Using Substrate Decoys to Characterize Glycosylation Pathways in *Helicobacter pylori*

Lucas DiCerbo, Class of 2023

The discovery and development of antibiotics has saved countless lives. However, the growing threat of antimicrobial resistance (AMR), along with the detrimental effects of nonspecific antibiotics, demonstrate the need for more targeted antibiotic treatments.[1] *Heliobacter pylori* is one such antibiotic-resistant pathogen which is linked to stomach ulcers and gastric cancer. Though not pathogenic in all cases, *H. pylori* colonizes nearly half the global population,[2] and current treatments use a combination of drugs that are often harmful to the host.[3] This urgent need for new treatment has earned it a place on the World Health Organization's list of global priority pathogens[4] and is the primary motivation behind my research in the Dube Lab.

Bacterial glycans – sugar-containing molecules found on bacteria – have previously been identified as potential antibiotic targets because of their species-dependent specificity.[5] Furthermore, these polysaccharide structures are frequently critical to cell fitness; they are implicated in functions such as host adhesion and immune cell recognition – or, in some cases, evasion.[6] These observations are especially true for pathogenic species; previous studies have shown that pathogens which cannot effectively synthesize glycans have reduced pathogenesis.[7] Bacterial glycans are clearly intriguing therapeutic targets, but their complexity makes them difficult to study. Mammalian glycans, upon which many analytical techniques are based, use 9 monosaccharide substrates. Bacterial glycans use over 700. This sheer number of monosaccharides makes bacterial glycans difficult to study by conventional methods.[6] Previous work in the Dube lab has utilized Metabolic Oligosaccharide Engineering (MOE) to ease this process: a fluorescent or otherwise visualizable monosaccharide is introduced into the cell, incorporated into its glycan structures, and visualized with a biorthogonal reaction (independent of host machinery) to confirm the presence or absence of glycans.[8] This can be employed following genetic mutation to measure the impact of different gene knockdowns upon glycan synthesis. In this way, at least 13 genes involved in glycoprotein synthesis in *H. pylori* have been identified by the Dube lab. The enzymatic products of these 13 genes are all potential therapeutic targets. However, the roles of their individual genetic products, as well as the ordered details of glycan assembly, remain unknown.

My work this summer built upon previous efforts in the Dube lab to determine this information. I employed a specific class of MOE probe: a fluorescent substrate decoy. These fluorescent molecules are known to *divert* endogenous glycan synthesis onto themselves (Figure 1), allowing the glycans they synthesize to be expelled from the cell instead of incorporated onto the cell surface. The added fluorescence makes the entire structure visualizable to analytical techniques which would not otherwise be able to detect the decoy. Using the fluorescent substrate decoy NBD-Fucose, I sought to isolate *H. pylori*'s glycans from the cell, purify them, and characterize them. To do this, I employed High Performance Liquid Chromatography and Mass Spectrometry, among other techniques. I hypothesized that comparing the glycans of different glycosylation mutants would reveal certain differences, indicating the different roles of the enzymes encoded by those genes. In the long term, this could shed light on how to target *H. pylori* glycosylation and treat infection.

While some interesting initial findings were revealed this summer, I still have only just scratched the surface. Most of my data pertained to the optimization of my analytical parameters to best understand this system. This optimization is not yet complete; it remains difficult to quantify differences in glycosylation between wildtype and mutant. While the fluorescence of these substrate decoys makes them easy to visualize, analyzing the full glycans themselves amidst a complex mixture of cultured media – even without the cellular components – is challenging. Additionally, the strong fluorescence of the compound makes subtle differences challenging to detect. For example, the fluorescence given by purification steps may have simply revealed the presence of the original, unmodified decoy in the conditioned media, but the signals given by the elaborated glycan may have been much harder to see. Future work will focus upon developing adequate mammalian cells with more commonly studied decoys as a reference point, such that more can be understood about the data I have obtained so far from this novel analytical technique.

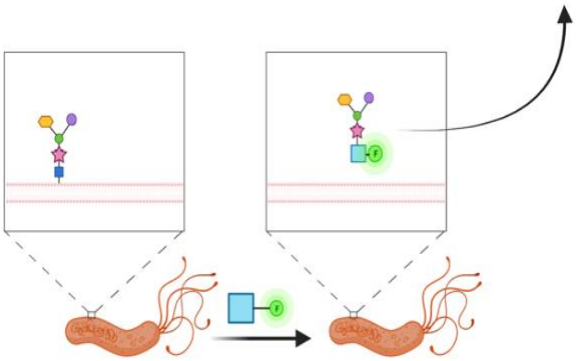

Figure 1. Fluorescent Substrate decoys divert endogenous glycan synthesis onto themselves. These elaborated glycans can be more easily isolated from cells, purified, and characterized.

Faculty Mentor: Danielle Dube

Funded by the Surdna Foundation Undergraduate Research Fellowship Program

References

(1) Allison, T. M.; Conrad, S.; Castric, P. The Group I Pilin Glycan Affects Type IVa Pilus Hydrophobicity and Twitching Motility in Pseudomonas Aeruginosa 1244. *Microbiology* **2015**, *161* (9), 1780–1789. https://doi.org/10.1099/mic.0.000128.

(2) Centers for Disease Control and Prevention (U.S.). *Antibiotic Resistance Threats in the United States, 2019*; Centers for Disease Control and Prevention (U.S.), 2019. https://doi.org/10.15620/cdc:82532.

(3) Malfertheiner, P.; Megraud, F.; O'Morain, C.; Bazzoli, F.; El-Omar, E.; Graham, D.; Hunt, R.; Rokkas, T.; Vakil, N.; Kuipers, E. J.; The European Helicobacter Study Group (EHSG). Current Concepts in the Management of Helicobacter Pylori Infection: The Maastricht III Consensus Report. *Gut* **2007**, *56* (6), 772–781. https://doi.org/10.1136/gut.2006.101634.

(4) Kudelka, M. R.; Antonopoulos, A.; Wang, Y.; Duong, D. M.; Song, X.; Seyfried, N. T.; Dell, A.; Haslam, S. M.; Cummings, R. D.; Ju, T. Cellular O-Glycome Reporter/Amplification to Explore O-Glycans of Living Cells. *Nat. Methods* **2016**, *13* (1), 81–86. https://doi.org/10.1038/nmeth.3675.

(5) Suzuki, H.; Iwasaki, E.; Hibi, T. Helicobacter Pylori and Gastric Cancer. *Gastric Cancer* **2009**, *12* (2), 79–87. https://doi.org/10.1007/s10120-009-0507-x.

(6) Luong, P.; Dube, D. H. Dismantling the Bacterial Glycocalyx: Chemical Tools to Probe, Perturb, and Image Bacterial Glycans. *Bioorg. Med. Chem.* **2021**, *42*, 116268. https://doi.org/10.1016/j.bmc.2021.116268.

(7) Thung, I.; Aramin, H.; Vavinskaya, V.; Gupta, S.; Park, J. Y.; Crowe, S. E.; Valasek, M. A. Review Article: The Global Emergence of *Helicobacter Pylori* Antibiotic Resistance. *Aliment. Pharmacol. Ther.* **2016**, *43* (4), 514–533. https://doi.org/10.1111/apt.13497.

(8) Tra, V. N.; Dube, D. H. Glycans in Pathogenic Bacteria – Potential for Targeted Covalent Therapeutics and Imaging Agents. *Chem Commun* **2014**, *50* (36), 4659–4673. https://doi.org/10.1039/C4CC00660G.