Developing Fluorescence Assays to Detect and Identify Glycan-Degrading Enzymes

Esteban Tarazona Guzman, Class of 2025

The human body hosts trillions of microorganisms; and of these, bacteria are the most prominent. The relationship between humans and bacteria is multifaceted, playing crucial roles in both health and disease. Within the gut, *Bacteroides fragilis* is a prime example of this complex interplay. On one hand, it plays a beneficial role in digestion by aiding to metabolize carbohydrates. However, if *B. fragilis* escapes the gut and infiltrates other parts of the body, it can have adverse and morbid effects.[1] Glycans, chains of monosaccharides that coat bacterial cells, are crucial for bacterial fitness as well as interactions with its surroundings. For example, the adhesion of *B. fragilis* to gut epithelial cells is facilitated by glycans. Without these glycans, the bacteria would not be able to coexist within its human host. These glycan chains are regulated by glycosidase enzymes which aid in glycan tailoring, degradation, recycling, and biosynthesis.[2] Despite their crucial role in maintaining bacterial glycans, glycosidases remain understudied due to their wide variety within bacteria. Selectively identifying and comparing glycosidase activity across a range of bacteria will give further insight into how glycans are modified and, more broadly, its implications on human health.

During the first half of the summer, I familiarized myself with essential laboratory techniques such as cell culture, lysing cells, and stock solution preparation. I then applied these skills to produce meaningful data by optimizing a fluorescence assay to screen for glycosidase activity in lysates from both *Bacteroides fragilis* and *Helicobacter pylori.* Fluorescent probes based on bacterial monosaccharides, DATDG and FucNAc, as well as a probe based on glucose (a ubiquitous sugar in human and bacterial cells) were employed to detect the presence of monosaccharide-specific glycosidases in bacterial lysates (Fig. 1A). Once introduced to these lysates, glycosidases cleave the sugar from resorufin, causing the resorufin to emit fluorescence at 585 nm (Fig. 2B). Through this method, we aimed to identify sugar-specific glycosidase activity by observing either the presence or absence of fluorescence.

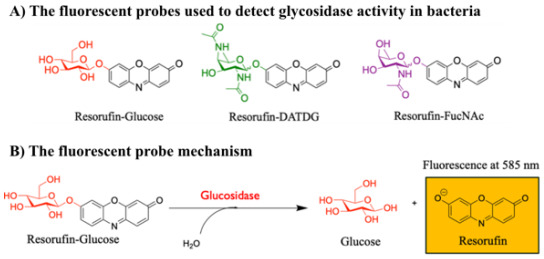

Figure 1. Fluorescent probes (A), synthesized by Dr. Suvarn Kulkarni at IIT Bombay, were introduced to bacterial lysates to detect glycosidase activity. Glucosidase is a known type of glycosidase that selectively cleaves glucose from glycans and causes the resorufin-glucose probe to fluoresce (B).

We hypothesized *B. fragilis* lysate would result in the most fluorescence given its known role in carbohydrate metabolism in the human gut, which requires extensive glycosidase activity to effectively break down sugars. We predicted that *H. pylori* would display some glycosidase activity since all bacteria need glycan-degrading enzymes to maintain their glycan structures, but not as much as *B. fragilis*. When screening *B. fragilis* with these probes, our hypothesis was confirmed as we saw significant activation of the Res-Glc probe, as well as significant activation of the Res-DAT and Res-Fuc sugar probes but to a smaller scale (Fig. 2A). The high signal from Res-Glc suggests a greater abundance of glycosidases targeting glucose than DATDG or FucNAc. This aligns with glucose's prominent role in the human gut where bacteria like *B. fragilis* are heavily involved in its breakdown, necessitating a high concentration of glucose-targeting glycosidases. When screening *H. pylori* with these probes, we saw *H. pylori* lysate was able to activate Res-DAT and Res-Fuc significantly despite displaying up to 3 times lower signal than the B. fragilis lysate (Fig. 2B). This suggests that *H. pylori* have fewer glycosidases targeting glucose than those targeting purely bacterial monosaccharides such as DATDG or FucNAc.

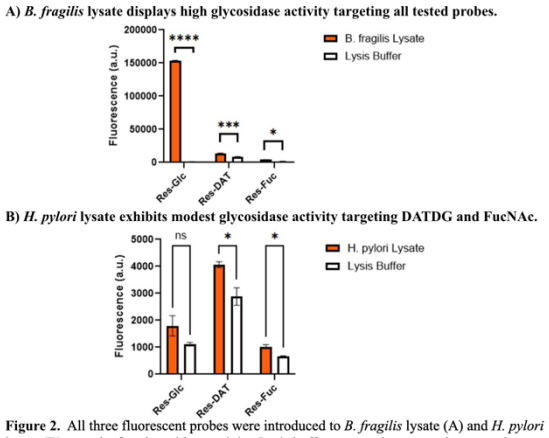

Figure 2. All three fluorescent probes were introduced to *B. fragilis* lysate (A) and *H. pylori* lysate (B) to probe for glycosidase activity. Lysis buffer was used as a negative control to distinguish background fluorescence from experimental fluorescence.

Faculty Mentor: Professor Danielle Dube

Funded by the William B. Sorabella '97 Global Health Fellowship

[1] Zafar, H., & Saier, M. H., Jr (2021). Gut *Bacteroides* species in health and disease. *Gut microbes*, *13*(1), 1–20. https://doi.org/10.1080/19490976.2020.1848158

[2] Luong, P., & Dube, D. H. (2021). Dismantling the bacterial glycocalyx: Chemical tools to probe, perturb, and image bacterial glycans. *Bioorganic & medicinal chemistry*, *42*, 116268. https://doi.org/10.1016/j.bmc.2021.116268