**Metabolic Labeling of glycans on common gut bacteria**

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The surface of bacteria is comprised of complex structures made up of monosaccharides termed glycans. Glycans play a crucial role in the survival of bacteria because they are closely linked to the bacteria's functions, communication, and overall fitness[1,2]. While many efforts have been dedicated to studying the glycan structures of pathogenic bacteria, the same cannot be said for symbiotic bacteria, which have been relatively neglected.

Symbiotic bacteria found within the human gut are composed of more than 3.8 * 10 species[3] and are directly related to human gut health and disease. Studies have shown that glycans on symbiotic bacteria play a major role in facilitating communication between gut bacteria and host cells[4], improving the immune system's ability to detect pathogenic bacteria[5], and reducing the pathogenicity of harmful bacteria[6]. The ability of common gut bacteria to uptake and breakdown polysaccharides and oligosaccharides also benefits the gut community by providing a source of nutrition[7]. The importance of common gut bacteria's glycans necessitates the understanding of these glycan structures and their connection to the bacteria's functions.

However, the complex structure of bacterial glycans, which consists of over 700 different monosaccharide building blocks[8], makes them challenging to study. A technique known as "Metabolic Oligosaccharide Engineering" (MOE), pioneered by Bertozzi[9,10], Reutter[11,12], and colleagues, is an effective chemical tool for addressing the difficulty in studying bacterial glycans. MOE uses azide-containing sugar analogs to incorporate into the bacteria's cellular glycans. Once incorporated, the azide acts as a chemical handle and can be tagged with a reactive partner. This method allows for a cellular readout of glycan biosynthesis, identifying glycans and even helping produce ways of inhibiting glycan biosynthesis. The information obtained from MOE is valuable for characterizing bacterial glycans, an area of need in common gut bacteria.

Earlier, aided by MOE, the Dube lab made a discovery, finding that *B. thetaiotaomicron* effectively integrated GlcNAz and GalNAz into their glycocalyx. However, the specific class of glycans into which the sugar analogs were incorporated remained ambiguous. This summer, with the assistance of a proteinase K assay and capsular polysaccharide (CPS) isolation, I successfully determined that GlcNAz and GalNAz were incorporated into the CPS of *B. thetaiotaomicron.* This piece of information opens the door to studying CPS's role in functionality and fitness of the bacteria. My next step is to inhibit the glycosylation of *B. thetaiotaomicron'*s CPS and probe for its functions.

To achieve this goal, I utilized small molecule inhibitors: Benzyl-α-GlcNAc and Benzyl-α-GalNAc, which mimic the structure of GlcNAc and GalNAc. I hypothesized that these small molecule inhibitors would inhibit the glycosylation of the bacteria's CPS. To test the hypothesis, western blot analysis was performed on *B. thetaiotaomicron* that was metabolically labeled with GlcNAz and treated with or without the inhibitors. Contrary to the hypothesis, the western blot analysis showed no significant decrease in CPS glycosylation when *B. thetaiotaomicron* was treated with the small molecule inhibitors.

Though the result was contrary to the hypothesis, it was not unexpected due to the limited knowledge of the chemical structure of *B. thetaiotaomicron*'s CPS. In the upcoming fall, I plan to continue this research by exploring additional small molecule inhibitors and testing their inhibitory effects on the glycosylation of *B. thetaiotaomicron*'s CPS. If any of the small molecules exhibit noticeable inhibition, I intend to study the downstream effects of the inhibitor on the bacteria's fitness and functions. Ultimately, this will provide valuable information about the role of CPS in the bacteria's survivability.

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