Assembling genes involved in *Helicobacter pylori's* glycoprotein biosynthesis

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Helicobacter pylori is a pathogenic bacteria notorious for causing stomach cancers and ulcers. It is already known to be resistant to common use antibiotics such as clarithromycin and metronidazole[1]. Like several other bacteria, *H. pylori* glycosylates some of its proteins using distinct monosaccharide building blocks (Fig. 1) which are great targets for the synthesis of new drugs that specifically target *H. pylori*. Previous studies in the Dube lab identified thirteen genes involved in *H. pylori's* glycosylation pathway by making insertionally inactivated glycosylation mutants of which thirteen displayed defects in biofilm formation, host cell adhesion, and motility[2]. However, the order in which the thirteen glycosylation genes act and the structure of each distinct oligosaccharide (or glycan) on each respective glycoprotein still remains unknown.

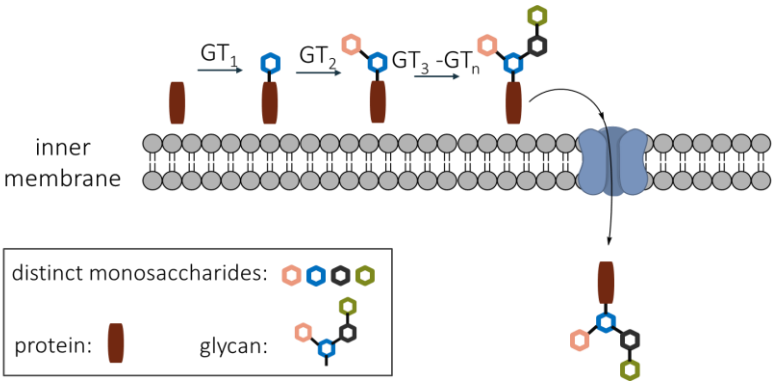

Figure 1: A simplified model of *H. pylori's* glycoprotein biosynthesis involving a stepwise glycosylation pathway mediated by a series of glycosyltransferase (GT) enzymes[2].

The goal of this project was to determine the order in which the thirteen glycosylation genes assemble *H. pylori's* glycoproteins by comparing urease from wildtype cells to glycosylation mutants. Urease is a glycoprotein in *H. pylori* [3] that catalyzes the hydrolysis of urea releasing ammonia which neutralizes the acidity of the surrounding pH in the stomach thus conferring *H. pylori* with the ability to thrive. We hypothesized that, when compared to wildtype urease, variations in the truncation of the glycan structure on urease from mutant strains will facilitate the construction of the pathway for *H. pylori*'s glycoprotein biosynthesis.

Approach: Several batches of wildtype and two glycosylation mutants of *H. pylori* were grown on horse blood agar plates and then inoculated into liquid culture. Cells were harvested by centrifugation to get at least 1g of starting material. Soluble proteins were extracted from harvested cells using sound energy. Urease was isolated from soluble proteins by running each respective sample through an ion exchange column (IEX) then a size exclusion column (SEC). IEX employs a low to high salt gradient to separate proteins in a sample based on their charge while SEC further separates proteins pooled from IEX based on their molecular weight. Each column chromatographic step was guided by the phenol red urease detection assay (PRUDA) to determine fractions testing positive for urease activity. Urease enriched samples from the wildtype and two glycosylation mutants will be analyzed for glycan structure using the Liquid Chromatography Mass Spectrometer (LC-MS).

Results: Urease was successfully purified from wildtype and two glycosylation mutants via IEX and SEC. It eluted halfway through the applied salt gradient in IEX chromatography confirming that it bound to the resin and was later displaced by the salt ions. Several distinct peaks were seen in SEC, one of which corresponded to urease activity as expected in the PRUDA assay. However, the resolution of the peaks from both chromatographic processes decreased with each successive run pointing to the need for a better system for re-equilibrating the column between runs.
Further studies will purify urease from the remaining glycosylation mutants and begin LC-MS analysis which would be done in collaboration with Nicholas Scott (University of Melbourne, Australia).
In the long-run, understanding how *H. pylori*'s glycoproteins are glycosylated will enable the synthesis of novel drugs containing small molecule inhibitors which specifically interfere with its glycosylation pathway, leaving the beneficial bacteria that reside in the human gut unharmed.

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References

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[3] Champasa, K., Longwell, S.A., Eldridge, A.M., Stemmler, E.A., Dube, D.H. (2013). *Molecular Cellular Proteomics.* **12**, 2568-2586.