ENGINEERING METABOLIC PATHWAYS IN E. COLI FOR INCREASED CARBON FLUX TOWARDS PHB PRODUCTION

(Technical Paper)

AN INVESTIGATION OF SUSTAINABLE AND BIODEGRADABLE PLASTIC PRODUCTION WITHIN THE BIOMANUFACTURING INDUSTRY TO REDUCE SINGLE USE PLASTIC POLLUTION

(STS Paper)

A Thesis Prospectus submitted to the

Faculty of the School of Engineering and Applied Science
University of Virginia | Charlottesville, Virginia

In partial fulfillment of the requirements of the degree
Bachelor of Science, School of Engineering

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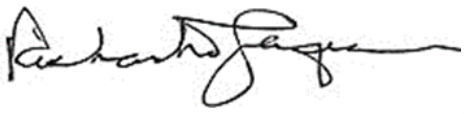
November 2021

On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.



Approved _____ _____ Date 24 November 2021____
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Introduction

Plastic pollution has recently become one of the most pressing environmental issues, especially as the rate of disposable plastic usage rapidly increases, overwhelming the world's ability to safely dispose of them. Single use plastics can include anything from bottles, bags and food packages and are the most commonly discarded type of plastic. This is more important now than ever, since half of all plastics ever manufactured have been made in the last 15 years. The plastics break down into microplastics, which are small particles, often less than one-fifth of an inch across. These microplastics, spread across the globe, are virtually impossible to recover, and have been found in municipal drinking water systems and drifting through the air.

Technical Discussion

Each year, 380 million tons of plastic waste is produced globally with an estimated half of that waste being for single use purposes only. Single-use plastic is manufactured to last forever, however is often only used for a few minutes before being thrown away, and not even recycled ("Plastic Pollution Issues | The Problems With Plastic," n.d.). The reliance on synthetic plastics prolongs the release of toxic emissions into the environment, not only polluting the environment but exposing all living organisms to carcinogens. For example, bisphenol A (BPA), found in polycarbonate bottles and the linings of food and beverage cans, can leach into food and drinks. The U.C Centers for Disease Control and Prevention reported that 93% of people had detectable levels of BPA in their urine. Exposure to high BPA levels in premature infants is an area of great concern and research suggests that BPA may cause cancer in people. BPA is a weak synthetic estrogen found in many rigid plastic products, food and formula can linings and cashier receipts. It has an estrogen-like activity that makes it a hormone disruptor, which is a commonly shared trait among other chemicals in plastics. Hormone disruptors can affect how estrogen and other hormones act in the body, by blocking or mimicking them, which can throw off the body's hormonal balance (*Exposure to Chemicals in Plastic*, 2020).

Although there are currently many different types of plastic that are in the market, the majority of them are not biodegradable and can hang around in the environment for hundreds of years and can cause you to be exposed to chemicals such as BPA. Therefore, a solution to this is to move towards a more sustainable option such as polyhydroxybutyrate (PHB). Biodegradable bioplastics, such as PHB, offer a sustainable alternative as they are natural byproducts of facilitated degradation of these synthetic plastics, such as styrene. PHB is a bio-based plastic that offers a more efficient and fully circular solution to plastic pollution.

Transfoam LLC, is a biomanufacturing platform that tackles single-use plastic pollution at the beginning and end of the product lifestyle. Transfoam has three main priorities they are

focusing on: remediating global plastic pollution, manufacturing fully biodegradable plastic, and harnessing the power of microorganisms. Specifically, Transfoam employs an engineered strain of *E. coli* to turn waste into PHB to make healthier consumer goods and packaging. The specific strains of *E. coli* employed in the bioprocess possess the central metabolic phenylacetic- acid (PAA) pathway, and therefore the ability to convert phenylacetic acid to acetyl-coA, which acts to bridge the engineered pathways. The acetyl-coA then goes through a plasmid called *pha* in order to produce PHB.

The optimization of the PAA pathway will be done in a laboratory environment by genetically engineering the pathway in order to maximize acetyl-coA production. The use of CRISPR-Cas9 and related technologies can be employed to make such modifications to the *E. coli* genome. Once the modifications are made and there is an increased efficiency of phenylacetic acid to acetyl-coA conversion, we are hoping that there will be a significant increase in PHB production suited for commercial applications. There will also be a modeling component of this project, where we develop a metabolic model to identify gene candidate modifications. To do this, a flux-balance model will be conducted to identify relevant gene pathways to knockout for increased carbon flux towards PHB production.

The aim for the flux-balance model is to design a genome-scale metabolic model (GEM) for *E. coli* strains TG1 and W involving the sty and pha plasmids to stimulate gene-protein reactions and associations to predict metabolic fluxes. In order to do this, an optimal set of metabolic reactions involved in the PAA and other pathways for a published *E. coli* genome-scale metabolic model to maximize the production of acetyl-CoA would need to be determined. Then, incorporate these metabolic reactions and metabolites from the sty and pha pathways into the published *E. coli* model. The flux-balance model will be constraint based and used to verify that the modifications follow the conservation of mass and energy by calculating the flux of metabolites through the network. The flux-balance model will then be used to model the consumption of styrene and production of biomass and PHB of the modified *E. coli* model on various growth mediums, and determine the effects of additional metabolites and gene modifications on these outcomes. The ideal output for this is a genome-scale metabolic model for *E. coli* strains TG1 and W that can be used to make model-based decisions on how to increase the flux into acetyl-CoA and eventually improve PHB production.

An additional aim for this project is to perform CRISPR/Cas9 editing on targeted pathways to maximize the acetyl-CoA output. The first step to this would be to identify the repressor genes and/or activator molecules to modify the expression of key genes involved in the metabolic pathways inherent to *E. coli* for gene knockout experiments. The pathways that should be explored are the PAA pathway, the AckA-Pta and fatty acid biosynthesis pathways which both utilize acetyl-CoA as reactants in the synthesis of acetate and fatty acids, respectively. The next step is to identify a possible molecular switch to limit the activity of metabolic pathways

consuming acetyl-CoA separate from the PHB production pathway. This is where CRISPR/Cas9 is used to perform all identified gene edits to the *E. coli* genome. The final step is to validate gene edits using Surveyor nuclease in a mismatch cleavage assay to detect indels, which are insertion-deletion mutations. Surveyor nuclease is an assay used to detect single base mismatches or small insertions or deletions. The assay consists of PCR amplification of the target region of the identified gene(s), hybridization to form heteroduplexes between mutant and wild-type reference DNA, Surveyor nuclease treatment of the annealed DNA to cleave heteroduplexes, and analysis of the DNA on an agarose gel or another method of DNA separation based on size. The ideal output of this is a genetically modified strain of *E. coli* TG1 or W that can maximize acetyl-CoA production from PAA as the growth medium with the ultimate goal of increasing PHB production (*Frontiers | The Impact of AckA, Pta, and AckA-Pta Mutations on Growth, Gene Expression and Protein Acetylation in Escherichia Coli K-12 | Microbiology*, n.d.).

In summary, the primary goal of this project is to utilize inexpensive waste as a raw material to reduce both the cost and carbon footprint of PHB production. To prove that this approach will work, we must confirm the production of PHB in engineered E coli TG1 and W strains in a medium that contains styrene as the primary carbon source.

STS Discussion

Large corporations are under constant scrutiny by the public for their role in environmental destruction on a global level because of their high consumption rate of single use plastics. In fact, just 20 companies are responsible for over half of the single use plastic consumption in the entire world. At the top of this list is U.S's energy giant ExxonMobil, contributing 5.9 million metric tons to global plastic waste (*20 Companies Responsible for 55% of Single-Use Plastic Waste: Study*, n.d.). However, ExxonMobil has made several public statements, claiming that they are taking action to address plastic waste by increasing plastic recyclability and supporting improvements in plastic waste recovery.

In addition, in the last five years, an increase in consumer and regulatory pressure for more sustainable goods and practices has led to the rapid emergence of the biodegradable plastics market. Biodegradable plastics can replace traditional materials in nearly 90% of consumer packaging applications, which is a $95B segment of the greater $200B plastic resins market. However, due to the infancy of the PHB industry, only a small handful of manufacturers around the world have successfully reached the full plant scale. In the last few years alone, investor interest in this opportunity has grown tremendously as recognizable brands like Bacardi and Nestle have publicly declared PHB integral to their sustainable transitions, creating a massive opportunity for new, innovative manufacturers to enter the rapidly growing market to help fill the unmet demand.

The general topic of my STS research will pertain to creating a more sustainable and biodegradable plastic, produced from waste products, that large corporations can replace current plastics with, which is tightly coupled to the technical project. I hope to optimize a biological process that will allow E. coli bacteria to more efficiently convert styrofoam into PHB, an alternative to single use plastics. PHB is a non-toxic plastic alternative and performs the same functionalities of conventional plastics, such as durability barrier properties, and shelf life. Maximizing the production of acetyl-CoA is crucial for the optimal production of PHB in order to compete with the mass manufacturing of conventional plastics. Contrary to our competitors, we are optimizing the yield and cycle time of turning waste plastics into PHB, creating a more scalable process. Using a synthesis of metabolic modeling and genetic modification techniques on the most studied natural machine, the goal of PHB production is to not only produce sustainable plastic, but to also produce it sustainably.

Conclusion

Single use plastics have been normalized and deemed acceptable because of their convenience and efficacy. However, the harsh effects of this are escalating and becoming increasingly apparent in the environment around us. That is why a new and more sustainable form of plastic, such as PHB, is needed now more than ever to ensure the safety of the environment and everyone involved. Not only is PHB biodegradable, it is able to be manufactured from styrene, a monomer of styrofoam, which is one of the largest sources of waste. If large corporations such as ExxonMobil began the switch over to PHB, it would take less energy to manufacture, and decrease the waste sent to landfills or incinerators, all while recycling old waste products (*Biodegradable Plastic Guide: Explore the Pros, Cons, and Uses - 2021 - MasterClass*, n.d.). This would greatly benefit the environment and help protect the living organisms by reducing the exposure to carcinogens.

References

https://www.cnbc.com/2021/05/18/20-companies-responsible-for-55percent-of-single-use-plastic-waste-study.html

https://www.plasticfreejuly.org/the-plastic-pollution-issues/

20 companies responsible for 55% of single-use plastic waste: Study. (n.d.). Retrieved October 13, 2021, from https://www.cnbc.com/2021/05/18/20-companies-responsible-for-55percent-of-single-use-plastic-waste-study.html

Biodegradable Plastic Guide: Explore the Pros, Cons, and Uses—2021—MasterClass. (n.d.). Retrieved October 13, 2021, from https://www.masterclass.com/articles/biodegradable-plastic-guide

Exposure to Chemicals in Plastic. (2020, September 11). Breastcancer.Org. https://www.breastcancer.org/risk/factors/plastic

Frontiers | The Impact of ackA, pta, and ackA-pta Mutations on Growth, Gene Expression and Protein Acetylation in Escherichia coli K-12 | Microbiology. (n.d.). Retrieved October 31, 2021, from https://www.frontiersin.org/articles/10.3389/fmicb.2020.00233/full

Plastic Pollution Issues | The Problems With Plastic. (n.d.). *Plastic Free July*. Retrieved October 13, 2021, from https://www.plasticfreejuly.org/the-plastic-pollution-issues/