Using *Sphingomonas* to Decrease Estrogen Levels in Water

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There has been a recent problem with large amounts of birth control pills being dumped into bodies of water. Thereupon, this is the only pathway for the estrogen from birth control to enter the water supply because water treatment plants already have a system for treating wastewater with estrogen from human excretions. The current wastewater treatment solutions may not always successfully remove excess estrogen that is added to the water supply from medications.This is contributing to high levels of estrogen in the water, causing North American men to experience low fertility. Albeit, agriculture accounts for 90% of estrogen in the environment, the gradual increased use of birth control pills is a readily current factor that has a greater chance of being eliminated or controlled. Water filtration techniques are unable to remove the estrogen because it is a hormone. Therefore, our goal was to research the types of organism, e.g. bacteria or plants, or other hormones that we could apply to potentially counteract this problem using synthetic biology techniques.

The high level of estrogen in the water is a great concern to public health because long-term exposure may negatively affect animal behaviour and physiology. The prevalence of estrogen is not only leading to infertility in humans but is also causing problems for aquatic ecosystems. For example, there have been cases of fish becoming intersexual after living in estrogen-contaminated water, and 37 species of feminized North American and European male fish have been reported over the past decade.

During our research, we came across *Sphingomonas*. A strain of this bacterium called KC8 has been found to be able to consume 17β-estradiol as its energy and carbon source. As of now, it represents the only known genome of an estrogen-degrading bacterium. Our team speculated that using this microorganism in the environment based on the concept of bioremediation. Using bioaugmentation and biostimulation, we could pump *E. coli* that has been modified with the genome of *Sphingomonas* strain KC8 into the water to counteract the surplus of estrogen.

Keywords: *Estrogen, KC8, Sphingomonas, PCR*

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Background

There has been a recent rise in estrogen levels in North American waters. Conspicuous quantities of birth control pills have been shown to have occured in higher concentration in various bodies of water. This increase has caused North American men to experience low fertility. Current water filtration techniques are unable to mediate the rise in estrogen due to the hormonal nature of estrogen.

We have developed a way to decrease the levels of estrogen in the water utilizing a modified strain of *Sphingomonas* called KC8 (the microorganism strand KC8 belongs to the domain Eukarya, kingdom Bacteria, phylum Proteobacteria, and class Alphaproteobacteria). This strain has the innate ability to rapidly degrade both 17(β)-estradiol and estrone, while KC8 (a strain of *Sphingomonas*) also dramatically reduces estrogen levels in men. This compound is a key element in elucidating or reducing the epidemic being addressed in this study. *Sphingomonas* is the only known genome of an estrogen-degrading bacteria as of now. The catabolic genes and enzymes of KC8 remain unknown (Chen et al. 2017).

We intend to use bioremediation to introduce KC8 into the water through integrating it into a species of bacterium commonly found in North American waters: *Escherichia coli* , which is found in large quantities and is well adapted to wastewater environments. *In situ* bioremediation means that microorganisms are pumped into the medium without needing to be isolated through initial removal.

Because the microorganisms with the intended genomes needed to degrade these hormones in the water are not preexisting, the microorganism must be genetically modified and introduced into the medium through the process of bioaugmentation. The process uses a pump to introduce the bacterium into the groundwater, allowing it to enter the affected bodies of water.

Systems Level

To solve the issue at hand, our system will be based on genetically engineered *E. coli* with a DNA sequence that is responsible for the degradation of 17β-estradiol and estrone in *Sphingomonas*. The *E. coli* will be genetically engineered through a process called "sequence knock in" using the CRISPR Cas9 gene editing system. The process involves the Cas9 protein carrying the target DNA sequence into the nucleus of the *E. coli* cell and inserting it into the DNA of the *E. coli* by making a double-stranded nick in a certain place in the chromosome and inserting the target DNA sequence ("Questions and Answers About CRISPR," 2019). Then, the whole chromosome is repaired by the cell's own DNA repair system. We shall obtain our copies of the target DNA sequence through the chemical process of the polymerase chain reaction, in which the targeted DNA sequence can be replicated in large quantities ("Polymerase Chain Reaction," 2015). We will make sure the genome sequence is accurate to our target genome by doing two rounds of PCR. In the first round, we will breakdown the target genome into smaller sequences and synthesize each one separately in order to maintain accuracy. Then, all the smaller sequence will be synthesis together through a process known as Mix PCR. We run the first product through a cycle of F/R PCR to single out and replicate the correct strand. Lastly, we run the product through gel to check if the sequence is correct.

Once we get enough copies of the target genome, we will insert the gene into a carrying prokaryotic double helix carrier molecule via CRISPR. The carrier molecule will enter the E. *coli* through the membrane by heating up the bacteria (in order to open up the pores) and sealing it with ice once the carrier molecule has entered. Once finished, the bacteria will be left to incubate and reproduce on agar plates for 1 day. The wanted bacteria will be picked out and will then be reproduced in our lab, resulting in a small population. After we have inspected the *E. coli* and confirmed its ability to degrade both 17β-estradiol and estrone properly without creating toxic byproducts, we will release it into the North American waterway system through bioremediation.

Device Level

For our system, it is necessary to have a sample of a small population of healthy genetically altered *E. coli* that can functionally degrade 17β-estradiol and estrone as its primary source of energy . The *E. coli* also needs to be able to survive and reproduce in the North America waterway system. Furthermore, we also need to develop a method to control the population of genetically engineered *E. coli*. We shall monitor and control the *E. coli* by inserting an additional genome sequence into its chromosome that makes it require access to a certain chemical that we shall provide; if it doesn't have this access, it will suppress all transcription in the cell. The chemical will not be harmful to other organisms or to the environment.

Parts Level

For our *E.coli* to be able to degrade estrogen, we need to obtain that trait through an organism that already has the ability to degrade it. This trait is present in KC8, the organism we have chosen , and we would use the genome sequence that is responsible for the degradation

of 17β-estradiol and estrone.This genome sequence has already been instructionalized (Hu et al. 2011). During the process of translating the genetic information, the other proteins in *E. coli* will be damaged. Therefore, we will insert it close to the promoter of a region for early access in order to check if it is operating correctly.

Safety

To safely test our hypothesis, we will first introduce the microorganism strand KC8 into a controlled environment. In doing so, we reduce the risk of any potential negative impacts that introducing this microorganism might cause. Using bioremediation, we will construct a transfer pump to safely introduce the KC8 strand into the water using indigenous *E coli*. Since the microorganism being used in our bioaugmentation system (*E. coli*) is already prevalent in North American waters, we do not need to introduce a new bacteria into the environment. We are only taking the *E. coli* that already exists in the water, and introducing *Sphingomonas* genome into them.

Our theoretical ideas of the issues that we might encounter in introducing KC8 relate to the potentially dangerous environmental effects of introducing a substantial population of KC8 into the waters. For example, we do not know if the native species will be affected by the sudden increase in bacteria concentrations. Our rising uncertainties introduce dispute regarding whether or not the KC8 strain being introduced with the E. *coli* will have any unique effects on animals in the waters. Furthermore, we are not sure if the genetically altered organism could affect the evolution of aquatic bacteria. All in all, introducing a new, genetically unnatural species to any place is risky, but we will make sure to minimize the potential for negative impacts through research and development.

Discussions

With the use of synthetic biology, the issue surrounding the significant amounts of estrogen in the waters, which is causing men across North America to experience lower fertility rates, could be solved. Although our conclusions are hypothetical as we did not have the chance to try out our experiment, we believe that the exercise of the genome from the *Sphingomonas* KC8 strain could revolutionize the various methods of estrogen removal in North American waters. The next steps that we plan on taking are trying the experiment and testing our hypothesis. This would be a certain indicator of our hypothesis as it will turn our hypothetical concept into a reality.

One of the problems that we may come across is possible mutations of the *E. coli* bacteria. This may result in an outbreak in North American waters, which would be more detrimental than helpful. A way to address this and think ahead is to use a particular compound that targets the mutant *E. coli strain*, resulting in its termination so that it is no longer harmful. This is another thing to consider for future steps when testing our hypothesis and making inferences about possible problems and solutions.

We will research a solution to the problems concerning the limit to the effectiveness of the KC8 strain. We are not sure how KC8 will affect the *E. coli* in the water and its environment. In the future, we plan to develop a bioaugmentation procedure that is adapted to our purpose. The aim of the procedure is to effectively bind KC8 to *E. coli* through methods involving the lytic and lysogenic cycles of bacteria. Our testing phase will also help us to test and determine a successful method of implementing the altered *E. coli* into the water supply.

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