Novel Synthetic Glycan for Targeted Microbiome Modulation

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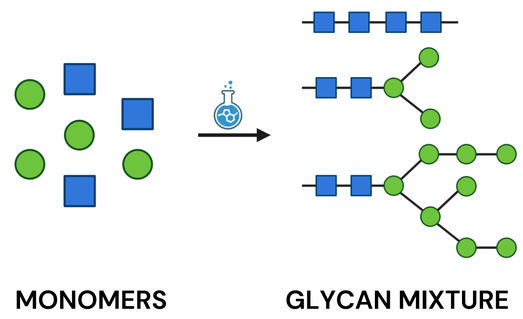
Introduction

Due to increasing evidence suggesting that gut-resident microbes can influence brain function through the production of neuroactive compounds such as gamma-aminobutyric acid (GABA), which plays a critical role in neural regulation and has been linked to a range of neurological and physiological conditions[1], we investigated the use of prebiotic glycans for targeted modulation of the gut microbiome to confer health benefits upon the host. We synthesized and identified a novel dietary glycan, SG-Glc, which significantly boosted GABA levels *ex vivo* by increasing *Bifidobacterium adolescentis* abundance. Further analysis revealed that *B. adolescentis* possesses a unique genetic profile enabling it to utilize SG-Glc for growth while producing GABA.



Methodology

Glycan Synthesis: Glycans were synthesized by heating and mixing monomer(s), catalyst, and DI water in a vial for a set time before quenching.

***Ex vivo* Fermentations:** Stool samples from 3 North American donors were incubated anaerobically at 37°C for 48 hours in a BioLector XT with a minimal media plus glycan or control. Catalyst and monomer were tested in the control groups.

GABA Quantification: GABA was quantified against a standard using uHPLC gradient connected in-line to a high-resolution MS.

Microbiome Profiling: Pelleted fermentation broth was sequenced using an Oxford Nanopore GridION. Microbial abundances were inferred using Kraken2[2] and Bracken[3] with a custom-made human gut bacterial genome catalogue.

Modeling: OLS linear models and correlation analyses were established using species abundances and GABA concentrations.

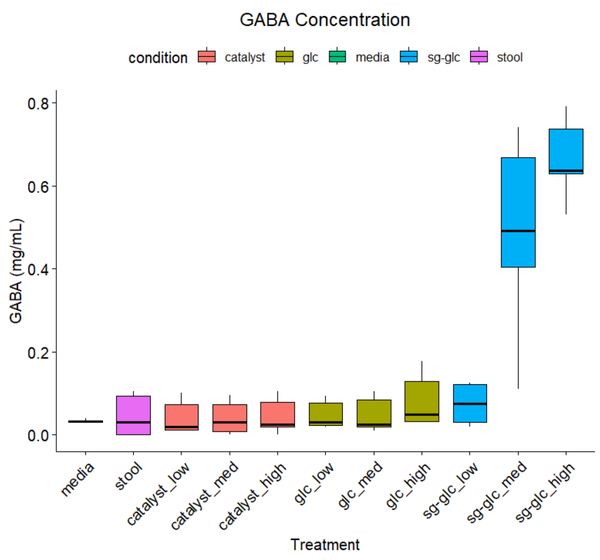
Pangenomics: Phylogenetic reconstruction rooted between *B. bifidum* and *B. breve* based on Bifidobacterium phylogeny was performed to explore the phylogenomic context of GABA-producing genes.

Key Results

1. Synthesized Glycan SG-Glc Increases GABA Production

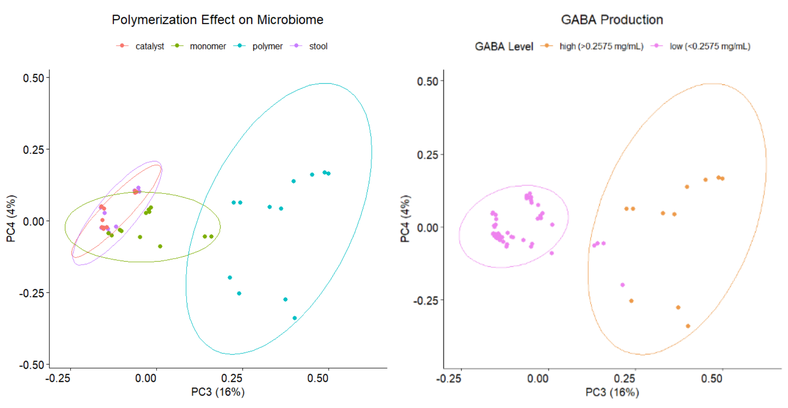
We synthesized >100 glycans with various combinations of monomers and catalysts. We then screened the glycans in an *ex vivo* stool fermentation model. Glycans were ranked by GABA concentration in the endpoint fermentation broth.

We identified glycan SG-Glc as the lead candidate. Dose-dependent increases in GABA production were observed alongside increasing concentrations of SG-Glc, while individual components used to synthesize SG-Glc did not exhibit the same effect.



2. SG-Glc Exhibits Targeted Modulation of the Gut Microbiome

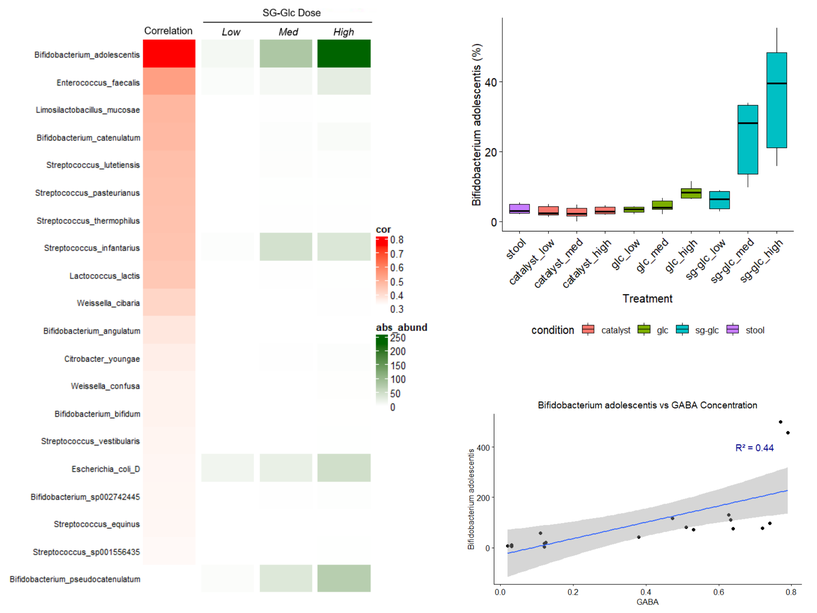
PCoA analysis of species-level microbiome profiles suggests that SG-Glc precisely modulates the microbiome toward a GABA-producing state. Polymerization and polymer components drive the separation of samples along PC3, along which high and low concentrations of GABA clearly segregate. This indicates a strong association between glycan structure and GABA production.



3. GABA Producing Strain Dominates Treated Communities

Bifidobacterium adolescentis exhibited the highest correlation with GABA amount and became the dominant species by relative abundance in medium and high concentration conditions of SG-Glc.

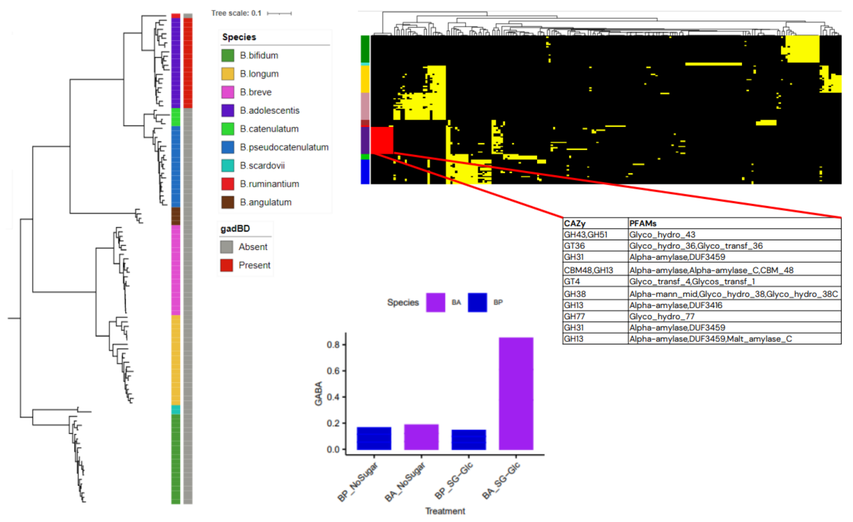
The dose-dependent impact of SG-Glc on *B. adolescentis* was seen with increasing glycan concentration.



4. *B. adolescentis* Uniquely Utilizes SG-Glc to Produce GABA

Among the species of Bifidobacterium present in the donor slurry, only *B. adolescentis* was found to have gadBD genes, which are responsible for GABA production.

We found that *B. adolescentis* contains a unique block of 10 enzymes (red) for SG-Glc-like polymer utilization.



CAZy	PFAMs
GH43,GH51	Glyco_hydro_43
GT36	Glyco_hydro_36,Glyco_transf_36
GH31	Alpha-amylase,DUF3459
CBM48,GH13	Alpha-amylase,Alpha-amylase_C,CBM_48
GT4	Glyco_transf_4,Glycos_transf_1
GH38	Alpha-mann_mid,Glyco_hydro_38,Glyco_hydro_38C
GH13	Alpha-amylase,DUF3416
GH77	Glyco_hydro_77
GH31	Alpha-amylase,DUF3459
GH13	Alpha-amylase,DUF3459,Malt_amylase_C

Conclusion

Our findings demonstrate the potential for synthetic glycans such as SG-Glc to modulate the gut microbiome and improve host health by targeting the gut-brain axis. SG-Glc and other targeted microbiome modulators could present novel ingredients for therapeutic or non-pharmacological intervention purposes, such as improving overall brain function, mental health, and physiological well-being.

References: 1) Nuss 2015 *Neuropsych Dis Treat, 11:165–175* 2) Wood *et al* 2014 *Genome Biol 15, R46* 3) Lu *et al* 2017 *PeerJ Computer Science 3:e104* 4) Mazzoli & Pessione 2016 *Front. Microbiol. Vol 7.*