Effects of Polyhydroxybutyrate Production on Cell Division

National Aeronautics and Space Administration

Kathleen Miller[1], Asif Rahman[2], and Masood Z. Hadi[2]

[1] Stanford University, Stanford CA

[2]Synthetic Biology Program, Space Biosciences Division, NASA Ames Research Center, Moffett Field, CA

Abstract

Synthetic biological engineering can be utilized to aide the advancement of improved long-term space flight. The potential to use synthetic biology as a platform to biomanufacture desired equipment on demand using the three dimensional (3D) printer on the International Space Station (ISS) gives long-term NASA missions the flexibility to produce materials as needed on site.

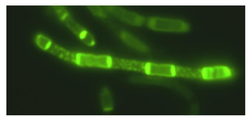
Polyhydroxybutyrates (PHBs) are biodegradable, have properties similar to plastics, and can be produced in *Escherichia coli* using genetic engineering. Using PHBs during space flight could assist mission success by providing a valuable source of biomaterials that can have many potential applications, particularly through 3D printing.

It is well documented that during PHB production *E. coli* cells can become significantly elongated. The elongation of cells reduces the ability of the cells to divide and thus to produce PHB. I aim to better understand cell division during PHB production, through the design, building, and testing of synthetic biological circuits, and identify how to potentially increase yields of PHB with FtsZ overexpression, the gene responsible for cell division.

Ultimately, an increase in the yield will allow more products to be created using the 3D printer on the ISS and beyond, thus aiding astronauts in their missions.

Introduction

During cell division FtsZ is the first protein to move to the cell division site and recruits other proteins that produce a new cell wall between the dividing cells. Interestingly the *ftsZ* gene is also present in other organisms making this study relevant to other synthetic biological systems. The goal of this project is to overexpress the *ftsZ* gene in PHB producing *E. coli* cells and determine if the yield of bioplastic production increases.



An elongated cell producing PHB

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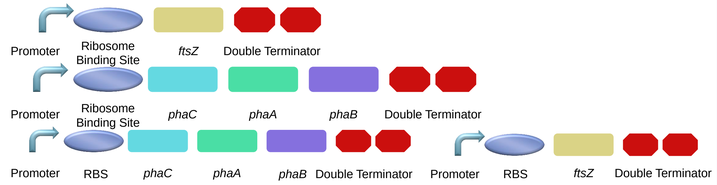
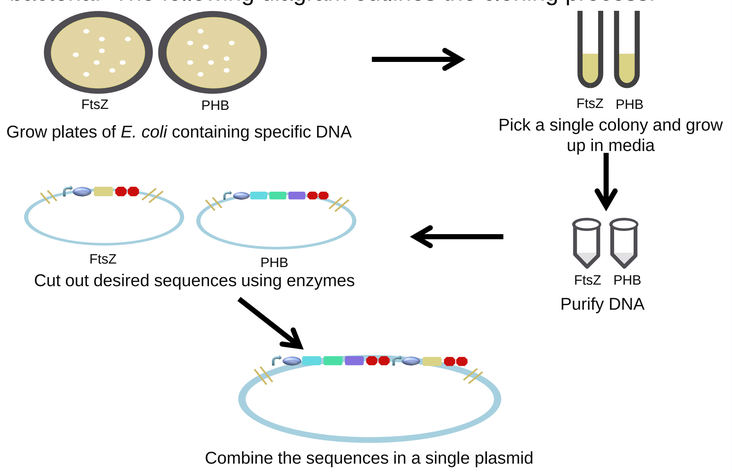
Design

DNA constructs are artificially constructed nucleic acid segments to be inserted into cells. This project utilizes three different constructs, one containing the *ftsZ* gene, one containing *phaC*, *phaA*, and *phaB*, which produces PHB, and one that is the combination of the first two constructs.



Methods

The major method utilized in this project is molecular cloning. This process is a gene amplification technique where recombinant DNA is constructed *in vitro* and amplified *in vivo* inside *E. coli* bacteria. The following diagram outlines the cloning process.

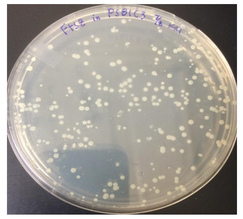


Primer Design

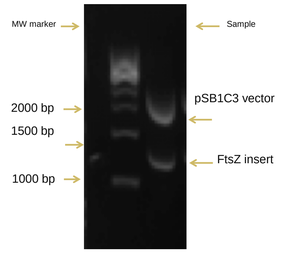
Performing a Polymerase Chain Reaction (PCR) allows billions of copies to be made of a specific DNA sequence. Primers are short pieces of DNA that are designed to match the segment of DNA to be copied. For this project primers were used to make various copies of the *ftsZ* gene.

Direction	Sequence	Description
Forward	atgtttgaaccaatggaacttaccaa	Used to amplify the *ftsZ* gene
Reverse	atcagcttgcttacgcaggaatgct	
Reverse	ttaatcagcttgcttacgcaggaatg	Amplification of *ftsZ* including stop codon
Reverse	gaattcgccagaaccagcagcgga	Amplification of *ftsZ* including linker
Forward	gacaggatctagaatgtttgaaccaatggaacttacc	Used to amplify the *ftsZ* gene in RFC23
Reverse	atctgcagcggccgctactagtatcagcttgcttac	
Forward	cgagatctatgtttgaaccaatggaacttaccaatgac	Used to amplify the *ftsZ* gene in RFC21
Reverse	atcagcttgcttacgcaggaatgctgggatatccagat	

Results



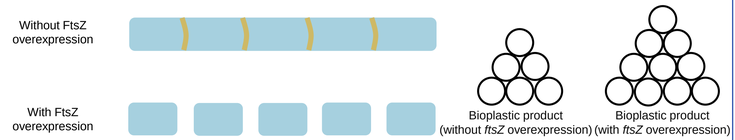
A chloramphenicol agar plate with colonies containing *ftsZ*



An agarose gel containing the *ftsZ* insert and pSB1C3 vector

Conclusions/Future Work

It is anticipated that overexpression of *ftsZ* will improve cell division. Improving cell division has the potential to increase the yield of PHB production in *E. coli* bacteria and thus allow for higher bioplastic production. In addition, understanding cell division will be important in a microgravity environment and across other species.



Applications

Bioplastic 3D printer at NASA SpaceShop Products

Acknowledgements

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