PETase and GFP based bioreporter for rapid detection of microplastics in water

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ABSTRACT

The problem of microplastics is ever growing due to their durability and as they become smaller and smaller, microplastics become harder to detect. Current methods like Visual inspection are time consuming and can be erroneous and rendered useless for very small plastic particles. Fourier transform infrared spectroscopy is much more sensitive but can be expensive and require skilled labor. Our solution aims to create a protein based bioreporter of microplastics using the PETase enzyme's affinity to PET plastics and the GFP protein to signal the presence of microplastics. It will be both cheap and easy to utilize.

INTRODUCTION

Large scale production of Plastics and poor management practices are the major contributors to microplastic pollution in aquatic environments which is environmentally problematic(Nkosi et al., 2023). Microplastics are defined as particles of plastic between 1um-5mm in diameter. Their spread in water ecosystems due to environmental factors and the inherent properties of microplastics, such as density, hydrophobicity, and poor biocompatibility eventually leads them into the marine ecosystem (Kye et al., 2023). Potential effects on living things include oxidative stress, DNA damage, organ dysfunction, metabolic disorders, immune response, neuron-toxicity, and reproductive or developmental toxicity(Pan et al., 2023). Despite recent studies, the research on prevalence of microplastics in Global South and North has lagged behind(Nkosi et al., 2023).Current methods of qualitative and quantitative analysis of microplastics include visual inspection and Fourier-transform-infrared spectroscopy. These methods are time consuming, labor intensive and unfeasible for on-site studies(Zhou et al., 2022). Considering these factors, the early detection of microplastics is key to early detection of the hazard and prevention of these side effects. Furthermore, diagnostic methods of microplastics like bioreporters are crucial for this purpose. Our project presents a solution by production of Bioreporter for a quick, onsite qualitative detection of PET microplastics in aquatic environments.

SOLUTION AND IMPLEMENTATION

General objectives:

We aim to produce a bioreporter which can detect the presence of microplastics of PET in the aquatic environment.

Objectives

Our project aims at constructing a PETase based bioreporter using GFP as signaling molecule. This will involve the construction of an expression cassette of the PETase gene to be fully expressed in E. coli coupled to the green fluorescent protein (GFP) through an appropriate linker sequence. The PET-GFP fusion protein will enable direct detection of microplastic degradation through activation of GFP fluorescence upon interaction of PETase and PET microplastics.

Experimental design

Our experiment aims to create a bioreporter based on PETase enzyme and GFP. In silico we will design an expression cassette using the pET11a+CYP119(Addgene: pET11a+CYP119) plasmid with an insert size of 1109 bp. The cassette will contain the T7 promoter (BBa_I712074), RBS 34 (BBa_B0034), and PETase fused with a His-tag, for purification obtained from Igem parts. Additionally, the GFP gene from *Aequorea victoria* will be added using linker genes to act as a reporter of PET presence. The linker will unbind the GFP once the PETase enzyme attaches the substrate therefore releasing fluorescence in water, indicating microplastic presence.

Using recombinant DNA technology, the E. coli host will be transformed using the recombinant pET11a+CPY119 plasmid which incorporates the cassette. The transformed E. coli will be selected based on Ampicillin resistance and then confirmed using PCR with specific primers.

Gene expression will be induced through the T7lac operator. It is achieved by introducing isopropyl β-D-1-thiogalactopyranoside (IPTG), a lactose analog, into the bacterial culture. IPTG binds to the lac repressor, releasing it from the lac operator and allowing RNA polymerase to transcribe our gene of interest. The broth culture will then be exposed to a known amount of microplastic to test whether it can act as an effective indicator of microplastic presence.

Expected results

We expect to observe fluorescence color in the sample broth with microplastics. Microscopic analysis will be used to asses fluorescent for tiny particles of plastics invisible to the naked eye.

Material and methods

method	**procedure and materials**
1. plasmid construction	plasmid: pET11a+CYP119 from addgene IsPETase gene with the size: 1109 bp with His-tag (obtained from Igem parts). Restriction enzymes: ZraI and AlwNI. (still not yet disseminated)
2. Gene cloning and Bacterial transformation	Bacterial strain:E.coli cells(DH5alpha). selectable marker: Ampicillin ressistance Ampicillin antibiotic: 100μg/mL PCR for confirmation of the transformed cells.
3. Gene expression induction	The optical density of 0.6 cultures will be induced by 0.1 mM IPTG and will be further incubated at 18°C for 24hrs(Seo et al., 2019).

4. <u>Gel electrophoresis</u>	used for the detection of the transformed cells due to the display of the band size including the gene of interest.
5. <u>protein purification</u>	Cell lysis will be done followed by exposure to the Nickel matrix that binds the His tag. Then the debris will be washed leaving the chimeric protein. Mass spectrometry and SDS-page will be used to confirm the presence of the PETase-GFP protein
6. <u>Microplastic preparation and exposure for PETase- GFP detection</u>	PET plastic will be purchased from a manufacturer and then crushed at low temperature to achieve size less than 100 um. Microscopic examination will be utilized to observe the level of fluorescence.

CONCLUSION

In a nutshell, our project outlines a promising approach to create a bioreporter capable of detecting PET microplastics in aquatic environments. It involves engineering E. coli bacteria to produce a PETase-GFP fusion protein that fluoresces in the presence of PET. The experimental design includes plasmid construction, gene cloning, protein expression, and exposure of microplastics for testing. Successful development of this bioreporter would offer a valuable tool for early detection and monitoring of microplastic pollution, contributing to environmental protection and health management.

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