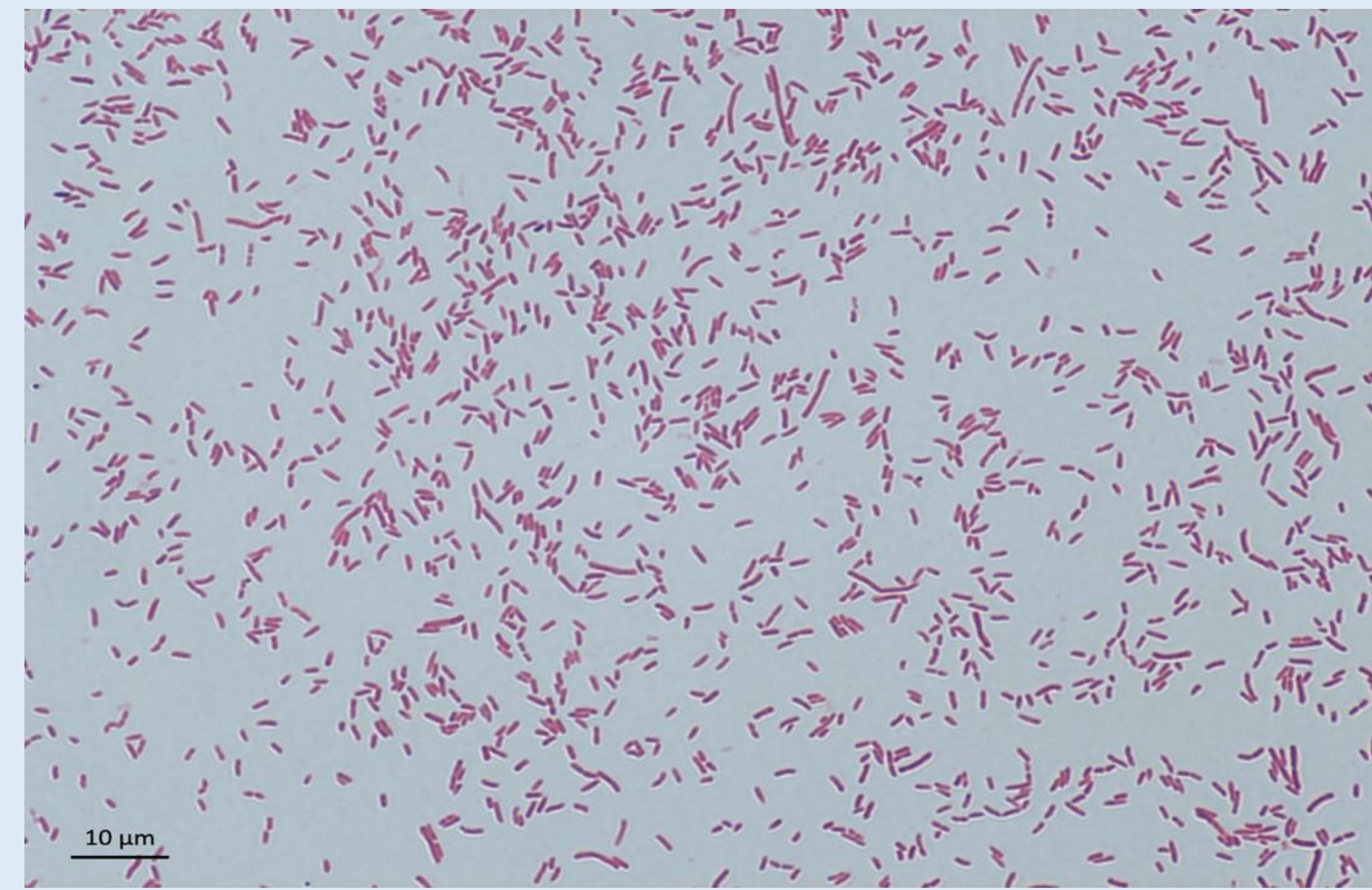# Genome annotation of a novel *Hafnia alvei* bacteriophage isolated from sewage

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Hafnia alvei

Introduction

- Each year, hundreds of thousands of people are infected by antibiotic-resistant bacteria and fungi (CDC, 2020).

- *Klebsiella pneumoniae* and *Hafnia alvei* are Gram-negative bacilli that posses the β-Lactamase gene, enabling antibiotic resistance (Hindiyeh et al., 2008).

- An isolated, antibiotic-resistant strain of *H. alvei* appears to have acquired resistance from *Klebsiella pneumoniae* via horizontal gene transfer (Singleton, unpublished results).

- The genus *Hafnia* has been associated with infections acquired from both community and hospital settings (Yin et al., 2019).

- A novel bacteriophage for has been recently identified for both *Hafnia alvei* and *Klebsiella pneumoniae.*

- Isolation of genomic DNA and next-gen DNA sequencing produced 4 contigs. It is not known whether these DNA contigs represent independent DNA segments, or a single DNA sequence.

- After genetic sequencing, genomic annotation of bacteriophage contigs were performed to identify the function of encoded proteins.

Objective

- Annotate the contig fragments of the isolated bacteriophage for *H. alvei* and reassemble the fragmented DNA into a single linear strand of double-sided DNA.

Results

- Contig Fragments 2 and 3 share DNA relatives, while Contig 1 more closely resembles host bacteria genome (Table 2, 4, 6).

- Genomic maps visualize the locations of ORFs across all three contig fragments (Figure 1).

- All three fragments encode some shared, but unique protein categories along with many hypothetical proteins (Table 1, 3, 5).

Methods

Isolation of Genomic DNA

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DNA purification & Sequencing

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Identification of open reading frames (ORFs)

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Placement of ORFs into DNA Master

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BLAST search of ORFs in DNA Master and NCBI database
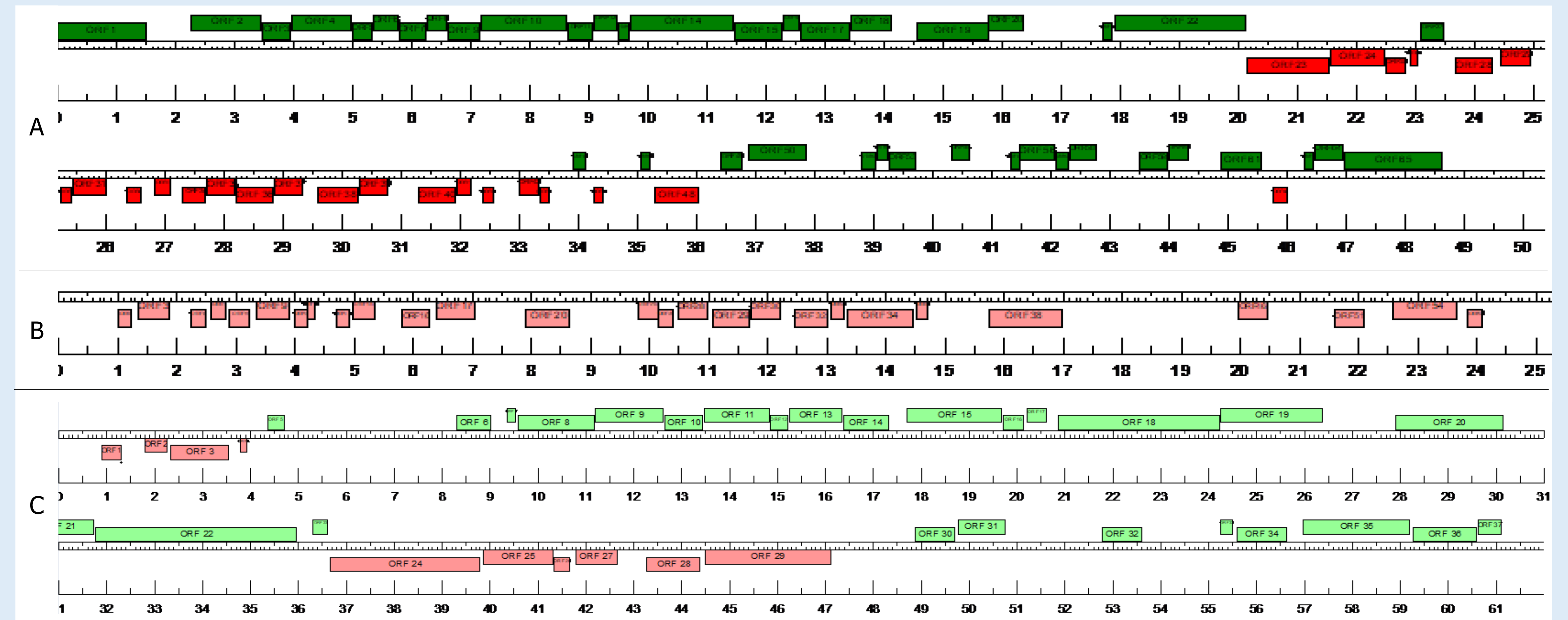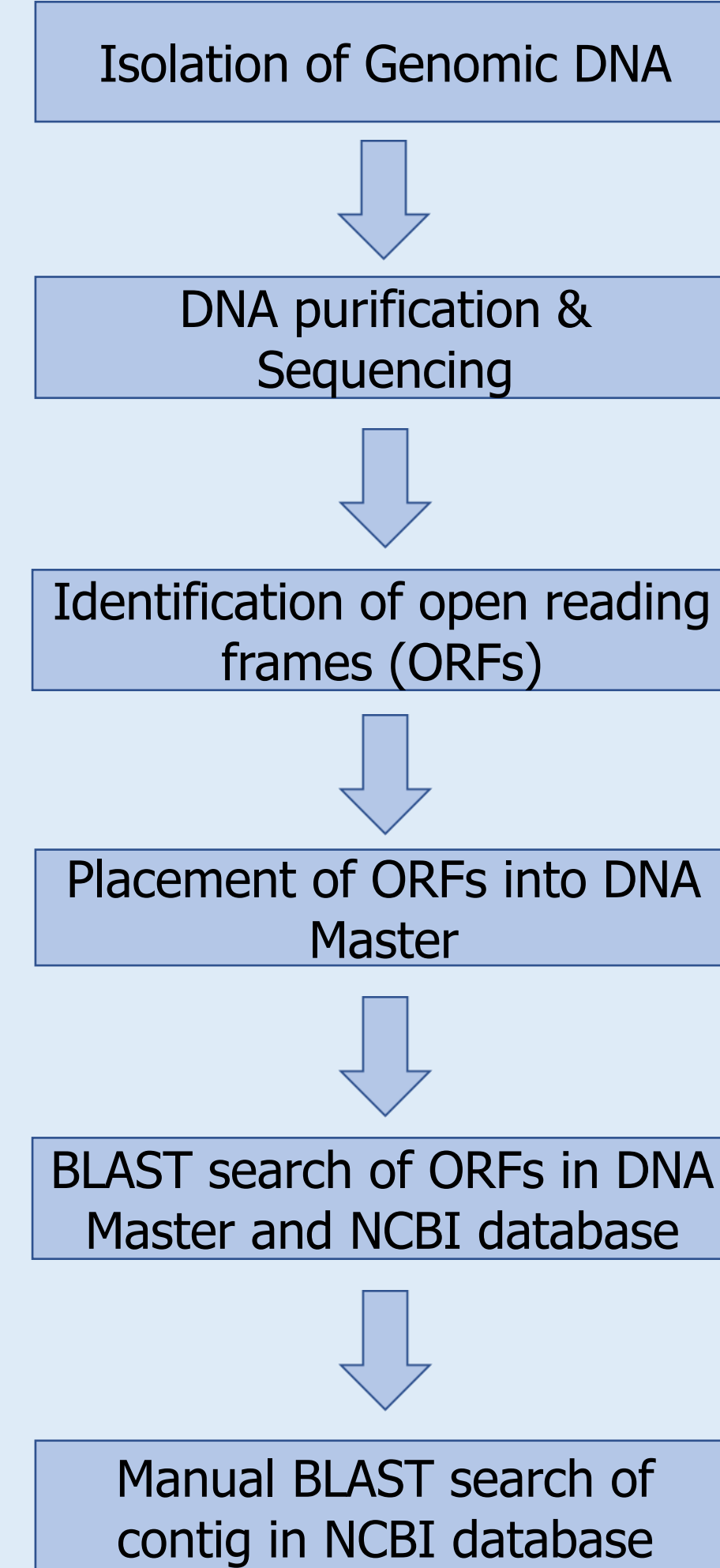
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Manual BLAST search of contig in NCBI database

Figure 1. A.) Genomic map of *Hafnia* bacteriophage contig fragment 1. B.) Genomic map of Hafnia bacteriophage contig fragment 2. C.) Genomic map of Hafnia bacteriophage contig fragment 3. ORFs are numbered from left to right in order of identification. ORF color is indicating the direction of transcription with green being read forward and red being read in reverse. The values below ORFs represent the number of kilobase pairs. ORFs are enclosed with braces based on their similarity in predicted function and purpose.

Table 1. ORF protein predictions for *Hafnia alvei* bacteriophage contig 1 organized into categories based on predicted function and purpose.

ORF Category	Number of ORFs
Hypothetical	41
Enzyme Catalysis and Metabolism	4
Structural Protein	3
DNA Transcription	4
DNA Packaging	2
Protein Synthesis	1
Cell Lysis	1
DUF	9
Total Number of ORFs	65

Table 3. ORF protein predictions for *Hafnia alvei* bacteriophage contig 2 organized into categories based on predicted function and purpose.

ORF Category	Number of ORFs
Hypothetical	28
tRNA	6
DNA Synthesis	5
DNA Replication	2
DNA Metabolism	1
DNA Hydrolysis	1
DNA Recombination	1
Cell Lysis	1
Conserved Phage	1
Total Number of ORFs	45

Table 5. ORF protein predictions for *Hafnia alvei* bacteriophage contig 3 organized into categories based on predicted function and purpose.

ORF Category	Number of ORFs
Hypothetical	23
DNA Packaging	1
Cell Lysis	2
Head Structural Components	3
Tail Structural Components	3
DNA Replication and Metabolism	5
Total Number of ORFs	37

Table 2. DNA relatives identified for contig 1 of the *Hafnia alvei* bacteriophage determined by a nucleotide search from the NCBI Basic Local Alignment Search Tool (BLAST). E values indicate high quality matches. The percentage of sequence alignment was determined utilizing the entire genome of DNA relatives and contig 1.

Scientific Name	E Value	Sequence Alignment
Hafnia alvei FB1	0	97.33%
Hafnia alvei strain FDAARGOS_1038 Chromosome	0	96.94%
Hafnia alvei strain PCM_1220 Chromosome	0	95.06%
Obesumbacterium Proteus strain DSM 2777	0	94.52%
Hafnia phage vB_HpaM_yong1	0	94.28%

Table 4. DNA relatives identified for contig 3 of the *Hafnia alvei* bacteriophage determined by a nucleotide search from the NCBI Basic Local Alignment Search Tool (BLAST). E values indicate high quality matches. The percentage of sequence alignment was determined utilizing the entire genome of DNA relatives and contig 2.

Scientific Name	E Value	Sequence Alignment
Erwinia phage phiEa 21-4	0	99.35%
Erwinia amylovara phage phiEa104	0	99.25%
Erwinia phage SunLIRen	0	99.19%
Salmonella phage VB_SenM-SB 18	0	98.83%
Salmonella phage SE 5	0	98.22%

Table 6. DNA relatives identified for contig 3 of the *Hafnia alvei* bacteriophage determined by a nucleotide search from the NCBI Basic Local Alignment Search Tool (BLAST). E values indicate high quality matches. The percentage of sequence alignment was determined utilizing the entire genome of DNA relatives and contig 3.

Scientific Name	E Value	Sequence Alignment
Salmonella Phage vB_SenM_SB18	0	99.10%
Erwinia Phage SunLIRen	0	98.10%
Salmonella Phage ST-3	0	98.04%
Erwinia Phage phiEa21-4	0	98.01%
Enterobacter Phage phi63_307	0	97.65%

Conclusions

- The majority of the identified ORFs for the novel *Hafnia* phage were hypothetical proteins.

- Novel *Hafnia* phage closely resembles bacteriophages that infect the Enterobacteriaceae family, such as *Erwinia* and *Salmonella.*

- Analysis and gene annotation of bacteriophages is crucial, as bacteriophages can be utilized in phage therapy to combat antibiotic-resistant *Hafnia alvei* infections.

- Due to variation in protein predictions and ORF interpretation, further investigation is needed to attempt to reassemble the genome as it is not clear where there is overlap within the contig fragments

References

CDC (Center for Disease Control and Prevention). 2020. About Antibiotic Resistant [Internet]. [Cited 24 April 2020.] Available from https://www.cdc.gov/drugresistance/about.html

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Yin, Z. et al. 2019. Comparative genomic analysis of the Hafnia genus reveals an explicit evolutionary relationship between the species alvei and paralvei and provides insights into pathogenicity. BMC Genomics, 20, 1-16.