**Analysis of *Helicobacter pylori* strains deficient in protein glycosylation**

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*Helicobacter pylori* is a gram-negative pathogenic bacteria that colonizes the human digestive tract and can cause duodenal ulcers, gastritis, and even some gastric cancers (1). *H. pylori* is growing increasingly resistant to current antibiotic treatments; therefore a novel approach for treating *H. pylori* is essential (2). *H. pylori*’s ability to sugar-modify, or glycosylate, its proteins is imperative for the function of many proteins involved in *H. pylori* pathogenesis; therefore, the protein glycosylation pathway is a good candidate for future drug targeting (3).

Hallie Carol ’14 worked to broadly identify enzymes involved in the glycosylation, or sugar coating, of *H. pylori* proteins. The *H. pylori* genome was screened for glycosylation genes similar to those found in other bacteria. Each gene screened was mutated in *H. pylori* to render it inactive, and the synthesis of sugar-modified proteins in *H. pylori* strains containing compromised versus intact glycosyltransferases was assessed. Out of the 20 potential glycosyltransferase genes studied, 13 mutant gene strains exhibited decreased synthesis of sugar-modified proteins, and one mutant strain showed no glycosylation (4).

I continued this research project by analyzing the mutant strains deficient in sugar-modified protein synthesis to determine if the mutated enzymes are essential for *H. pylori* protein glycosylation. I conducted molecular cloning of the functional *H. pylori* glycosyltransferase genes whose mutants displayed diminished or nonexistent glycosylation profiles. These genes were incorporated into plasmids, which are small sequences of DNA that can be taken in and expressed by bacteria. I was able to successfully create four types of plasmids that will be reinserted into the mutant strains that display decreased protein glycosylation in order to create “complemented” strains. These complemented strains will be analyzed for the recovery of glycoprotein synthesis using metabolic oligosaccharide engineering (MOE). MOE utilizes the bacteria’s natural glycosylation machinery to incorporate unnatural sugar analogs into complex sugar structures (5). These sugars are then covalently reacted with Phos-FLAG probes via Staudinger ligation and the glycosylation profiles will be analyzed using western blot analysis (5-7).

Interestingly, some of the putative *H. pylori* protein glycosylation genes that Hallie Carol ’14 identified are implicated in glycosylating lipids to produce lipopolysaccharides (LPS) in related bacterial species (4). LPS are class of molecules that are crucial for *H. pylori’s* pathogenesis (7). I am investigating the potential overlap in the protein glycosylation and LPS biosynthesis pathways. I conducted two experiments to assess whether LPS is labeled in MOE experiments: binding-affinity column chromatography to remove LPS and a hot phenol-water extraction to isolate LPS from metabolically labeled *H. pylori*. The glycosylation profiles of these samples were analyzed using MOE. Western blot analysis showed equivalent labeled glycans in both the control and LPS-depleted samples; however, no labeled glycans were seen in the isolated LPS sample. These results indicate that the signaling that we have seen in our *H. pylori* samples in past experiments was not purely signaling from metabolically labeled LPS molecules, and did indeed show us labeling of glycosylated proteins (4). The lack of signaling from purified LPS may be due to a low concentration of LPS in the sample, which may have been insufficient to show signaling. I plan to repeat this experiment using a greater amount of metabolically labeled *H. pylori* for the LPS isolation in order to increase my yield of purified LPS. This work has the potential to elucidate *H. pylori*’s protein glycosylation pathway, which could ultimately reveal novel drug targets for *H. pylori*.

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References