SELECTED TALK:

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Identifying glycan consumers in human gut microbiota using metabolic labeling coupled to fluorescence-activated cell sorting

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Complex glycans derived from our diet are a major driver of the composition and metabolism of the human gut microbiota, with downstream effects on host physiology and health. In order to advance our understanding of glycan metabolism by human gut bacteria, we need methods to link bacterial taxa and carbohydrate-active enzymes (CAZymes) to their glycan substrates. We used a functional method to identify gut bacteria that uptake specific glycan structures in stool samples. The method combines *ex-vivo* metabolic labelling using fluorescently labeled oligosaccharides with fluorescence-activated cell sorting (FACS) and amplicon sequencing. Three healthy human volunteers were interrogated with three different glycan structures. Abundance analysis of the exact sequence variants (ESVs) identified using the ANCHOR pipeline highlighted the ability of the Bacteroidetes phylum to consume a wider variety of glycans than Firmicutes. We observed metabolic labeling in various taxa such as *Collinsella aerofaciens* (Actinobacteria) and *Blautia wexlerae* (Firmicutes), that was supported by CAZymes in their genomes consistent with the glycans. Moreover, we demonstrated that sorted metabolically labeled cells could be cultured to isolate glycan consumers from a human gut microbiota sample. Cultures of galactomannopentaose-positive cells led to the isolation of a *Bacteroides xylanisolvens* strain capable of growing on this substrate as a sole carbon source. Similarly, we could isolate *Bacteriodes uniformis* and *Bifidobacterium angulatum* strains growing on fructo-oligosaccharides.

Furthermore, we used this method to explore the metabolism of maltodextrin by gut bacteria and the impact of amylase inhibitors. The clinically used human α-amylase inhibitor acarbose is used to treat type 2 diabetes and is known to inhibit the growth of some bacteria on starches. In contrast, montbretin A has been reported to be more specific for human α-amylase. Yet, the extent of both molecules on glycan metabolism remains to be fully appreciated. Here we used *ex-vivo* metabolic labeling of gut bacteria with fluorescent maltodextrin in the presence or absence of the amylase inhibitors. FACS and amplicon sequencing led to the identification of new species that are inhibited by acarbose. In contrast our results support the fact that montbretin A is more selective and does not significantly impact metabolic labeling in a microbiota sample.

Metabolic labeling is a valuable tool to characterize glycan metabolism in gut bacteria and could help understand the impact of drugs on the human gut microbiota composition. The method also has the potential to provide a mechanistic understanding of prebiotic glycans by identifying and isolating specific gut microbiota they support in patient-derived stool samples.