

Introduction

Isolate 38 is a novel bacterium isolated from the hindgut of a herbivorous fish called *Kyphosus sydneyanus*, which is found in rocky reefs in Australasia. The entire genome of Isolate 38 was sequenced for genomic analysis. Hindgut microbial communities are especially important in herbivorous hosts as they can digest plant components that are resistant to the host's enzymes.² Diverse carbohydrate-active enzymes (CAZymes) allow microbes to break down complex carbohydrates in their environment³. *Kyphosus sydneyanus* has been found to only produce amylases, which break down starch, leaving many polysaccharides intact as they enter the hindgut. It is there that microbial fermentation, mainly by phyla *Bacteroidota* and *Bacillota*, continues the metabolic pathway. ⁴ The goal of this study is to place Isolate 38 within a genus and determine how it's metabolically unique in its use of CAZymes.



Methods

Bioinformatics

Software called dbCAN was run on Isolate 38 and 21 other Rikenellaceae reference genomes from NCBI. Using R Studio 2023.12.1+402 and Python a heatmap, presence absence plot, and counts table were produced.

Identifying a more comprehensive dataset

GTDB-Tk (Genome Taxonomy Database) software was run to produce a phylogenetic tree with Isolate 38 and the Rikenellaceae family which included 81 genomes. The clade including Isolate 38 and 46 other genomes was combined with the NCBI reference genomes and run to produce a more comprehensive phylogenetic tree. Figure 2 shows one clade from that tree. Using the NCBI database the species and sample information were recorded.

CAZyme presence in novel hindgut bacterium *Rikenellaceae* Isolate 38 Lin Sensenbrenner **Advisor: Emily Wollmuth, Ph.D.** Department of Biology, Carthage College



Legend: Alistipes Tidjanibacter Unspecified Rikenellaceae Our Isolate

Bacterial Genomes were sourced from humans (34%), non human mammals (43%), birds (11%), and termites $(9\%)^6$.



Figure 2. A phylogenetic tree showing a subset of the comprehensive dataset

Figure 1. Pilot CAZyme Presence Absence from reference *Rikenellaceae* genomes

1v1 genomic
05.1 chick38 bin11 genomic
245 1 ASM1911624v1 genomic
525 1 020 14 316316 idba bin 58 genomic
260851 BRZ ME bin86 genomic
575.1 Δ SM78657v1 genomic
605 1 PR JEB16355 denomic
385.1_ASM1793538v1_genomic
1375 T ASM1763137vT genomic
17628675 1 ASM1762867v1 genomic
7936605.1 ASM1793660v1 genomic
0174663451 ASM1746634v1 genomic
017648105 T ASM1764810vT genomic
176301151 ASM1763011v1 genomic
D17390585.1 ASM1739058v1 denomic
_017628305_1_ASM1762830v1_denomic
5059945 1 ASM1505994v1 genomic
17396085 1_ASM1739608v1_genomic
7547065 1 ASM1754706v1 denomic
2293665 1 ASM229366v1 denomic
SCA 0022934251 ASM229342v1 genomic
A 002293505 1 ASM229350v1 genomic
002293535 1 ASM229353v1 genomic
5.1 ASM2200991v1 genomic
5060105.1 ASM1506010v1 genomic
017464745 1 ASM1746474v1 genomic
017936385.1 ASM1793638v1 genomic
SM1793562v1 genomic
5.1 ASM1742565v1 genomic
95.1 ASM1737989v1 genomic
5.1 SRS476346 3 genomic
likenellaceae bacterium 38
54465.1 ASM15446v1 genomic
106542665.1 Acom 1.0 genomic
3193205.1 Alistipes sp nov DSM112343 genomic
10095975.1 ASM4009597v1 genomic
0624755.1_81E7_genomic
100083545.T PRJEB13794 genomic
00107675.1_TMG-taxon_2693429854_annotated_assembly_genomic
018362775.1_Alistipes_kh20_genomic
25145845.1_ASM2514584v1_genomic
_900604385.1_PRJEB28786_genomic
006542685.1_Adis_1.0_genomic
6542645.1 Aond 1.0 genomic
16//115.1_ASM2/6//11V1_genomic
ASM2085987V1_genomic
STERRIISSSS DINS METAVVRAP VIJ MAG denomic
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Conclusions

- GH107
- GH150 codes for 1-Carrageenase uses H₂O to break Carrageenan backbone⁵
- GH38 participates in the breakdown of *N*-glycans
- GH107 hydrolyses $(1 \rightarrow 4)$ - α -L-fucoside linkages in fucan. Fucans are primarily found in seaweed⁵

Tidjanibacter⁵

Next Steps

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- 1.Pardesi, B., Roberton, A. M., Wollmuth, E. M., Angert, E. R., Rosendale, D. I., White, W. L., & Clements, K. D. (2023). Chakrabartyella piscis gen. nov., sp. nov., a member of the family Lachnospiraceae, isolated from the hindgut of the marine herbivorous fish Kyphosus sydneyanus. International journal of systematic and evolutionary microbiology, 73(10), 006100. 2.Moran, D., & Clements, K. D. (2002). Diet and endogenous carbohydrases in the temperate marine herbivorous fish Kyphosus sydneyanus. Journal of Fish Biology, 60(5), 1190-1203.

- 5.Elodie Drula, Marie-Line Garron, Suzan Dogan, Vincent Lombard, Bernard Henrissat, Nicolas Terrapon (2022) The carbohydrate-active enzyme database : functions and literature Nucleic Acids Res 50 : D571–D577.



Isolate 38 has three unique CAZymes including GH150, GH38 and

- Carrageenan and fucan can both be found in seaweeds in the *Kyphosus* sydneyanus diet.⁵
- Isolate 38 is most closely related to the genera *Alistipes* and

• Run POCP (Percentage of Conserved Protein) and AAI (Amino Acid Identity) software to confirm placement within a genus and identify genomic similarity between Isolate 38 and its closest relatives • Rerun dbCAN on genomes within that genus to evaluate the CAZyme presence of Isolate 38 compared to most related genomes.

- 3.Wardman, J. F., Bains, R. K., Rahfeld, P., & Withers, S. G. (2022). Carbohydrate-active enzymes (CAZymes) in the gut microbiome. Nature Reviews Microbiology, 20(9), 542-556.
- 4.Facimoto, C. T., Clements, K. D., White, W. L., & Handley, K. M. (2024). Bacteroidia and Clostridia are equipped to degrade a cascade of polysaccharides along the hindgut of the herbivorous fish Kyphosus sydneyanus. *ISME communications*, 4(1), ycae102.
- 6.National Center for Biotechnology Information (NCBI)[Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2025 Apr 09]. Available from: https://www.ncbi.nlm.nih.gov/