Discovering Yucky: Annotating its Genome and Finding Gene Function

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Outline

1.What is a bacteriophage?

2. Methods

3. Results

4. Future directions

What is a Bacteriophage?

A bacteriophage is a virus that infects bacteria.

Yucky, our bacteriophage, infects *Gordonia rubripertincta*.

More than 10^31 bacteriophages exist in the biosphere (Pope et. al,2017).

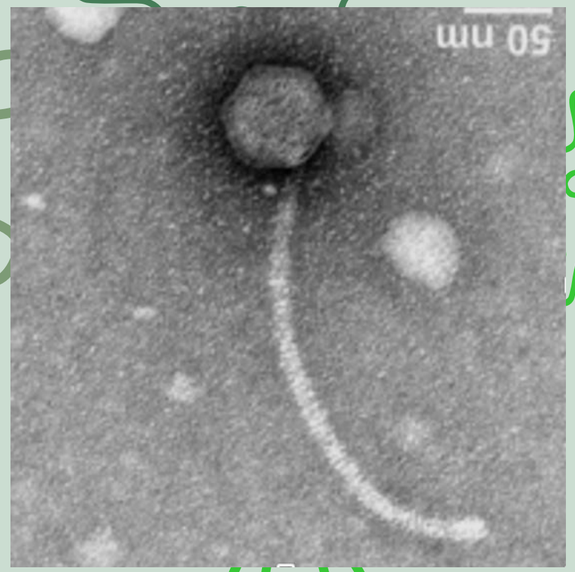
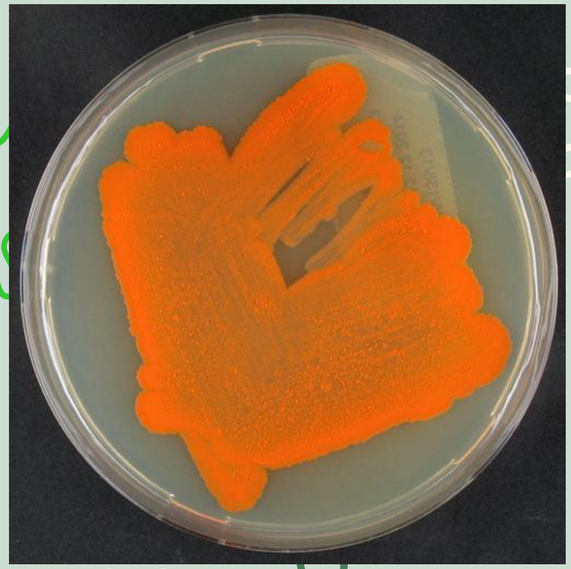


Image of Yucky



Schober et. al 2025

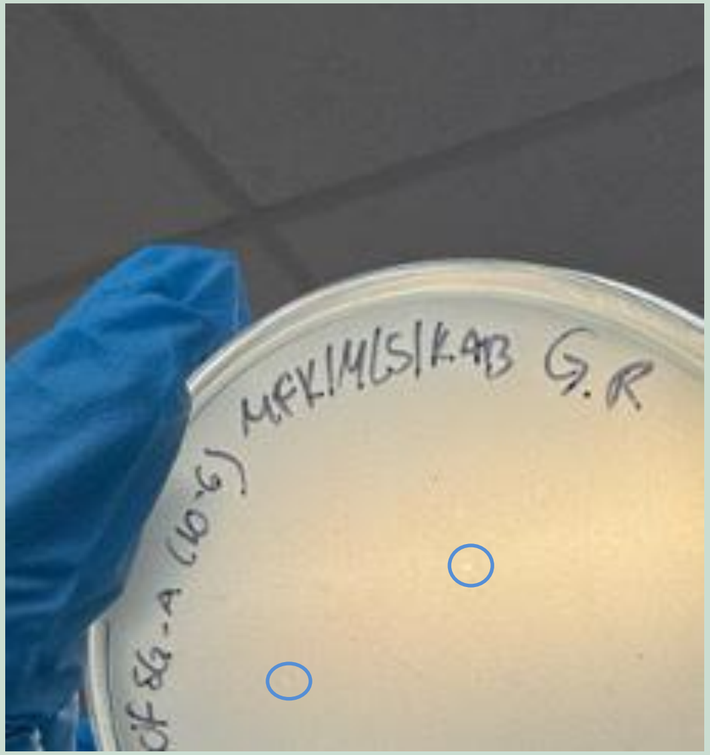
Background

Yucky was discovered and isolated in the fall semester.

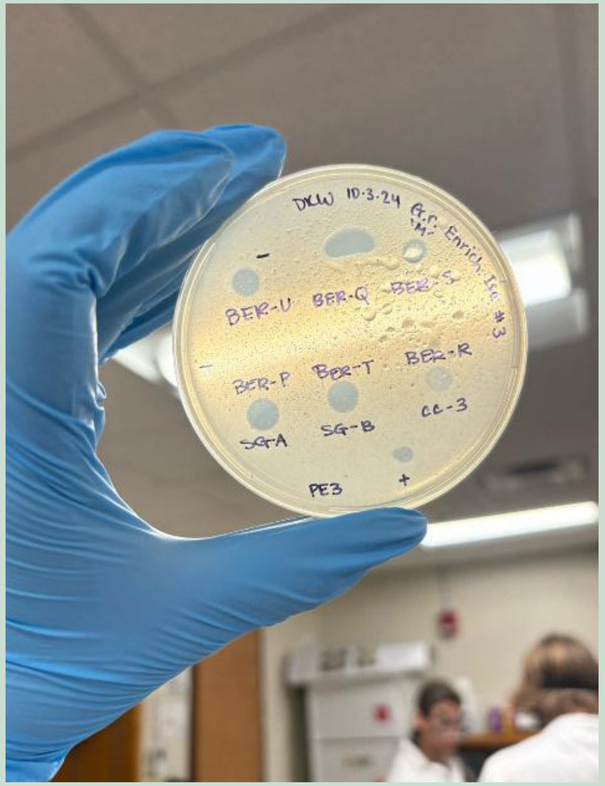
Collection
- Soil samples

Isolation
- Plaque assays

Characterization
- Spot tests
- Gel electrophoresis
- TEM



Plaque assay



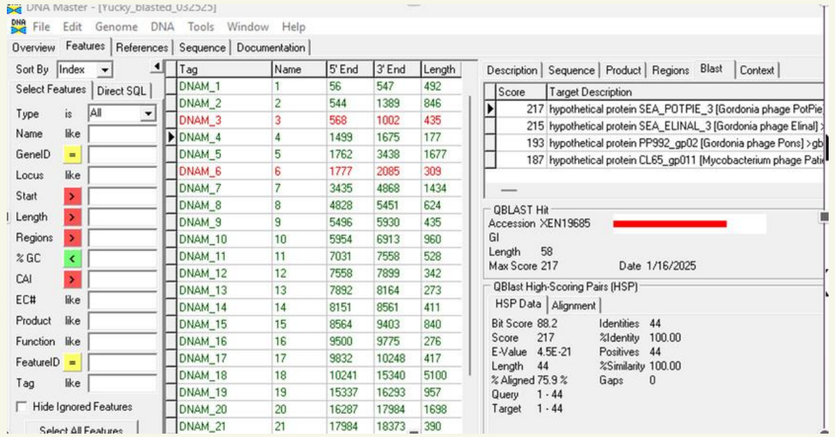
Spot test

Annotation Approach

Is it a Gene?

DNA Master

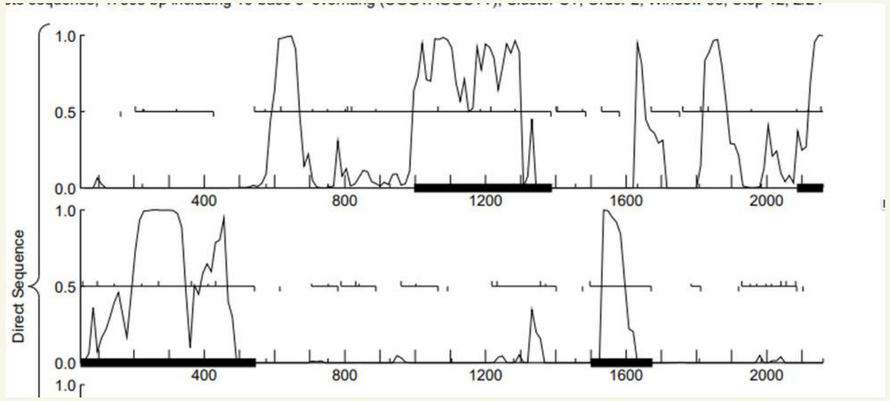
BLAST: Determines how similar genes are in other phages compared to Yucky's.
Gap/overlap: determines if there is a gap between two genes or if they overlap.
Start/end site: start site can change, end site is fixed.



DNA Master

GeneMark

Shows coding potential for a feature.



Where Does it Start?

DNA Master

BLAST: shows alignments to similar genes.
Gap/overlap: comparing gap/overlap between different start site can determine which start is the best.
RBS: shows how likely it is to have a certain start site.

NCBI: shows alignments for start sites not called in DNA Master.
Starterator: shows how other phages in the same pham compare.
GeneMark: shows how much coding potential is cut off for certain start site.

Gap/Overlap

DNAM_7	7	3435	4868	1434
DNAM_8	8	4828	5451	624

RBS

#	Score	Z Value	Distance	Score	Upstream of the Start	Codon	Position	Length
1	-3.453	2.238	17	-5.453	GGAGAGGGCGGGTGATGATCTC	GTG	3435	1434
2	-4.013	1.970	8	-5.235	GAACAAGAATTACAGCAAGCTC	ATG	3459	1410
3	-5.205	1.400	5	-7.205	CGTCGGCTGGCCAGCCACGTGT	GTG	3603	1266
4	-5.205	1.400	11	-5.962	CTGGCCAGCCACGTGTGTGGAC	GTG	3609	1260
5	-4.717	1.633	9	-5.492	ACTTGACTTCCGCGGGTACGAC	ATG	3645	1224
6	-4.299	1.833	7	-5.822	CCAGTCGACCATCCAGAAGATC	GTG	3681	1188
7	-3.964	1.994	16	-5.760	GATCGTGGACGACAATCAACTG	GTG	3699	1170

NCBI

Gene: Yucky_7 Start: 3435, Stop: 4868, Start Num: 116
Candidate Starts for Yucky_7:
(Start: 116 @3435 has 54 MA's), (133, 3459), (195, 3603), (196, 3609), (217, 3645), (232, 3681), (236, 3699), (246, 3732), (252, 3756), (305, 3825), (327, 3858), (450, 4014), (467, 4065), (475, 4095), (483, 4119), (496, 4161), (521, 4230), (531, 4248), (554, 4296), (578, 4368), (631, 4539), (643, 4572), (664, 4632), (670, 4653), (685, 4686), (707, 4746), (709, 4749), (781, 4860),

Starterator

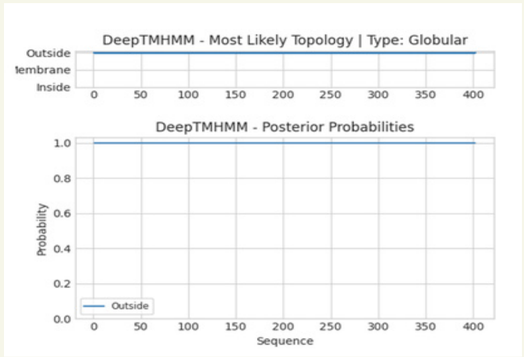
What is its Function?

DNA Master

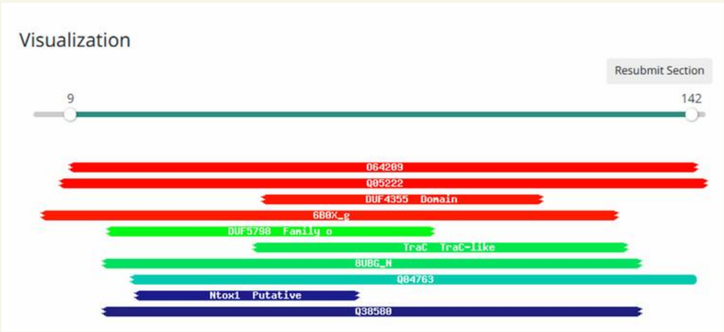
BLAST: shows functions of highly similar genes.

NCBI: shows functions of highly similar genes for start sites not called by DNA Master.
HHpred: shows conserved domains and top hits of possible functions.
Phamerator: shows the genes, functions, conserved domains, and how they relate to our phage.
Deep TMHMM: shows if a hypothetical protein is inside/outside of the membrane, or if it is a transmembrane protein.

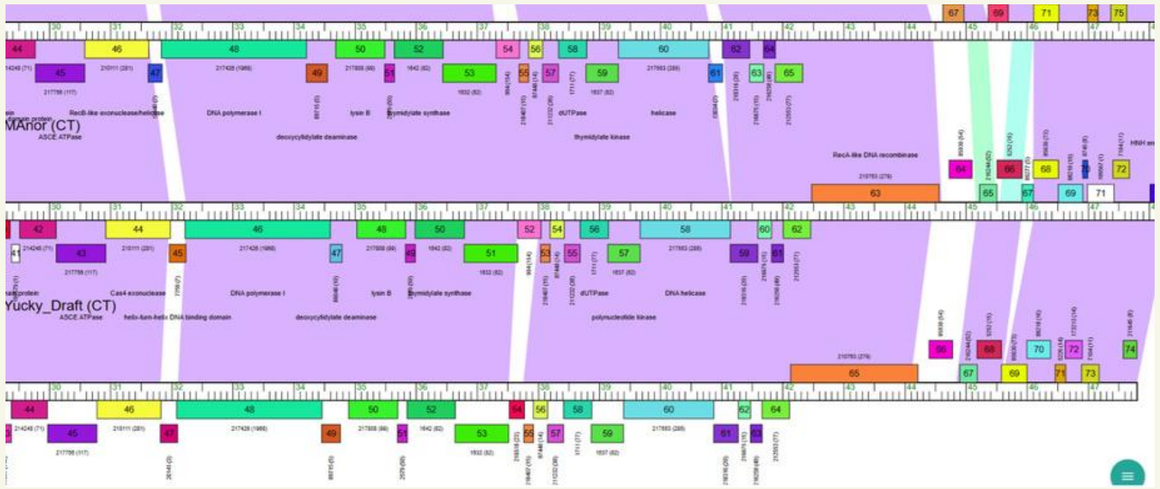
Deep TMHMM



HHpred



Phamerator

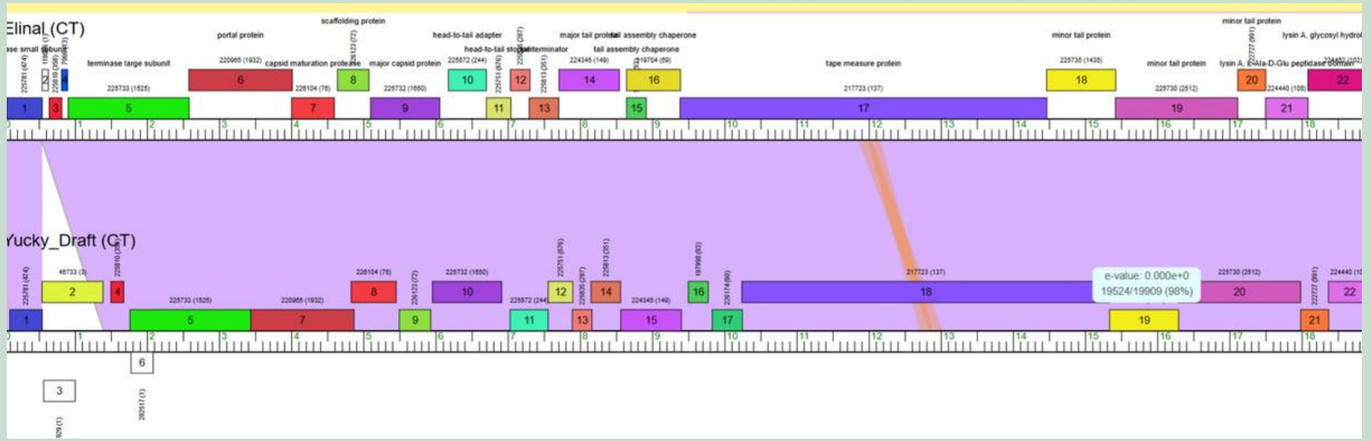


Results

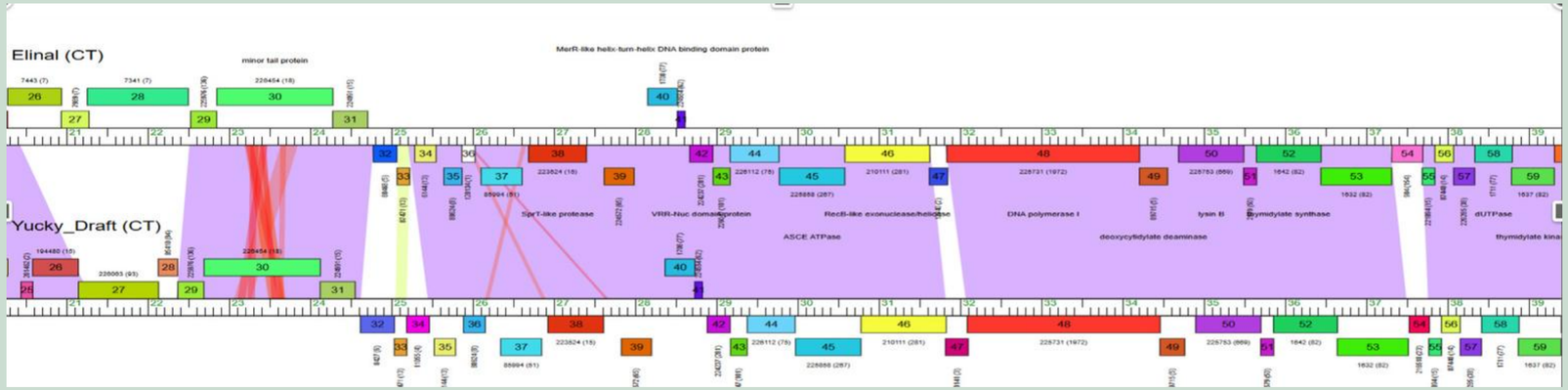
Results

74 genes

- 41 forward.
- 33 reverse.
- ~ 25 hypothetical proteins.
- ~ 49 with function assigned.



Phamerator



Phamerator

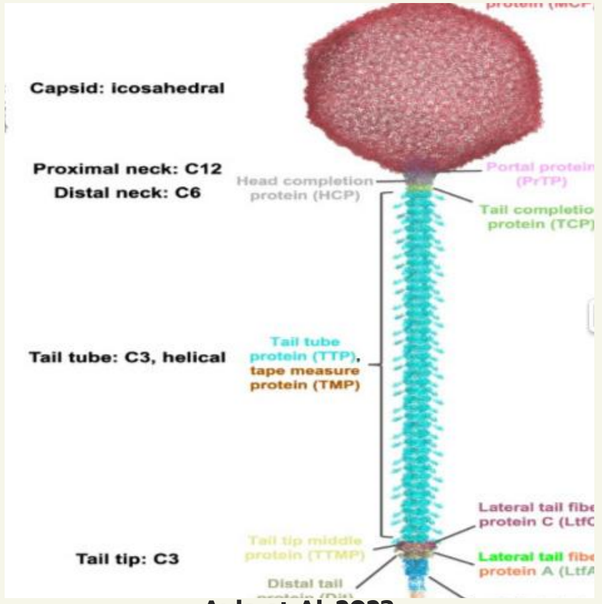
Results: Tape Measure Protein (TMP)

- **Feature 18 of Yucky.**
- **What is a TMP?**
  - This protein helps in forming the tail structure of a bacteriophage. (Poisson et al., 2011).

What does a TMP do?

- TMPS help move the phages' genetic material into the host cell's cytoplasm so the phage can use the cell's machinery to replicate. (Poisson et al., 2011).

- **What is the structure of a TMP?**
  - A TMP is made of repeated sections of two amino acids tryptophan (W) and phenylalanine (F). (Cumby et al., 2015).

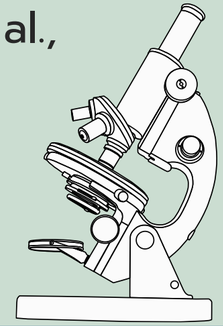


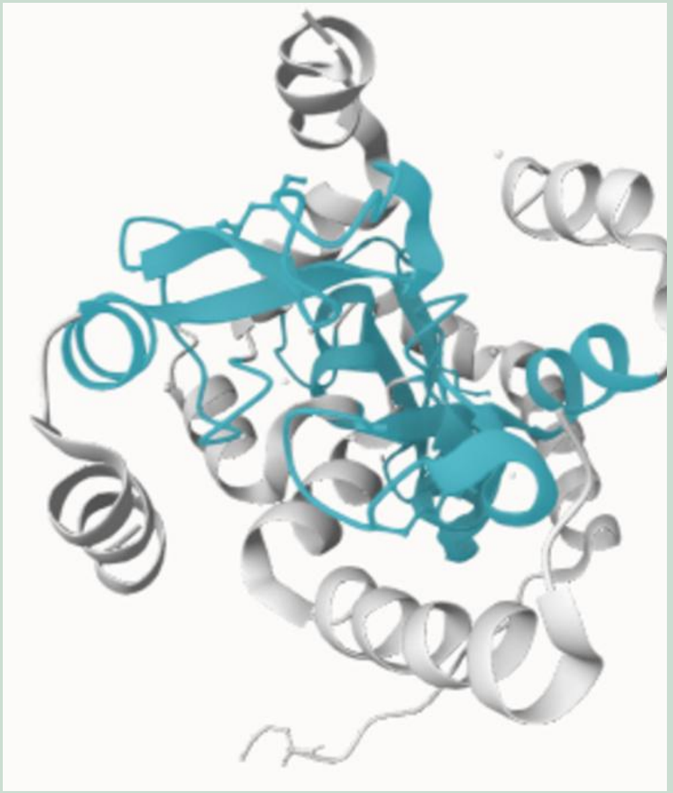
Ayla et Al. 2023

Mahoney eat al. 2016

Results: HNH Endonuclease

- Feature 74 of Yucky.
- Histidine-Asparagine-Histidine motif.
- Located near the end site.
- Stimulates homologous recombination (Zhang et al., 2017).
- Function as DNA packing machines (Zhang et al., 2017).





HHPred: InterPro

Template alignment | Template 3D Structure | PDBe

1. **5H0M_A HNH endonuclease; Thermophilic bacteriophage, HNH Endonuclease, DNA nicking, HYDROLASE; 1.52A {Geobacillus virus E2}**

Probability: 95.9%, E-value: 0.043, Score: 32.55, Aligned cols: 67, Identities: 25%, Similarity: 0.288, Template Neff: 11.1

```
Q ss_pred            ChhHHHHHHHHHHHHHhhcCCCcccCCCCC----CCCccccccccHHH--CCCCCCcccHhhCHHHHHHHhHHHHHH
Q Q_Feature74   10   PRATRRRIRRRGRCEHKDAGGTVCRAVVPP----GTGGVDHIIPRAE--GGTNADDNLQLLCEDHHSVKSKAESARG   80 (105)
Q Consensus     10   ~~~~~~~~~~~~~~~~~~~~~~~c~~~~~~----~~~~~~~~~~~~~--~~~~~~n~~~~~~~~~~~~~~~~~~~~   80 (105)
                     ....|..++..++.+    ..+.|..|+..    ....++|+.+...  ++.....|+..+|..||.........+
T Consensus     57   ~~~~w~~~r~~~~~~~----~~~~c~~c~~~~~~~~~~~~Hi~~~~~~~~~~~~n1~~1c~~ch~~~~~~~~~~   129 (130)
T 5H0M_A        57   HSREWERTRLAVLAK----DNYLCQHCLKEKKITRAVIVDHITPLLVDWSKRLDMDNLQSLCQACHNRKTAEDKRRY   129 (130)
T ss_dssp            TSHHHHHHHHHHHHH----TTTBCHHHHHTTCCCBCCEEEESSCTTTCGGGTTCGGGEEEECHHHHHHHHHHHHHH
T ss_pred            cCHHHHHHHHHHHHH----cCCCchhhchhcCCCceEEeeeeecccCHHHcCChhHHHhcHHHHHHHhHHHHHhc
```

HHPred

Summary

Summary

In Fall 2024, Yucky was discovered and isolated, and shipped off to University of Pittsburgh to be sequenced.

In Spring 2025, Yucky's genome was annotated and 3 questions were answered:

- Is it a gene?
- Where does it start?
- What is its function?

Next, SEAPHAGES will be confirming the annotation work that was done this semester.

The rest of the semester we will be working on a manuscript.

Future Directions

Future Directions

Phage therapy

•Felix d'Herelle is credited with starting phage therapy over a hundred years ago (Hatfull, 2020).

•Phages are used to treat bacterial infections and can be used as alternatives to antibiotics (Lin et al., 2017).

•A wide variety of bacteriophages can be utilized, as many phages only infect certain bacteria.

• Phage therapy has only been approved for human use in special cases (Swenson et al., 2024).

Future Directions

Food safety

- Specific phages can be used to prevent decay of food products and stop the spread of food borne illnesses making environments in food production safer. (Sillankorva et al., 2012)
- They are a natural way of controlling pathogens in food production and different phage products have been approved be the USFDA and USDA. (Sillankorva et al., 2012)

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