Detection and Tracking of Estrogenic and Toxic Substances in the Quinnipiac River

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INTRODUCTION

There are numerous chemicals that are present in the environment that have harmful effects on humans. Some of those substances may be endocrine disrupting chemicals, which are a concern due to their harmful effects on wildlife (Sorokin, 2006). These chemicals mimic human hormones and may cause an increase in cancers, decrease in sperm counts, and even early puberty. Some of these chemicals reside in waterways accessible to locals.

The Quinnipiac River is surrounded by numerous factories and wastewater treatment plants. These factories have a long history of discharging waste into the river. After the Connecticut Clean Water Act of 1967 and the Water Pollution Control Act of 1972, waste addition into the river has been banned for the industries but not for wastewater treatment plants (DEEP). The purpose of researching possible estrogenic substances in the Quinnipiac River is to assess the water quality and identify any dangerous substances that may still remain. This is of great concern because the Quinnipiac River has become a center of recreation, including fishing. If endocrine disruptors are present, the fish eaten will contain them and have detrimental effects on locals who may eat it. It is important for the river to be uncontaminated, especially if it is open to fishing. In addition, the presence of estrogenic (or toxic) substances will affect the amount of fish present as male fish become feminized and have reduced sperm counts in the presence of these substances. This could have dramatic effects on the entire ecosystem (Sorokin, 2006).

BLYES, genetically engineered yeast, is one of the tools used to detect estrogen in water samples. The yeast contains the human estrogen receptor gene (hERα) as well as bioluminescence-producing plasmids under control of human estrogen response elements. When estrogen is present in the sample the reaction will produce light (Sanseverino, 2008). The Quinnipiac River was tested for the detection of estrogenic substances and those that are toxic to eukaryotic cells to determine if there is a seasonal nature to their input into the river and to track sources of the contamination.

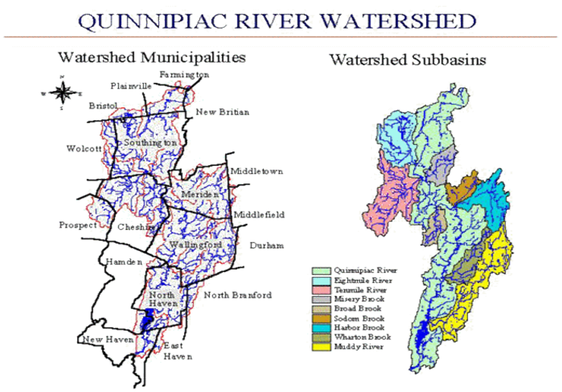

Figure 1. Quinnipiac River Watershed

MATERIALS & METHODS

All glassware was rinsed thoroughly with soap and water, foiled and baked at 500˚C for 5 hours to remove any unwanted chemicals or compounds.

Sample Collection: Water samples were collected in 1 liter glass bottles at 6 different sites along the Quinnipiac River (Figure 2). The 6 sites were located in North Haven, New Haven, Meriden, Southington, Plainville and Plantsville Connecticut. The samples were stored on ice and then filter through a Whatman 0.22 Glass microfiber.

Solid-Phase Extraction (SPE): All 6 samples were extracted according to a modified version of the EPA Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS. The samples were loaded at a flow rate of 5-10 mL/min through SPE Hydrophilic-Lipophilic Balanced disks (Figure 3). The disks were dried and the analytes were eluted by adding 12ml of Methanol. The extract was dried by using a nitrogen evaporator, re-dissolved in 1000 µl of deionized (DI) water and then stored in a -20˚C freezer.

17B-Estradiol Standards: 17B-Estradiol was used to perform dilutions (Figure 4). Four sets of dilutions were added to a 96 well-plate at a time. After the wells were dry, 200 µl of *Saccharomyces Cerevisiae* (BLYES) was added to the wells. The plate was sealed and placed in the incubator at 30˚C for 3-4 hours. The plate was placed in the pate reader, and the data was graphed (Figures 5 and 6).

Figure 2. Sample Collection

Figure 3. Solid-Phase Extraction

Figure 4. Dilutions

RESULTS

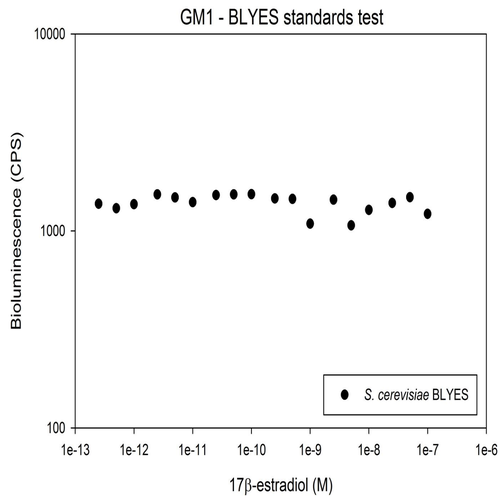

Figure 5. Standards Test 2017-06-08

(17β-estradiol incubated with 2017 BLYES for 4 hours)

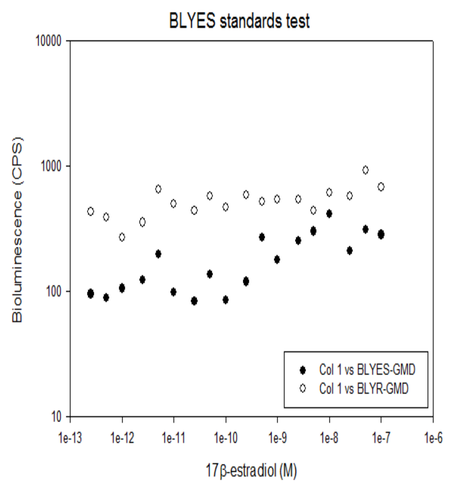

Figure 6. Standards Test 2017-07-28

(17β-estradiol incubated with 2015 BLYES and BLYR for 4 hours)

Figure 5 demonstrates the first set of dilutions incubated with a strain of BLYES from the year 2017. The graph in figure 5 indicated that the yeast strain was not successful in detecting bioluminescence. A yeast strain that can detect bioluminescence should produce a graph with a sigmoidal shape. To troubleshoot the yeast, different variables were manipulated. The yeast that was loaded into the 96 well plate was left to dry in the dark instead of the light, the plates were ran for 13 hours to determine whether they needed to be incubated longer, a new bottle of 17β-estradiol was ordered and used, and lastly yeast strains from 2015-2017 was isolated and regrown to detect the most efficient strain. Figure 6 demonstrates the last set of dilutions incubated with a strain of BLYES from the year 2015. The graph in figure 6 displays a sigmoidal shape, indicating that the yeast strain was successful in detecting Bioluminescence.

CONCLUSION

The standard that was established shows the functionality of the genetically engineered *Saccnaromyces Cerevisiae*. The 2015 BYLES that was tested on the 28th of June was proved to be able to emit bioluminescence in the presence of estrogen. These results allows further application of the yeast on the samples that were collected during the 2 month interval. Further application of this research includes more sample collection, dilutions, and data analysis of estrogenic substances in the Quinnipiac River. The data will lead to the determination of sources of contamination in the river.

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