Saving Oysters in the Chesapeake Bay Using Synthetic Seaweed

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Oysters in the Chesapeake Bay provide an economic and environmental benefit to our region. Unfortunately, there has been a decline in the oyster population due to loss of habitat, over harvesting, and parasitic disease. Our project focuses on the parasitic protist, *Perkinsus marinus*, which is killing oysters in the bay. We want to protect the oysters by creating an artificial seaweed comprised of cellulose with embedded proteins that will bind to and capture the parasite. Key components of our system include *Gluconacetobacter xylinus*, which will produce the cellulose, *Escherichia coli*, which will manufacture our protist binding protein, and the operon which codes for a cellulose binding domain/galectin fusion protein. Although we use living bacteria to produce the cellulose and the protein, we will test our samples before exposure to the environment to ensure our final product will not contain these organisms. We hope that one day oyster farmers will be able to float our synthetic seaweed in their oyster beds to reduce the number of *P. marinus* harming their catches.

Keywords: Oysters, Chesapeake Bay, cellulose, *Perkinsus marinus*, galectin

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Watch a video introduction by the authors at
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Oysters are an important ecological and economic factor in the Chesapeake Bay area. These mollusks are considered the "vacuum cleaners" of the Chesapeake Bay; that is, they filter the water to remove excess inorganic and organic molecules (Horn Point Oyster Hatchery [HPOH], n.d.), which positively affects the living conditions of almost all other aquatic species in the bay. Oysters feed on microscopic phytoplankton and algae, preventing algal biomass from accumulating and becoming harmful to other organisms. Also, when oysters die, their shells become new habitats, so-called "oyster reefs", for worms, fish, crabs, and other mollusks (HPOH, n.d.). Their hard shells also create a suitable surface for barnacles, mussels, and anemones (HPOH, n.d.). On an economic level, oysters provide a large market for food and jobs for many fishers and watermen (HPOH, n.d.). It is evident that oysters are a keystone species of the Chesapeake Bay.

However, the population of oysters in the Bay has been declining. The population present in the Chesapeake Bay today is

only 1% of what it was in the 17th century (NOAA, n.d.). This is associated with factors such as over-harvesting, loss of habitat, high and low salinity, high temperatures, low dissolved oxygen, loss of spawning due to low density, and predation (NOAA, n.d.). Historically, over-fishing has been the greatest contributor to the Eastern oyster decline in the Bay (Rothschild, 1994). Over the past few years, Maryland and Virginia have experienced more than $40 billion in annual losses due to less profit from industries based on oyster harvesting (Pelton & Goldsborough, 2010). This decline has also negatively affected water quality in the bay. In the late 1800s, the Chesapeake Bay's oysters could filter the entire bay in approximately three to four days, whereas today's population takes nearly a year to filter this same amount (Chesapeake Bay Program, n.d.). More recently, since the 1950s and 1960s, diseases such as MSX (Multinucleated Sphere Unknown) and Dermo have also had increasing effects in the decreasing population of the Eastern oyster (Rothschild, 2010), also known as *Crassotrea virginica*. Our project focuses on reducing the effect of disease on the oysters, specifically the disease Dermo, which is caused by the parasitic protist *Perkinsus marinus*. Dermo is especially harmful because once it infects an oyster, more spores containing the parasite are released into the environment and can affect many other oysters.

The purpose of this project is to create a synthetic seaweed to capture this protist and prevent the oysters from getting infected. In the future, the seaweed can be used by oyster farmers and applied to oyster restoration sites along the Chesapeake Bay to reduce the effect of Dermo on *C. virginica*.

Systems Level

The oyster population in the Chesapeake Bay has been declining over the past several years due to the protist *P. marinus* whose spores infect the oysters by attaching onto the galectin receptors on the oysters' surface (Figure 1). Once attached, the protist progresses through the oyster and causes the oysters' cells to lyse. Eventually, the oyster dies and the *P. marinus* spores can now spread through the water to infect other oysters. We plan on using *G. xylinus* to synthesize a cellulose sheet, which will act as artificial seaweed, with galectin receptor proteins embedded into sheet. When placed in the water near an oyster bed, the sheets will attract the *P. marinus* spores and prevent them from infecting the oyster population.. With our seaweed submerged in the bay, alongside cultivated oyster beds, there will be less spores in the water, and fewer infected oysters. There are many factors attributed to the decline in the oyster population, but our device focuses primarily on trying to alleviate the problem involved with the *P. marinus* spores that cause Dermo. Our device will be most applicable for oyster farmers dealing with *P. marinus* spores infecting their oysters, and hopefully eradicate the disease in some areas of the bay. The method in which our cellulose sheet will be implemented in the aquatic environment has not yet been tested. We do however, have a possible method in mind, in which a counterweight is attached to the cellulose sheet to submerge it into the water. A buoy is also going to be attached to the cellulose sheet to allow for the seaweed sheet to be located and easily accessible.

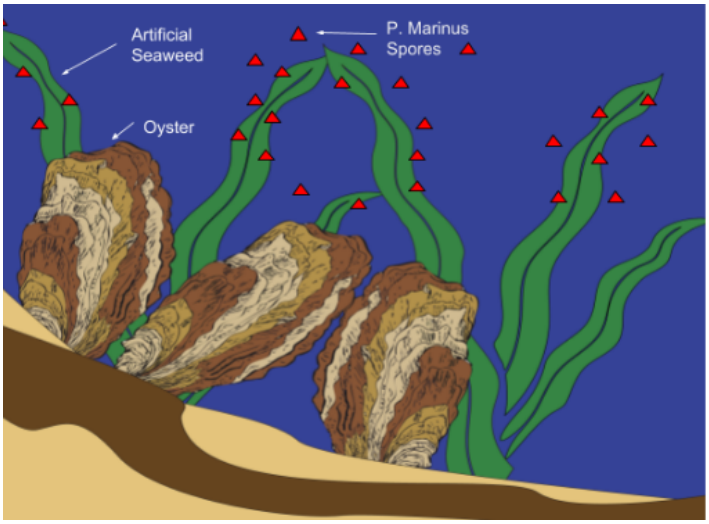

Figure 1: The G. xylinus produced cellulose sheets with the embedded galectin receptor protein will surround the oysters. The artificial seaweed will act as a barrier preventing the P. marinus spores from infecting the oysters.

Device Level

We will use two primary components to manufacture our protist-catching seaweed. The first component, *G. xylinus*, is a bacterial species that produces the cellulose used for our artificial seaweed. The second component, *E. coli*, is another species of bacteria that is used for synthesizing the fusion protein which captures the protists.

One of the main devices was the *G. xylinus*, which produces cellulose. Cellulose is a great material for holding our synthetic galectin. We have been propagating the bacteria in liquid media for over a year. We obtained the bacteria and growth protocol from Imperial College, London. Then, we used the *G. xylinus* to cultivate tough cellulose sheets in the shape of a container.

E. coli is the second major device that we use in our system. We designed an operon that will regulate the expression of the fusion protein gene, and then transform it into *E. coli*. The fusion binding protein, which combines Galectin and Cellulose Binding Domain (CBD) will be mass produced by the *E. coli*. The CBD part of the fusion protein will attach to the cellulose, as proven by Imperial College. The second part of the fusion protein, Galectin, attaches with the *P. marinus* spore (Figure 2). Later, we plan to kill the bacteria and keep the cellulose that we will use to create an artificial seaweed. We will be using a sterilization method created by the IGEM team from Imperial College in London to inactivate any viable *G. xylinus* and *E. coli* bacteria that remain in the cellulose sample.

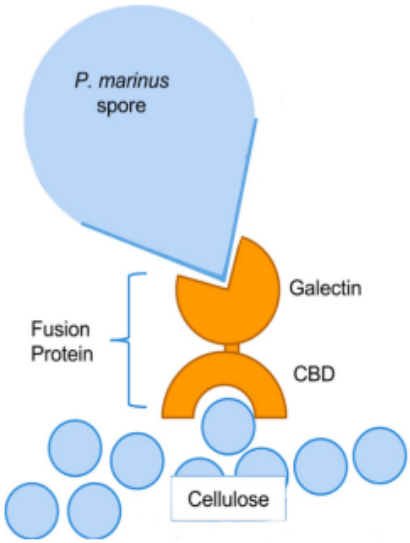

Figure 2. We will form a fusion protein consisting of a CBD, which Imperial College has found to connect to cellulose sheets. With a linking protein, the CBD will connect to Galectin, which will be used as a receptor to in order to attract and connect the P. marinus spore.

Parts Level

Within our experiment, we have an operon used to produce a fusion protein for capturing the oyster parasite *P. marinus* (Figure 3). At the promoter (BBa_R0010), the transcription factors bind to this 200 base pair sequence, enabling the RNA Polymerase to bind to the DNA so mRNA can be transcribed. The ribosome binding site (RBS; BBa_B0034), allows for the ribosome to attach to the mRNA sequence, initiating translation of the mRNA to create the fusion protein. The fusion protein is made up of the cellulose binding domain (CBD; BBa_K1321340) and galetin receptor protein (BBa_K1489005). The CBD is responsible for binding to the cellulose. The gene for the cellulose binding domain is 366 base pairs long. The galectin protein, which is responsible for capturing the *P. marinus* spores, is encoded by a gene that is 1686 base pairs long. Normally, the galectin protein is the receptor for *P. marinus* spores on the Chesapeake Bay oysters and is expressed intracellularly and extracellularly. The University of Maryland's 2014 iGEM team created and provided us with the galectin gene. Our operon contains two separate terminator sequences (BBa_B0010 and BBa_B0012) . The first terminator is a 64 base pair stem-loop sequence. The second terminator is a rather inefficient sequence that acts as a promoter in the opposite direction; it is 41 base pairs long.

Figure 3. This is the operon used to produce the CBD/Galectin fusion protein. The Promoter site will be used to initiate transcription and the ribosome binding site (RBS) will be used to start translation. The terminator sequence, which ends ribosome transcription is downstream from the CBD/Galectin coding region.

Safety

Throughout the course of our experiments, we enforced general lab safety protocol. Each member of the team wore appropriate lab gear, sterilized all the lab materials pertaining to the experiment, and disposed of hazardous waste appropriately. In addition to lab safety, environmental safety was also taken into consideration. Since we are using live bacteria to produce our cellulose and proteins, we must take certain precautions to guarantee the inactivation of the bacteria for the safety of the bay. The 2014 IGEM team of Imperial College in London developed a method of removing both *G. xylinus* and *E. coli* from the cellulose sample. To ensure that there are no living bacteria present on the cellulose, we will follow their example of heating the cellulose at temperatures between 60☐ and 80☐ for at least two hours since the removal of *G. xylinus* is dependent on time and temperature. Another heat treatment will be used on our *E. coli* sample in order to retrieve our manufactured proteins and ensure the bacteria's inactivation. To confirm that there are no living bacteria present, we will take a sample from both the cellulose and *E. coli* culture, streak them onto separate media, and observe the formation of any bacterial colonies of either *G. xylinus* or *E. coli*. If no colonies develop, the heat treatment would have been a success and the combining of both the cellulose and proteins would be permitted. On the off chance that bacteria do survive, we will repeat the treatment until no colonies develop. After combining the samples and ensuring that the proteins are successfully attached to the cellulose, we will wash the cellulose to rid it of debris, guaranteeing the removal of any unnecessary by-products. The final product will be safe to place into the bay, and there will be no living organisms present on its surface that could potentially affect the entire ecosystem. We hold environmental safety in high regard, and we will take every measure to achieve the safest result for the well being of the bay and the organisms within it.

Discussions

As of now we are still in the process of making this system fully functional. Our current cellulose sheets, unfortunately, are too delicate and thin to insert the galectin receptor via the fusion protein. We plan on formulating a way to produce stronger and thicker sheets of cellulose so that they can survive the currents of the Chesapeake Bay. In addition to cellulose production, we have made our two test plasmids, one with CBD-BFP and the other with BFP. Now, we are in the process of validating whether our plasmid transformations were a success through gel electrophoresis.

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