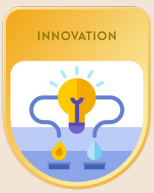
Detection of oxybenzone via production of LacZ gene induced by GEM protein in engineered *Saccharomyce cerevisiae*



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Oxybenzone is a chemical compound found in sunscreens that serves as an active ingredient to block ultraviolet (UV) rays. Recently, concerns have arisen due to the adverse effects of sunscreens containing oxybenzone on coral reefs. Oxybenzone contributes to coral bleaching by accumulating in coral tissues and reducing sunlight availability for photosynthetic organisms due to its UV-absorbing properties. Through the genetic engineering of *Saccharomyces cerevisiae*, oxybenzone concentration levels in water bodies can be detected to determine the significance of this problem. The estrogenic properties of oxybenzone allow it to bind to a synthetic fusion protein containing a human estrogen receptor. This protein will induce a standard pGAL promoter and lead to the expression of the LacZ gene. When expressed, the LacZ gene will code for the synthesis of beta-galactosidase an enzyme, which breaks the substrate, para-aminophenol-beta-d-galactopyranoside (PAPG), down into galactose and para-aminophenol (PAP), producing an electrochemical signal which can be measured using the Field-Ready Electrochemical Detector (FRED) and analyzed to determine oxybenzone concentration. The detection of oxybenzone will allow for greater understanding of the issue of coral bleaching, and provide a tool to assess the impact of further steps being taken to protect these aquatic ecosystems.

Keywords: Oxybenzone, Human Estrogen Receptor (hER), *Saccharomyces cerevisiae*, estrogenic, coral bleaching

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Background

Oxybenzone is a common chemical component in sunscreen with multiple properties that allow it to inflict harm on aquatic habitats. Oxybenzone is a petrochemical derived substance that is easily absorbed into the epidermal linings of coral. The absorbed oxybenzone causes stress on coral, leading them to expel zooxanthellae, the algae they rely on for energy, and lose pigments, causing them to become white in an effect known as "coral bleaching" (National Oceanic and Atmospheric Administration, 2021). The process of coral bleaching, as well as damage to the genetic material of marine organisms, can occur due to high concentrations of oxybenzone in aquatic ecosystems (Downs, 2021). Furthermore, oxybenzone has estrogenic properties, which enables it to attach to estrogen receptors and disrupt the endocrine system of marine organisms (Morohoshi et al., 2005).

Bleaching of coral reefs worldwide has led to a reduction in the biodiversity of species populations in aquatic ecosystems, causing detrimental effects on coastal communities. Coral reefs have cultural and spiritual significance in many places, such as Hawaii, and are a global source of economic revenue for coastal regions through recreational activities and tourism (Ige, 2018). Coral bleaching has recently become a globally recognized issue, several legislation to ban products containing oxybenzone have been passed in five different countries, as well as in four US states (Lenahan, 2020). Further legislation is being considered in many other coastal countries, with very similar aims to the legislation that have been previously passed. The aim of act SB 2571, a law passed in Hawaii to ban sunscreens containing oxybenzone, was to reduce the damage and harmful ecological effects oxybenzone has on coral reefs and additional aquatic life. Another aim of this act was to support research on the management of these toxins; highlighting the importance of monitoring these levels of oxybenzone for the protection of coral reefs (The Senate, Twenty-Ninth Legislature, State of Hawaii, 2018).

At low concentrations, oxybenzone easily enters food chains and quickly magnifies due to its properties that allow it to be readily absorbed by gastrodermal linings (Downs, 2021). In order for oxybenzone contamination in marine ecosystems to be managed, a system to detect and measure oxybenzone concentration levels is necessary. A two plasmid system expressed in *Saccharomyces cerevisiae* was developed to detect oxybenzone concentrations in order to address these issues. This system will provide a greater understanding of the location and extent of oxybenzone contamination in coral reefs. Consequently, detection of oxybenzone will provide information on the state of aquatic environments in regards to chemical pollution.

Systems Level

The system used to implement the testing of oxybenzone concentration levels in water is based upon the Field-Ready Electrochemical Detector (FRED), which was developed and used by mentors at FREDsense technologies. The system includes a cartridge, containing the engineered *S. cerevisiae* and a para-aminophenol-beta-d-galactopyranoside (PAPG) substrate. Water is collected and added to the cartridge, which is then placed in a FRED device. From there, the device incubates the test and the results will be available for analysis (Sanchez et al., 2021). The signal detected by the system is a current produced by the oxidation of 4-aminophenol, which is produced as a result of the cleavage of the PAPG substrate via beta-galactosidase by the LacZ gene (Tschirhart et al., 2016). A linear voltage sweep is then applied to an electrode immersed in a solution containing the engineered *S. cerevisiae*, the PAPG substrate, and the water sample. The voltage sweep encompasses the voltage at which 4-aminophenol undergoes an oxidation reaction to form 4-iminoquinone. Due to this, when the voltage sweep occurs, the current produced by the oxidation of 4-aminophenol is proportional to the signal produced by the engineered *S. cerevisiae*, and therefore proportional to the sample of interest (Tschirhart et al., 2016). A study done by Aranda-Diaz et al. (2017), concluded estradiol was detected by their construct in concentrations ranging from 5-125 nM. Results may differ between estradiol and oxybenzone and differences in the gene used for detection may change results, however; the range and sensitivity of the estradiol detection may be used as a benchmark for ruther lab work and mathematical modeling.

Device Level

A two plasmid system has been developed for this project, with both *Escherichia coli* and *S. cerevisiae* as chassis. The plasmids will be transformed in *E. coli* for replication, then put into *S. cerevisiae* after miniprep and transformation. Due to the inability of *E. coli* to express the human estrogen receptor (hER), all oxybenzone detection will occur when the plasmids are in *S. cerevisiae,* which for the purposes of this experiment will be of the cell line cen.pk (Zhang et al., 2000).

Using a synthetic fusion-protein estradiol detection system developed for *S. cerevisiae* by Aranda-Diaz et al. (2017) as reference, a two plasmid system will detect oxybenzone through its binding to a human estrogen

receptor (hER). When oxybenzone is present, it will bind to a protein expressed by the first plasmid (Plasmid One) that induces the promoter of the second plasmid in the system (Plasmid Two), leading to the expression of the LacZ gene (Figure 1).

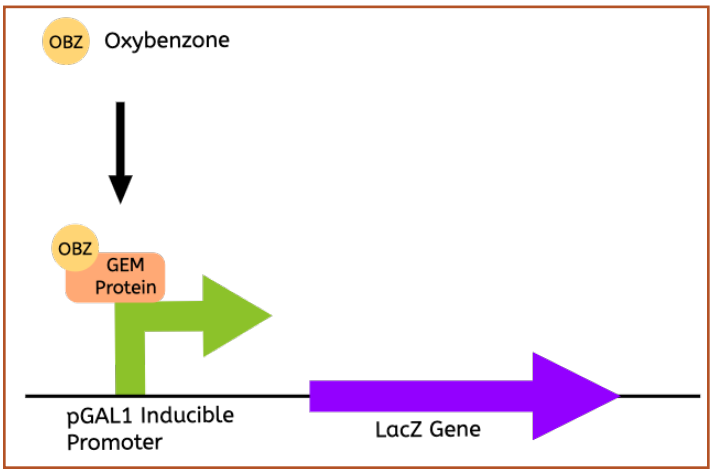

Figure 1. *When oxybenzone (OBZ) binds to the GEM protein from Plasmid One (pHES839), it will induce the pGAL1 promoter of Plasmid Two (pHES875), leading to the expression of the LacZ gene.*

Parts Level

A two plasmid system has been developed to allow for the detection of oxybenzone by *S. cerevisiae* (Aranda-Diaz et al., 2017). Plasmid One (pHES839) contains the constitutive promoter pADH1 and a standard ribosome binding site B0034. Both plasmid sequences end with the double terminator B0015.

Plasmid One codes for the GEM protein, a synthetic fusion protein named for its three main components: a DNA binding domain (**G**al4p-DBD), a human estrogen receptor ligand binding domain (h**E**R-LBD), and a strong activator (**M**sn2p-AD). When hER-LBD binds to estrogen or an estrogenic compound, such as oxybenzone , GAl4p-DBD takes DNA and begins transcription with the help of Msn2p-AD (Figure 2) (Molina-Molina et al., 2008; Morohoshi et al., 2005).

Plasmid Two (pHES875) contains the pGAL1 promoter, which is inducible by the GEM protein produced by Plasmid One when estrogenic compounds bind (Aranda-Diaz et al., 2017). It also contains the LacZ gene (Figure 3), which when expressed will signal the production of beta-galactosidase, an enzyme which breaks down the substrate, para-aminophenol-beta-d-galactopyranoside (PAPG), into galactose and para-aminophenol (PAP), allowing for electrochemical detection (Biran et al., 1999).

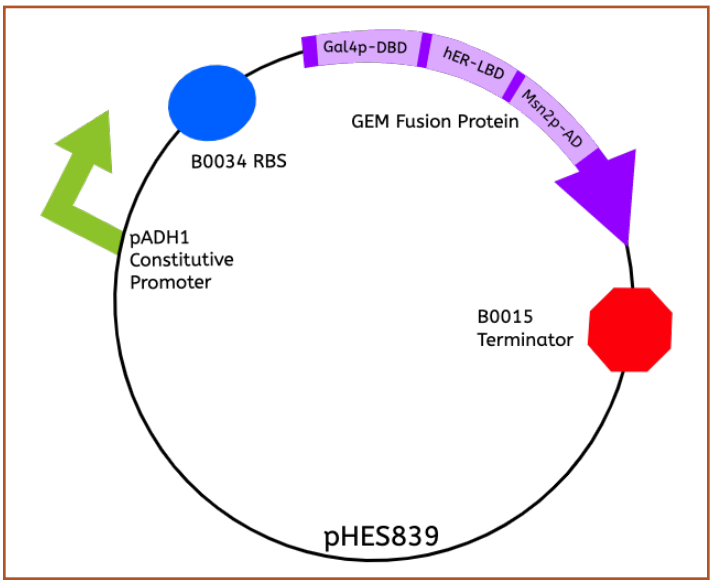

Figure 2. *Plasmid One (pHES839) contains an operon consisting of the constitutive promoter pADH1, the B0034 ribosome binding site, the GEM fusion protein and the B0015 terminator.*

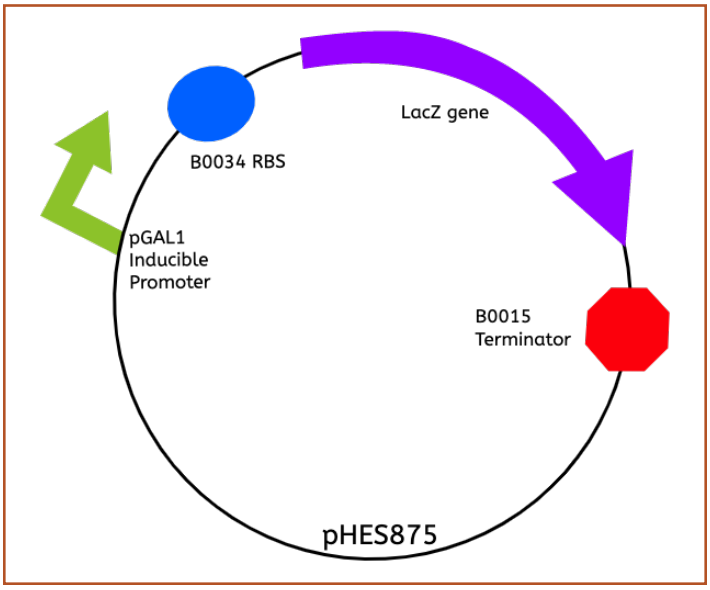

Figure 3. *Plasmid Two (pHES875) consists of the pGAL1 inducible promoter, the B0034 ribosome binding site, the LacZ gene and the B0015 terminator. The pGAL1 promoter is induced by the oxybenzone-GEM complex.*

Safety

Common safety precautions, such as wearing lab coats, goggles, gloves and other necessary PPE, will be taken in the lab when handling experiments. All lab work will be done in a lab with a biosafety level of 1, meaning there is little risk to those performing experiments. Extensive research on oxybenzone has been done by team members to understand the potential concerns of working

with this compound. Oxybenzone is commonly used in skincare products, such as makeup and sunscreen, and will be handled in low concentrations so as to not pose a risk to those working in the lab (National Center for Biotechnology Information, 2021). When the testing of oxybenzone concentrations in water takes place, all water samples will be taken out of the aquatic ecosystems and all the testing will occur in a lab with proper safety precautions. The engineered *S. cerevisiae* will not come into direct contact with the environment, and all water samples with *S. cerevisiae* will be handled with necessary precautions. Once used for testing oxybenzone concentrations, the engineered *S. cerevisiae* will be autoclaved and disposed of using standard procedures. Furthermore, any water samples containing oxybenzone will be disposed of by the professional lab experiment will be performed in, or sent back to the supplier for proper disposal as recommended on the safety data sheet for oxybenzone to prevent oxybenzone re-entering the environment (National Center for Biotechnology Information, 2021).

Discussions

Detection of oxybenzone is the first step in addressing coral bleaching and protecting aquatic ecosystems (Allinson et al., 2018). Without understanding the severity of this issue in coral reefs, nothing can be done to manage this problem. A benefit to this system is that the electrochemical signal produced as a result of the LacZ gene will make data collections accurate and accessible on many devices. Due to the nature of the system, no engineered organisms will come into direct contact with marine ecosystems. A challenge that presents itself in the designing of this project is the accessibility of water samples. While coral bleaching is an important issue, it is not a prominent concern in the landlocked province of Alberta. Once testing and lab work have proven the system, device, and parts levels of this project to be successful, travel may be needed to fully understand and develop real-world applications. Further directions for this project include developing a system to manage oxybenzone contamination in coral reefs, once it has been detected by the current project.

Next Steps

In the future, 3D modeling must be done to further understand the bonding of oxybenzone molecules to the hER. Using computer programming, the fitting of the oxybenzone molecule into the hER will be visually demonstrated to further prove the effectiveness of oxybenzone detection by hER. Mathematical modeling must also be used to predict the electrochemical signal levels produced in relation to certain oxybenzone concentrations. This data will be used for the further development of the project's system level and can be used in future lab work done with *S. cerevisiae*.

Additional lab work to further prove the effectiveness of the developed plasmids will need to be done to expand on and develop this project. The two plasmids will be transformed into *E. coli* and replicated. From there, a miniprep will take the plasmids out, where they will then go into *S. cerevisiae* using a standard lithium acetate transformation (Kawai et al., 2010). For testing purposes, samples of engineered *S. cerevisiae* will be introduced to water samples with specific, known quantities of oxybenzone concentrations. This will allow for information on the system's range and sensitivity to be collected, allowing for accurate data to base analysis of oxybenzone concentrations.

Author Contributions

N.D. carried out literature review of safety information regarding this project. M.L. developed, filmed and edited the video, and O.O. carried out literature analysis for the background of this article. D.H. took the lead in planning and organizing the writing of the manuscript, and wrote the systems level, device level, parts level, discussions and future steps of this article. D.H. also graphically designed all figures used in the manuscript. All authors contributed to and provided feedback on the planning, research and writing of this manuscript.

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