Sequencing and Hybrid Assembly of Antibiotic Resistant Bacteria from an Undergraduate Microbiology Course

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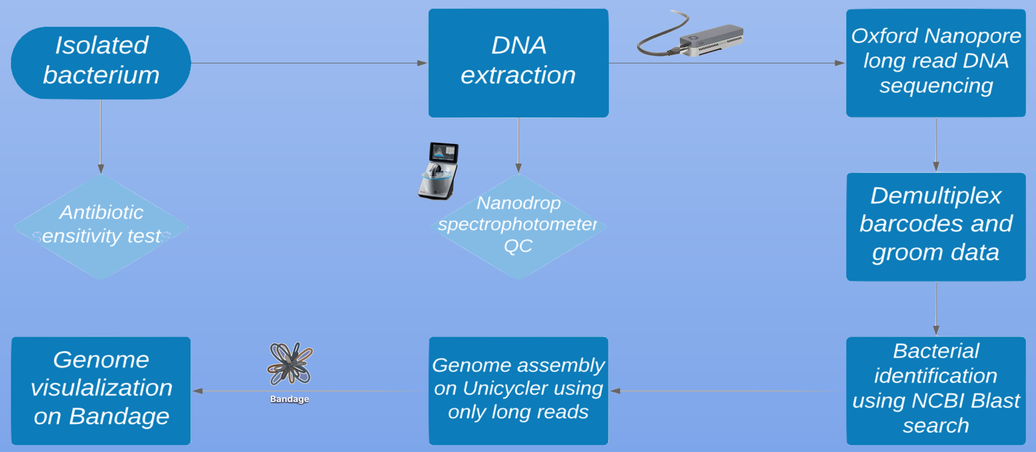
Abstract

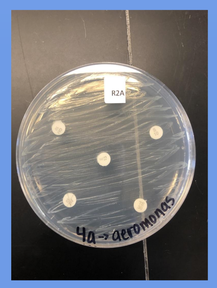
Here we present the full assembled genomes of three bacterial isolates using a hybrid assembly approach. Students in an upper division medical microbiology course isolated antibiotic resistant organisms from various environmental sources. Three isolates were identified as being from the genera Aeromonas, Comamonas, and Acinetobacter and were chosen for further investigation. All three of these genera are gram-negative proteobacteria known to include pathogenic species. The three microorganisms were isolated and confirmed to have resistance to several antibiotics. An internal region of the 16s rRNA gene was amplified by Polymerase Chain Reaction and sequenced using traditional Sanger sequencing. High concentration and high molecular weight preparations of genomic DNA were created using a modified alkaline lysis protocol. These preparations were sequenced using the MinION system (Oxford Nanopore). Additional genomic DNA was prepared using the Wizard Genomic DNA Preparation Kit (Promega), and short read sequencing was performed using the iSEQ 100 system (Illumina). The Illumina iSeq yielded accurate but short DNA sequences and the Oxford Nanopore MinION yielded long but error prone DNA sequences. We used various tools on Galaxy (usegalaxy.eu), an open source web-based bioinformatic database, to trim and check the quality of the DNA sequences. To assemble the genomes, we used Unicycler. Unicycler is a tool found on Galaxy that combines data from the two sequencing technologies we used in this research to create a hybrid assembly. The genomes were compared to already assembled genomes of our three genera to confirm whether or not our species have been identified previously. Antibiotic resistance was previously observed so we annotated the genomes to search for antibiotic resistance and other virulence genes, as well as the presence of mobile elements to facilitate transfer of these genes.

Isolation, DNA Preparation, and Sequencing



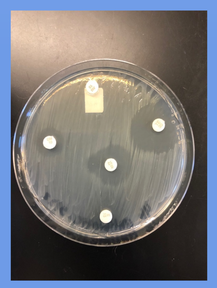
Antibiotic Resistance

Aeromonas



ERYTHROMYCIN	resistant
AMPICILLIN	resistant
GENTAMICIN	resistant
TETRACYCLINE	sensitive
STREPTOMYCIN	resistant

Comamonas



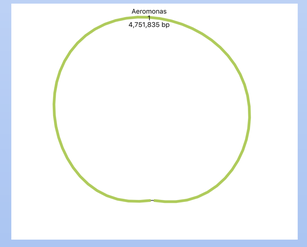
ERYTHROMYCIN	resistant
AMPICILLIN	resistant
GENTAMICIN	sensitive
TETRACYCLINE	sensitive
STREPTOMYCIN	sensitive

Acinetobacter

ERYTHROMYCIN	sensitive
AMPICILLIN	resistant
GENTAMICIN	sensitive
TETRACYCLINE	sensitive
STREPTOMYCIN	unknown

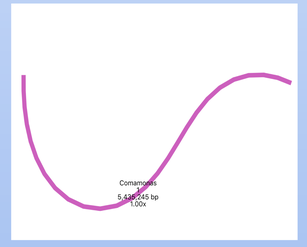
Bandage Plots of Genome Assemblies

Aeromonas



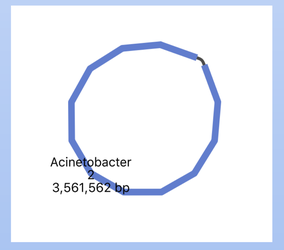
4.7 Million base pair genome
Depth:

Comamonas



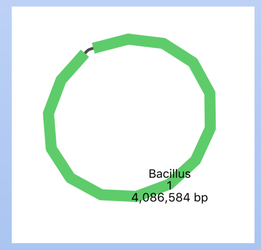
5.4 Million base pair genome
Depth:

Acinetobacter



3.5 Million base pair genome
Depth:

Bacillus



4 Million base pair genome
Depth:

Data Summary

Isolate	DNA Concentration (ng/uL)	260/280 ratio	260/230 ratio	Sequence Data (GB)	N50
Aeromonas	484	2.12	2.28	1.0	10954
Comamonas	481	1.92	2.32	1.7	7350
Bacillus	2127	2.15	2.30	5.7	15741
Acinetobacter					

16s rRNA Gene Comparison

Our **aeromona**s isolate

Aeromonas hydrophila: 93%

Aeromonas salmonicida: 93%

Aeromonas vivipollenous: 92.98%

Aeromonas lacus: 91.44%

Our **comamonas** isolate

Comamonas aquatilis: 96.69%

Comamonas testosterone: 99.93%

Comamonas sediminis: 96.82%

% match of our isolate's 16s rRNA gene sequence to known species' 16s rRNA gene sequence

Our **acinetobacter** isolate

Acinetobacter baumani: 97.35%

Acinetobacter calcaceticus: 96.33%

Acinetobacter viviani: 97%

Our **bacillus** isolate

Bacillus alkalitolerans: 93.16%

Bacillus fermenti: 93.43%

Bacillus subtilis: 99.1%

Bacillus cereus: 93.85%

Future Directions

- The next step in this research is to sequence the DNA on the Illumina sequencer. This will provide us with the more accurate short read DNA sequences to improve the genome assembly
- Once the assemblies are polished, we will annotate the genomes and identify the bacteria on a species level

Acknowledgements

I would like to thank Dr. Paul Orwin for mentoring me in this research project and Chris NeVille for his help with analyzing the sequence data and assembling the genomes.