

Introduction

Gut microbes play an important role in metabolism. *Kyphosus sydneyanus* is an herbivorous fish native to Australasia that consumes brown seaweed including the invasive Undaria pinnatifida. Using bacteria isolated from the hindgut of K. sydneyanus, we aim to understand the role of novel bacteria in the digestion of seaweed in the fish diet. Seaweed contain carbohydrates, such as carrageenan, beta-galactan, and cellulose, which are broken down by carbohydrate-active enzymes (CAZymes) produced by gut bacteria. Determining which CAZymes the novel isolates encode for will lead to a greater understanding of carbohydrate breakdown and the role of bacteria in the digestion of seaweed in the fish diet. Seaweed contains unique carbohydrates absent in terrestrial plants, so we are interested in identifying which CAZymes are encoded by our isolates compared to isolates from terrestrial organisms.



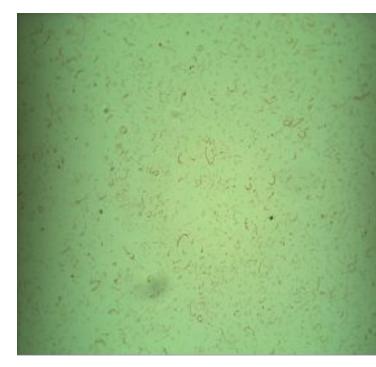


Figure 1. *Kyphosus sydneyanus* (left), Microscopy image of Isolate (right) Images from *Fishes of Australia* (left) and Bikiran Pardesi (right).

Methods

Genome Sequencing Reads were sequenced using Illumina and Nanopore technology

Genome Assembly Genome assemblies created using MaSuRCA and UniCycler

Genome Annotation

Annotations were generated using dbCAN4 and Prokka

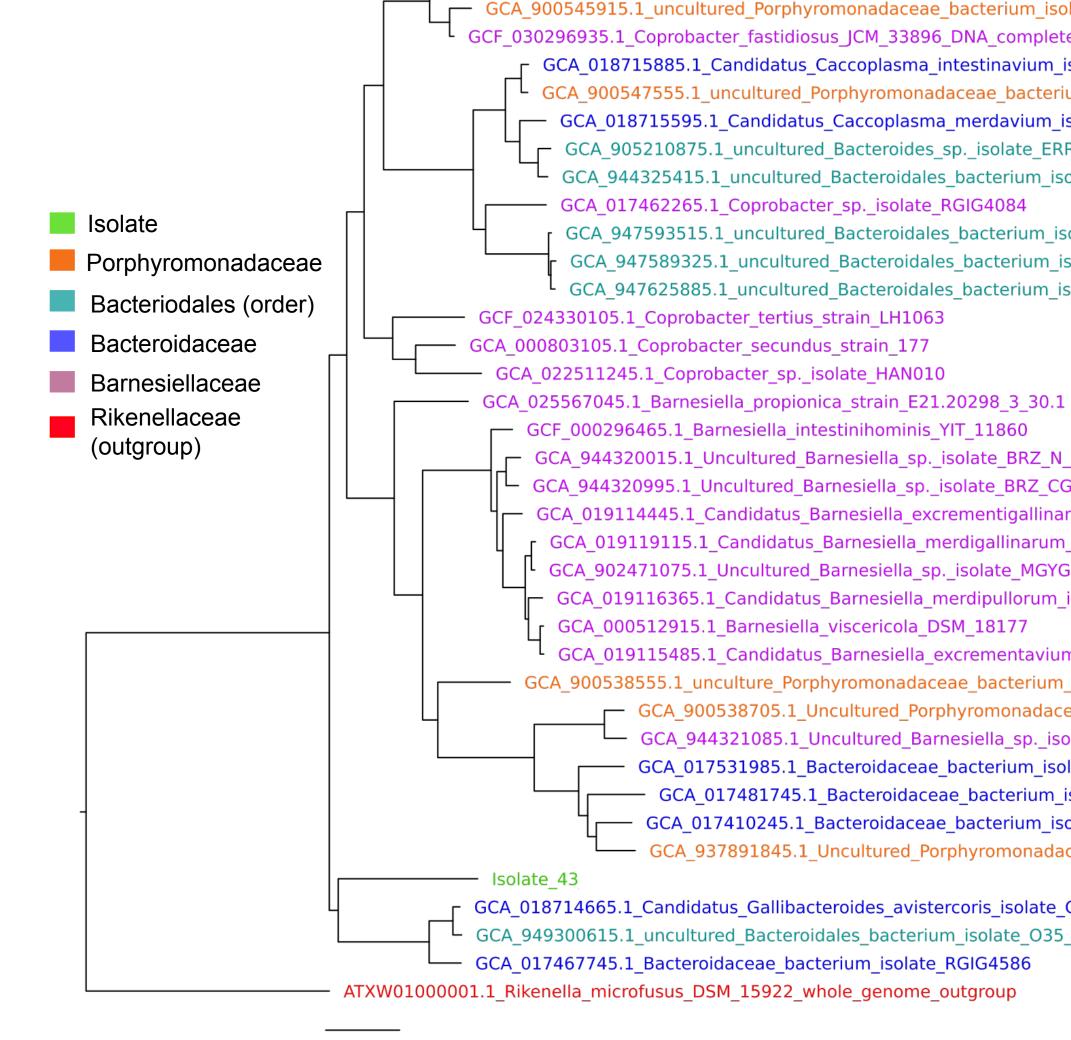
Analysis

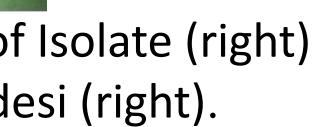
Phylogenetic and genome analysis to identify CAZymes and substrates

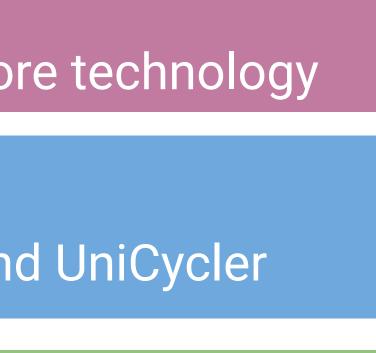
Table 1. Genome features from final assemblies of bacterial Is

	Contigs	Completeness	Contamination	Genes
Isolate 8	76	98.49%	0.5%	3,365
Isolate 43	13	98.68%	0.13%	3,009

Genome Analysis of a Novel Genus of Bacteria Grace Krueger, Damanpreet Khaira, Dr. Emily Wollmuth Department of Biology, Carthage College







sola	tes	8	and 43	
n			Gonos	

Figure 3. Phylogenetic tree displaying the closest related organisms (Bacteroidales order) to the novel bacterial isolates, using NCBI and GTDB databases.



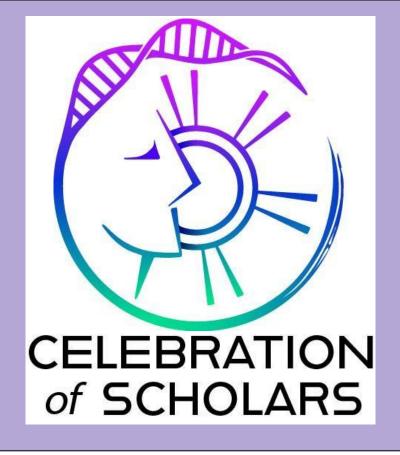
Results

- GCA_949297285.1_uncultured_Bacteroidales_bacterium_isolate_CO.094.metabat.bins.172 GCA 900545915.1 uncultured_Porphyromonadaceae_bacterium_isolate_UMGS835 GCF_030296935.1_Coprobacter_fastidiosus_JCM_33896_DNA_complete_genome GCA_018715885.1_Candidatus_Caccoplasma_intestinavium_isolate_21143 ¹ GCA 900547555.1 uncultured_Porphyromonadaceae_bacterium_isolate_UMGS999 - GCA_018715595.1_Candidatus_Caccoplasma_merdavium_isolate_6821 GCA 905210875.1_uncultured_Bacteroides_sp._isolate_ERR321649_bin.9 GCA 944325415.1_uncultured_Bacteroidales_bacterium_isolate_BRZ_AF_bin46 ----- GCA_017462265.1_Coprobacter_sp._isolate_RGIG4084 GCA 947593515.1 uncultured_Bacteroidales_bacterium_isolate_Lapin005_bin58 GCA_947589325.1_uncultured_Bacteroidales_bacterium_isolate_Lapin001_bin46 GCA_947625885.1_uncultured_Bacteroidales_bacterium_isolate_Lapin011_bin66 GCF 000296465.1 Barnesiella intestinihominis YIT 11860 - GCA 944320015.1 Uncultured Barnesiella_sp._isolate_BRZ_N_bin151 ndidatus Barnesiella excrementigallinarum isolate CHK169-14362 GCA_019119115.1_Candidatus_Barnesiella_merdigallinarum_isolate_CHK136-6590 GCA_902471075.1_Uncultured_Barnesiella_sp._isolate_MGYG-HGUT-01604 GCA 019116365.1_Candidatus_Barnesiella_merdipullorum_isolate_5648 GCA 000512915.1 Barnesiella viscericola DSM 18177 GCA 019115485.1 Candidatus Barnesiella excrementavium isolate 4398 05.1 Uncultured Porphyromonadaceae bacterium isolate UMGS48 GCA_944321085.1_Uncultured_Barnesiella_sp._isolate_BRZ_AF__bin112 GCA 017531985.1 Bacteroidaceae_bacterium_isolate_RGIG6483 GCA_017481745.1_Bacteroidaceae_bacterium_isolate_RGIG4690 — GCA 017410245.1 Bacteroidaceae bacterium isolate RGIG2076 GCA 937891845.1 Uncultured Porphyromonadaceae bacterium isolate SRR6425810
- GCA_018714665.1_Candidatus_Gallibacteroides_avistercoris_isolate_CHK158-818 GCA_949300615.1_uncultured_Bacteroidales_bacterium_isolate_O35_L1_319319_idba_bin.174

- organisms ranged from 52.92-61.39%.
- seaweed and other plants.

Genome	Glycoside Hydrolases	Glycosyl- Transferases	Polysaccharide Lyases	Carbohydrate Esterases
Isolate 43	61	38	9	6
GCA_017467745.1	82	26	0	7
GCA_018714665.1	99	21	0	6
GCA_949300615.1	104	24	0	7

We would like to acknowledge the SURE program at Carthage College for funding our research and for funding of the larger project, the New Zealand Ministry of Business, Innovation, & Employment.



Results

 \rightarrow The isolates are on their own branch in the phylogenetic tree (Figure 3).

→ Running BLAST on 16S rRNA sequences indicated the possibility of a novel family of bacteria. The average taxonomic cutoff for individuals to be in the same family is a percent identity of 90% for the 16S rRNA gene. The top BLAST hit for both isolates was *Coprobacter fastidiosus*, with an 89.10% identity.

→ Sequencing data for Isolate 8 contained fungal genes, indicating fungal contamination. No further research was done on Isolate 8.

→ Average Amino Acid Identity (AAI) values were calculated, confirming that the Isolate 43 belongs to a novel genus. The established threshold for organisms to be in the same genus is an AAI value ≥65%. Values from Isolate 43 compared to other

→ CAZyme encoding genes were identified for Isolate 43, which are potentially involved in breaking down the seaweed. These CAZymes target substrates such as carrageenan, beta-galactan, and cellulose, which are found in the cell walls of

→ Isolate 43 is the only organism in its clade with a class of CAZymes called polysaccharide lyases, which break down sulfated carbohydrates. An example of this is heparin, which is structurally similar to fucoidan, common in brown seaweed. Sulfated carbohydrates, like fucoidan, are found in the cell walls of brown seaweed, but typically not in terrestrial plants.

Table 2. Counts of CAZyme-encoding genes in Isolate 43 and related organisms

Future Directions

Our next steps include comparative genomics and doing further analysis of the novel bacterium in comparison to the most closely related organisms. Understanding these mechanisms can have important industrial applications, allowing researchers as part of a larger collaborative project to create natural fertilizers, products for animal feeds, and vitamin supplements without the need for the host fish.

Acknowledgements