Discovery of genes required for glycolipids biosynthesis in the gastric pathogen *Helicobacter pylori*

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Abstract

Helicobacter pylori (H. pylori) is a Gram-negative disease-causing bacteria that causes sores in the lining of the stomach, called ulcers and gastric cancer. *H. pylori* infects about 50% of the human population and has increasing resistance to antibiotic treatment like triple therapy making it a high priority pathogen by WHO for which new antibiotics are needed. Lipopolysaccharide (LPS) is a molecule found in the outer membrane of Gram-negative bacteria that plays an important role in *H. pylori*'s ability to colonize and infect the human body. However, the genes responsible for the biosynthesis pathway of LPS in *H. pylori* remain unknown. Based on previous experiments, the Dube lab created mutant *H. pylori* strains by doing an insertional inactivation in genes encoding for putative glycosyltransferases. Comparison of LPS biosynthesis in wild type *H. pylori* strains versus mutant *H. pylori* strains revealed genes that are potentially involved in LPS biosynthesis in *H. pylori.*

Project Objective

LPS is a molecule that is found in the outer leaflet of the outer membrane of Gram-negative bacteria. LPS is a key factor in colonization and resistance of *H. pylori* because it is responsible for the outer membrane permeability barrier, *H. pylori's* resistance to immune cells, mediates interactions between the bacterium and its environment, and is a key factor in infectivity. LPS is composed of three main domains: lipid A, a hydrophobic domain embedded in the membrane; O-antigens, responsible for host mimicry that facilitates immune escape and contributes to infectivity; and core oligosaccharide, a chain of sugar residues that controls the permeation properties of the outer membrane. LPS biosynthesis is well characterized in other bacteria but remains uncharacterized in *H. pylori*. The genes required for LPS biosynthesis in *H. pylori* remain largely unknown. Due to the importance of LPS in *H. pylori*'s infectivity, my goal is to shed light on how LPS structures are made by identifying keys genes involved in their biosynthesis. If we can have information on how structures work, we can begin to think about creating small molecules, antibiotics, that inhibit those key genes from functioning by preventing the proper sugar coating from being made.

Methodology Used

To find out which genes are required in LPS biosynthesis, I sought to compare the LPS profile in wildtype *H. pylori* versus mutant *H*. p*ylori*. Mutant *H. pylori* strains were constructed in previous experiments done in the Dube lab by doing an insertional inactivation in genes encoding for putative glycosyltransferases. I expected that wildtype *H. pylori* would make fully elaborated LPS while mutant *H*. p*ylori* would have a defect in making LPS. I plated my bacteria on soft agar horse blood plates and grew them at 37°C in an incubator for four days. After the fourth day, I set up liquid cultures by inoculation the bacteria in 50ml of brucella both at 37°C in an incubator shaking for four days. Then, I harvested my cultures by centrifuging them at 3500rpm for 20minute to get about 0.4g pellet. I extracted LPS from the pellet by using the hot-phenol water method to separate LPS from other biomolecules. Upon addition of heated phenol to the pellet, it gets separated into three layers: the aqueous phase which contains LPS and contaminants, the interphase which contains unwanted proteins, and the organic phase which contains nucleic acids. The aqueous phase is pooled and get purified through dialysis and proteinase k treatment. LPS extracted from the pellet was dialyzed against water for four days with 1,000 MWCO (molecular weight cut off) dialyzing tube. The dialyzing tube filters the LPS by letting molecules like salt and ions that are 1000 molecular weight or less go through, while molecules greater than 1000 molecular weight like LPS stays in the tube. After the dialysis, the samples were ultracentrifuge at 40000rpm to collect the LPS. Then LPS samples were further purified with the treatment of proteinase K.

Proteinase K helps degrade contaminating proteins. Samples were then visualized for their LPS profile by running an electrophoresis gel and staining LPS. Electrophoresis gel separates samples by molecular weight, smaller molecules down the gel and bigger molecules up the gel. I stained the LPS samples and visualized under a UV transilluminator.

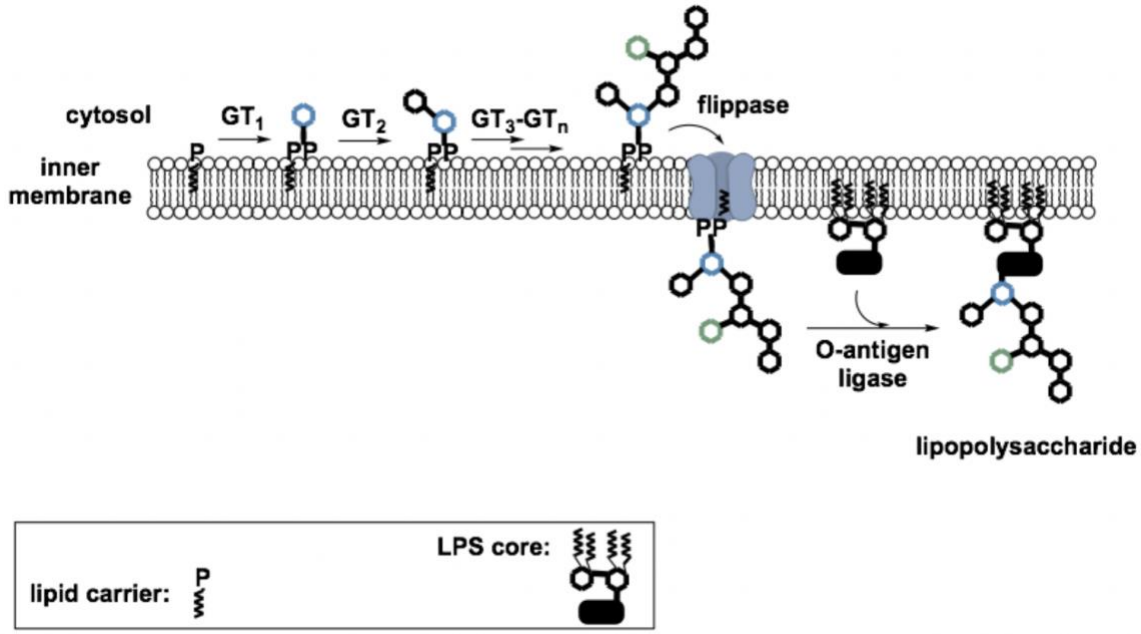
Result Obtained

Based on LPS stain, it appears that wildtype H. pylori synthesize fully elaborated LPS. Several of the mutants, including Δ607, Δ1236, Δ761, and Δ785, also appeared to synthesize fully elaborated LPS. By contrast, mutant 94 and mutant 1518 did not synthesize fully elaborated LPS. These results indicate that mutant 94 and mutant 1518 appears to be involved in LPS biosynthesis in *H. Pylori.*

Significance and Interpretation

Mutant 94 and mutant 1518 did not have fully elaborated LPS like wildtype (WT) *H. pylori* which tells me that both mutants might be involved in LPS biosynthesis pathway. This information is important because we can use it to figure out the order in which LPS glycosylation genes function and ultimately, create small molecule inhibitors that target key genes from functioning by preventing the proper sugar coating from being made.

Figures and charts



LPS biosynthesis pathway

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References

Li et al. *Lipopolysaccharide Structure and Biosynthesis in Helicobacter pylori* 2016, 21, 445-461.
Hug et al. *Helicobacter pylori Lipopolysaccharide is Synthesized via a Novel Pathway with an Evolutionary Connection to Protein N-Glycosylatio*n 2010, 6.
Dube et al. *Discovery of genes required for glycoprotein biosynthesis in the gastric pathogen Helicobacter pylori, (in preparation)*
Alba C et.al *Antibiotic resistance in Helicobacter pylori.* 2017 Oct;30(5):489-497
Davis, Charles Patrick. "*Helicobacter Pylori (H. Pylori)* Treatment, Diagnosis & Tests." *MedicineNet*,

Antibiotic resistance threats in the United States, 2013. Centers for Disease Control and Prevention
Tra, V. N., and Dube, D. H. (2014) Glycans in pathogenic bacteria --potential for targeted covalent therapeutics and imaging agents. *Chemical Communications* **50**, 4659-4673