Structural and functional characterization of human gut microbial sialidases

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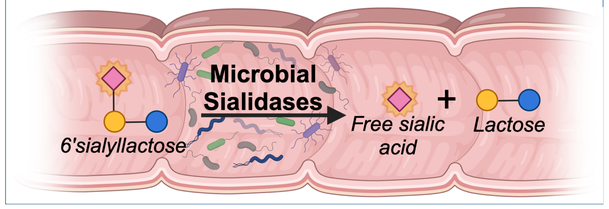
Background

The gut microbiome plays an important role in human health, and its development begins at birth. One factor contributing to infant gut microbiome development is feeding method[1]. Breastfed and formula fed infants have distinct microbiome compositions, but the reason for these differences is not well-understood[2]. Interestingly, sialylated human milk oligosaccharides (HMOs) are found in significantly higher concentrations in breastmilk than formula. These molecules remain undigested by the infant until reaching the gut microbiome, where the sialic acid can be cleaved from the oligosaccharide by microbial sialidases[3]. Here, we investigate the diversity of microbial sialidases and characterize their interactions with sialylated HMOs to better understand the role that these enzymes play in early gut microbiome colonization.



Results

Identifying microbial sialidases

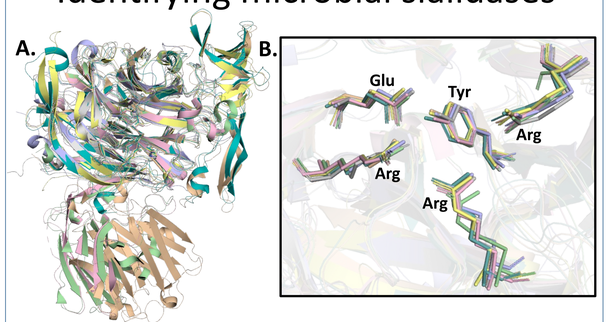


Figure 1: Structure-based rubric for GH33 microbial sialidases. GH33 sialidases were defined as any structure having at least 25% sequence similarity to one of the solved sialidase structures (A) and containing all five conserved active site residues (B). PDB: 4BBW, 5F9T, 2BF6, 6MNJ, 1EUR, 7LBU, 4X47.

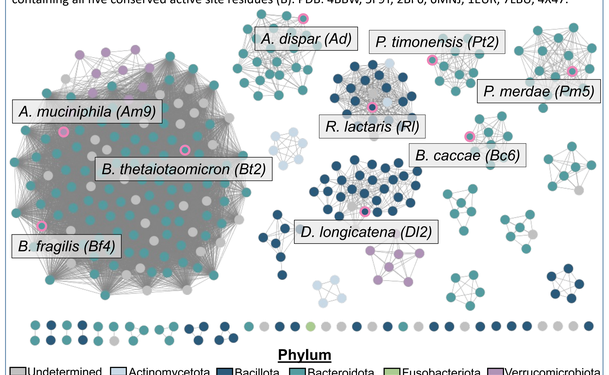


Figure 2: Sequence similarity network of potential microbial GH33 sialidases. The Integrated Gene Catalogue was mined using the structure-based sialidase rubric, identifying 290 unique microbial sialidase sequences. These sequences were organized using EFI-EST to create a sequence similarity network from which nine sequences were chosen for recombinant protein expression and purification.

Sialidase activity characterization

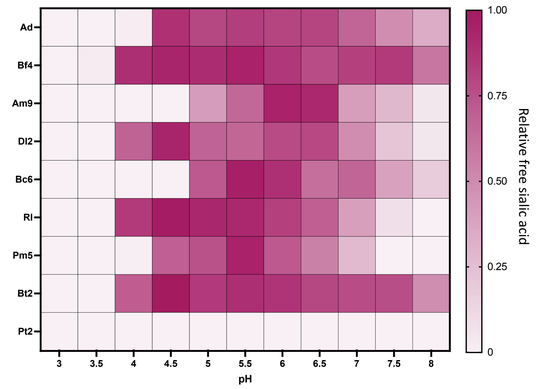


Figure 3: pH screen of purified GH33 sialidases. Activity of purified sialidases was assessed 4MU-Neu5Ac by measuring produced free sialic acid after 25 minutes. All functional enzymes were active at the biological pH of 6.5. N=3 biological replicates.

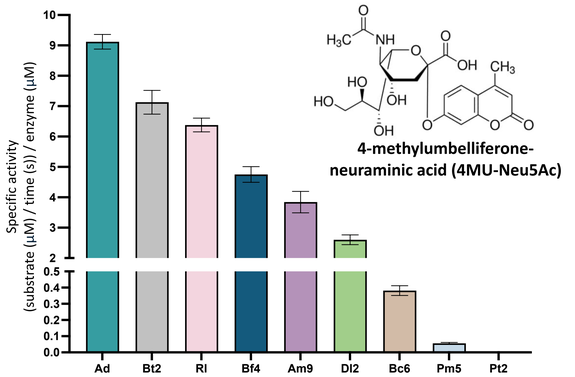


Figure 4: Specific activity of purified sialidases with 4MU-Neu5Ac. Purified sialidases were confirmed to be active using the reporter substrate 4MU-Neu5Ac, which fluoresces after the cleavage of sialic acid. Diversity was observed in activity, generally corresponding to clustering in the SSN. N=3 biological replicates, error bars represent SEM.

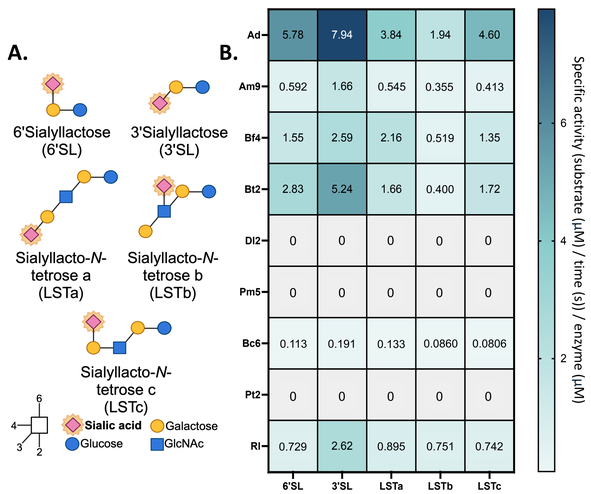


Figure 5: Specific activity of purified GH33 sialidases with monosialylated HMOs. Five monosialylated HMOs found in breastmilk (A) were assayed with all nine purified sialidases (B). Slight preferences for 3' linkages were observed, as well as diversity in overall processing abilities. N=3 biological replicates.

Novel sialidase structure analysis

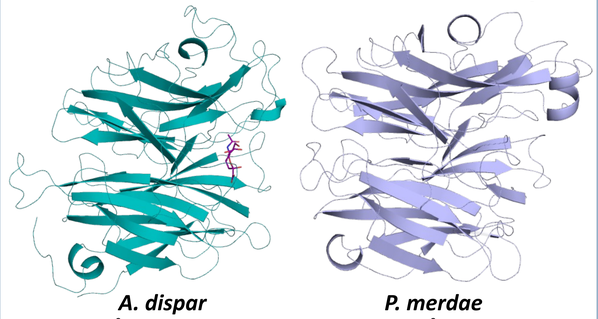


Figure 6: Novel structures of microbial sialidases. Structures of the characterized proteins from *A. dispar* and *P. merdae* were solved using X-ray crystallography.

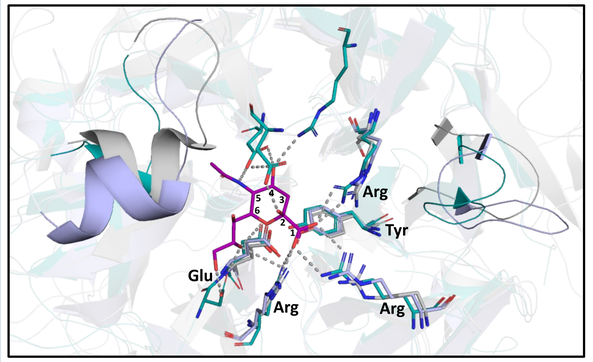


Figure 7: Active site comparison of sialidase structures. Comparisons of the active sites of *A. dispar*, *P. merdae*, and *B. thetaiotaomicron* (4BBW) sialidases reveal conservation of the catalytic residues used to create the sialidase rubric. Sialic acid bound to *A. dispar* sialidase makes several polar contacts to active site residues. Because of the conserved nature of the catalytic residues, two loops adjacent to the active site were analyzed for structural differences.

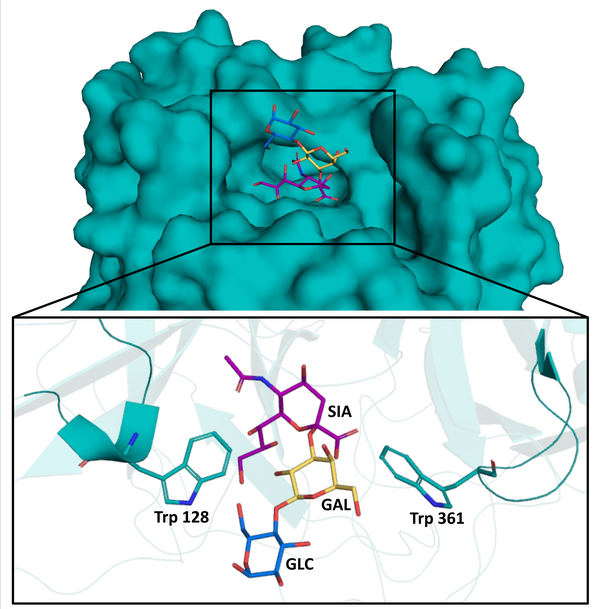


Figure 8: Modeling of 3'SL in novel *A. dispar* sialidase structure. The structure of 3'SL bound to a sialidase was pulled from PDB: 1SOI and overlayed with the location of free sialic acid in the structure of *A. dispar* sialidase. This revealed two unique tryptophan residues on the variable loops near the active site that potentially influence substrate binding.

Anaerobic culturing with HMOs

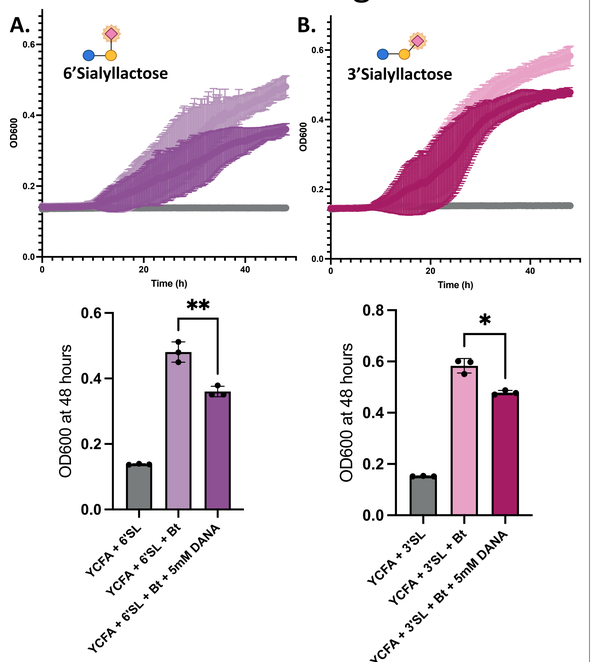


Figure 9: Anaerobic culturing of *B. thetaiotaomicron* with sialylated HMOs. The commensal gut microbe *B. thetaiotaomicron* (ATCC 29148) was grown with 6'SL (A) and 3'SL (B) as the sole carbon source. *B. thetaiotaomicron* was able to grow with both of these HMOs, but growth was decreased in the presence of the sialidase inhibitor 2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid (DANA). N=3 biological replicates, error bars represent standard deviation. Statistics performed by unpaired *t*-test with Welch's correction, *p<0.05, **p<0.01.

Future directions

- Modeling with 6'SL
- Mutagenesis of unique tryptophan residues in *A. dispar* sialidase
- Bioinformatic analysis of infant metagenomics
- Additional expression and purification of sialidases from the infant microbiome

Acknowledgements

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References

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3. Bell, A.; Severi, E.; Owen, C. D.; Latousakis, D.; Juge, N. Biochemical and Structural Basis of Sialic Acid Utilization by Gut Microbes. *J. Biol. Chem.* **2023**, *299* (3), 102989.