Metabolic glycan labeling of bacterial glycans using rare azide-containing L-sugars

Phuong Luong, 2022

The rapid rise of antibiotic resistance demonstrates the ineffectiveness of current antibiotics. As a result, there is a need for novel molecular targets to develop new antibiotics. Glycans, high-ordered molecules made of sugars that coat the cell surface, are compelling therapeutic targets. Particularly, bacterial glycans contain extremely rare monosaccharides absent from mammalian glycans and are linked to pathogenesis.[1] These characteristics suggest bacterial glycans can be harnessed to develop selective therapeutics without harming host cells.

However, bacterial glycans are difficult to study due to their complex structures. To accelerate their analysis, Bertozzi and colleagues pioneered metabolic oligosaccharide engineering (MOE), a method to probe glycans without full structural information.[2] In brief, MOE consists of supplying bacteria with unnatural, azide-containing sugars, which are incorporated into endogenous glycans and undergo selective chemical reactions to produce detectable, azide-dependent signals. From conducting MOE using rare D-sugar analogs on a few bacterial species, the Dube group discovered that bacteria incorporated rare sugars in a species-selective manner.[3] Because bacterial glycans incorporate a wide range of rare sugars including L-sugars,[4] expanding the panel of rare sugar analogs is requisite to studying glycans in more species.

This research profiles the incorporation of newly developed L-sugar analogs into various bacterial species. These species include the symbiotic *Bacteroides fragilis* and the pathogenic bacteria *Campylobacter jejuni*, *Plesiomonas shigelloides*, and *Vibrio vulnificus*. First, bacteria were labeled with a panel of L-sugars and an azide-free sugar (Ac) as the negative control. One D-sugar was included as a positive control for species with known incorporation (*C. jejuni*).[3] Then, labeled bacteria were screened for incorporation of sugar analogs into surface glycans via flow cytometry and cellular glycans via Western blots. Consistent results were observed in both flow cytometry and Western blots: *B. fragilis*, *C. jejuni*, and *V. vulnificus* showed no appreciable incorporation of L-sugars, while *P. shigelloides* showed slight incorporation of L-PneuNAz (Figure 1). These findings reinforce the observation that bacterial species incorporate rare sugar analogs, L-sugars in this case, in a species-selective manner. This trend sets the stage for the development of glycan-based therapeutics that potentially target pathogenic bacteria with selectivity.

Faculty Mentor: Danielle H. Dube