Finish results section and ask mentor for feedback on paper	16
17	18	19 Results section due Hand in mentor edited version Individual Meeting	20	21	22	23
24	25	26	27	28	29 Good Friday	30

31 Easter Sunday	1	2	3	4	5	6
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