CAZyme presence in novel hindgut bacterium *Rikenellaceae* Isolate 38

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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 environment [3]. *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

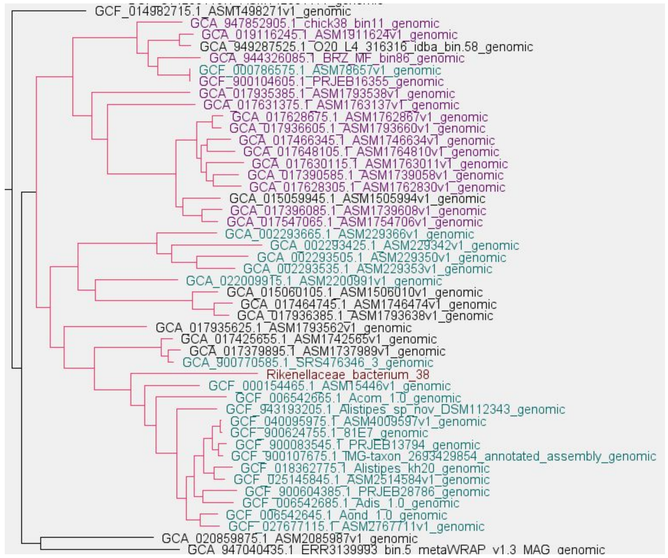
Software called dbCAN was run on Isolate 38 and 21 other Rikenellaceae reference genomes from NCBI. Using R Studio 2023.12.1+402 and Python a heatmap, presence absence plot, and counts table were produced.

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.

Results and Discussion

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



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

Conclusions

Isolate 38 has three unique CAZymes including GH150, GH38 and GH107

- GH150 codes for 1-Carrageenase uses H_2O to break Carrageenan backbone[5]
- GH38 participates in the breakdown of *N*-glycans
- GH107 hydrolyses (1→4)-*α*-L-fucoside linkages in fucan. Fucans are primarily found in seaweed[5]

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 *Tidjanibacter*[5]

Next Steps

- 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.

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References

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.
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.
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.
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/