# *Helicobacter pylori* novel detection and inhibition via bacteriophages, 1961P and KHP30

Campbell, E., Ly, L., Atwood, M., Phillips, B., Paiva-Filho, F., Kroll, Z., Robert, D., and Palmer J.
University of Massachusetts Dartmouth, Synthetic Biology Club

## Problem Statement

The intention of this research project is to develop a novel mechanism of detecting and eradicating the carcinogenic pathogen, *Helicobacter pylori,* from its stomach dwelling microbiota. *Escherichia coli* will be engineered to detect the presence of *H. pylori* and produce a phenotypic response, such as the production of green fluorescence, *in vitro*. With the success of this detection mechanism, the *E. coli* will be further engineered to produce *H. pylori* bacteriophage 1961P and/or KHP30, two well-known bacteriophages with specificity for eliminating *H. pylori*. The results of this project will provide the scientific community with a new potential approach of *H. pylori* bacteria therapy. This concept could further be extended as a possible treatment option for any pathogenic infection via engineered probiotics.

## Background Information

*Helicobacter pylori*, a Gram-negative bacterium, is classified as a class I carcinogen. An *H. pylori* infection causes gastric duodenal ulcers, gastric lymphoma, and chronic gastritis, which is a precursor to gastric carcinoma. *H. pylori* infects about half of the human population, with high prevalence in developing countries. *H. pylori*-induced gastric inflammation usually does not cause symptoms in the infected person, meaning the prevention of gastric ulcers is virtually impossible[1]. The current method of treating an infection of *H. pylori* is with the use of antibiotics, including Protein Pump Inhibitor (PPI), amoxicillin, clarithromycin, and metronidazole[2]. The rising complication with using antibiotics, however, is the propagation of antibiotic resistant bacteria, as well as the eradication of predominantly beneficial gut bacteria. In the absence of antibiotic therapy, *H. pylori* can live in the stomach for decades, or even an entire lifetime. What is most notable about *H. pylori* is its ability to secrete products that are linked with its ability to evade the host's innate immune system[1]. There is a great need for a new approach of eliminating *H. pylori* from the body.

Phage therapy is a progressively new method to eliminate and prevent bacterial infections. Phage therapy exhibits advantages over other biological agents, such as a high specificity for the target bacteria and clinical safety with minimal side effects. Additionally, there is higher frequency of phage mutation than bacterial mutation, so if a bacterium undergoes a mutation that results in phage resistance, phage mutation is likely to allow for adaptive evolution and continued inactivation of *H. pylori*[2]. There are a few bacteriophages for *H. pylori*, including 1961P and KHP30. 1961P and KHP30 are lysogenic phages that are well-known and frequently studied, and thus will be the phages used in our project to inhibit *H. pylori* through *in vitro* studies. This project will encompass the novel method of using a bacteriophage to eradicate an *H. pylori* infection via a probiotic delivery system.

Because of the highly acidic environment of the stomach (pH ~2), *H. pylori* requires various mechanisms to adapt. One mechanism is the use of urease, an enzyme that catalyzes the conversion of urea to ammonia and carbon dioxide. Once urease is synthesized in *H. pylori* cells, autolysis occurs – a process in which the cell lyses to release urease and other proteins into the extracellular environment (Figure 1). Neighboring *H. pylori* cells then bind the urease to their membrane to take advantage of ammonia's buffering capacity, allowing *H. pylori* to create and proliferate in a pH-tolerable microenvironment[3].

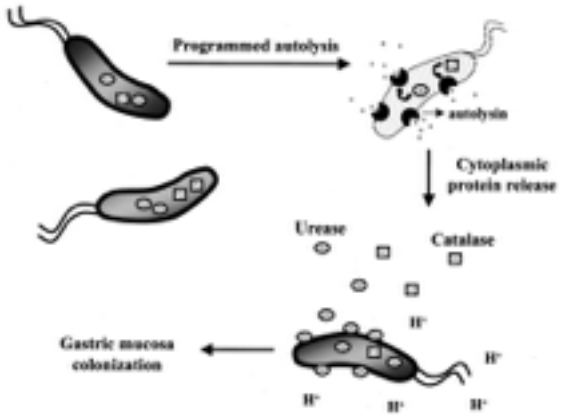


**Figure 1.** *H. pylori* autolysis of urease[3]

**Objectives**

1. *Engineer E. coli to detect H. pylori and produce response*

Competent cells of *E. coli* will be transformed with an engineered plasmid encoding a two-component regulatory system, actively promoted according to nickel ion concentration and pH. Therefore, the extracellular environmental conditions of the human stomach will act as the signaling mechanism for the binding of positively promoting proteins to bind our regulatory system triggering the expression our reporter gene, Green Fluorescent Protein (GFP).

2. *Verify and quantify the detection with microplate reader*

In a 96-well microtiter plate, *H. pylori* and the engineered *E. coli* will be co-cultured at varying concentrations and incubated in an environment similar to the stomach (37°C and pH of 2). If specific detection of the simulated stomach environment by *E. coli* is successful, wavelengths emitted by the green fluorescent proteins will be measured by the microplate reader. Quantitative analyses will be carried out to assess the efficiency and specificity of detection. Modifications to the engineered *E. coli* will be made at this time, as necessary.

3. *Engineer E. coli to inhibit H. pylori via bacteriophage, 1961P and/or KHP30*

Transformation of commercially available competent *E. coli* cells will be completed with an engineered expression vector containing known chromosomal integration points, isolated from *H. pylori* via PCR, and our bacteriophage genome The natural integration points for 1961P and KHP30 will then allow our plasmid vector to become the newly transformed extrachromosomal chassis for phage integration, which will be conducted via *in vitro* exposure of the vector to 1961P and KHP30. This specifically designed plasmid will then be introduced to the competent *E. coli* cells and tested.

4. *Quantitatively and qualitatively verify the inhibition*

A similar verification assay to objective 2 will be carried out to quantitatively measure the inhibition of *H. pylori*. The flux in population densities of both *E. coli* and *H. pylori* will be measured over a span of 24 hours in a microplate reader. Bacteriophage inhibition can be qualitatively visualized via plaque assays.

5. *Integration of engineered promotor with bacteriophage*

The final step in our process will then be to combine our engineered and quantified DNA parts onto a single plasmid, and transform *E. coli* with via the introduction of this plasmid. This will accomplish the regulated expression of bacteriophage based on the activation of our pH and nickel ion concentration dependent promoter, thus completing our environment specific eradication method of *H. pylori* using an *E. coli* probiotic.

**Anticipated Outcomes**

To our knowledge this would be the first time this type of bacteriophage therapy would function as both a sensory mechanism and an inactivation mechanism. From the verification assays, we will determine the time span that is required for our engineered *E. coli* to sense *H. pylori* and the minimal population densities of both bacteria needed for detection. The next and most important outcome of this experiment will be to successfully incorporate the whole genome of a bacteriophage into a plasmid. If production of the 1961P and KHP30 phage and elimination of *H. pylori* from the media is successful, we will be able to quantify the rate of death for *H. pylori* caused by the phage inhibition in our engineered *E. coli* compared to natural bacteriophage inactivation rates. Results from this project will shed light on possible in vivo studies, which may potentially lead to a novel treatment of numerous pathogenic infections.

**References**


1. Algood, Holly M. Scott and Cover, Timothy L. "*Helicobacter pylori* Persistence: an Overview of Interactions between *H. pylori* and Host Immune Defenses." *Clinical Microbial Review.* 2006; 19(4): 597-613. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1592695/.
2. Zou, Quing-Hua and Wei, Wen. "Phage Therapy: Promising For *H. pylori* Infection." *Clinical Microbiology.* 2013; 2: 112. doi: 10.4172/2327-5073.1000112.
3. Blanke, R Steven and Ye, Dan. "Chapter 20 Alternative Mechanisms of Protein Release."*ASM Press.* 2001. http://www.ncbi.nlm.nih.gov/books/NBK2422/.