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Bioproduction of Molecules for Structural 3D Printing Filaments

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Bioproduction of Molecules for Structural 3D Printing Filaments

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Abstract

In our laboratory, we are focused on the study of plant cells and their use in daily, real-world applications. Our main goal is to produce organic, conductive, and biodegradable material to be used by KAMPERS collaborators. *Physcomitrella patens* is the model organism we have used. We have created a *ggb* knockout mutant line of *P. patens* which is long lasting (immortal) and advantageous over wild-type strains for use in bioreactors. Our laboratory has identified several different metabolic pathways that have potential uses in creating conductive material for use in 3D printing. These pathways are the polyisoprene pathway, the polyacetylene pathway, and the polythiophene pathway. These pathways will be manipulated in *P. patens* to maximize the production of the monomers needed for polymerization of these materials. Our model systems will be optimized to efficiently create these materials and increase their biomass. We have also found that Eumelanin is a promising conductive material.

Introduction and Objective

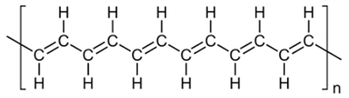
Due to the increasing demand of biodegradable and biorenewable materials in our society today, finding a cheap, green, and renewable source for use in industrial settings is an important challenge to overcome. We will be utilizing unique and different model systems to find materials that can be broken down by enzymes found in nature from bacteria and fungi. Multiple different model systems can be utilized for heterologous expression of many different types of materials, compounds, and products that could be used as green resources. Common model systems that have been studied include Arabidopsis, tobacco, and maize. DNA is inserted randomly into these model systems in a variety of ways in which insert foreign DNA into a host. Specifically, in *Physcomitrella patens* homologous recombination gene replacement can be used to insert foreign DNA. This system has been studied extensively over the past decade and we plan to utilize this system to produce green materials for industry. The goal of this project is to use the modified *P. patens* to produce novel materials such as to three-dimensional printing fibers which could greatly impact society today.

Objective:

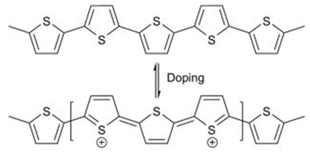
Objective 1.1.2 – "Explore to what extent new materials synthesized through synthetic biology can yield superior structural polymers or resins with programmable lifetimes."

Molecules of Interest and Polymers

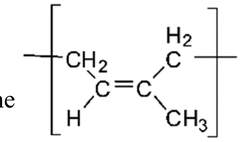
Conductive Organic Materials



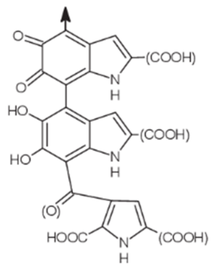
Polyacetylene



Polythiophene



Polyisoprene



Dihydroxyindoles

Eumelanin

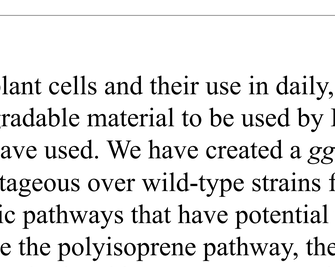
Model Systems and Manipulations

Moss (*Physcomitrella patens*)

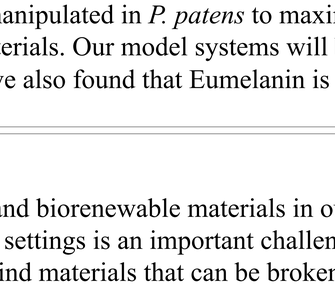
- Due to its unique ability to use homologous recombination gene replacement, Wildtype *P. patens* has been greatly studied as a model system.
- The compound terpenoids, biopharmaceuticals, and cosmetic products have all been produced from the use of *P. patens* in bioreactors.

ggb Mutant (*Physcomitrella patens*)

- *ggb* mutant *P. patens* generated in our lab is unique and important due to its capability of sustaining an undifferentiated cell line.
- It can also still use homologous recombination for transformation of foreign DNA as in the Wildtype.

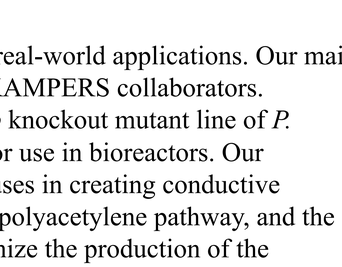


P. Patens ggb mutant



P. Patens Wild type

Plasmid Vector Designs



Cloning Vectors

- This was used to make copies of certain genes.

T1OG Plasmid Vector

- This vector is used to insert specific segments of DNA containing gene of interest into plant systems. The T1OG and PGX8 plasmids will be used for both constitutive and inducible systems.

Transfection and Cloning Genes

Escherichia coli cells will be transfected with the cloning vector once the vectors have been created. These cells carrying the new vector will be formed to have antibiotic resistance thus will be able to survive on the complemented media. As each *E. coli* cell undergoes cell division, the vector will also undergo reproduction thereby making copies of our gene of interest. After the vectors have been harvested from the cells, restriction enzymes will be used to ascertain our gene of interest from the cell. Finally, these genes of interest will be placed into destination vectors which will undergo transformation into our model system and therefore homologous recombination as well.

Experimental Design

Genetically Modify Model Systems

PEG mediated transformation will be utilized to transform *P. patens*. The antibiotics that we will use will selectively produce cells with our genes of interest which will be grown in a liquid media for several days. RNA extraction, agarose gel electrophoresis, and PCR will each be used to evaluate the extent of the genetic modification in the *P. patens*. To ensure and confirm that genetic modification did, in fact, take place, sequencing will be done to identify the genes of interest in their specific frames.

Assess Biomolecule Production and Gene Expression

The quantity of the production of the desired materials from our research will be obtained through gas chromatography. Liquid chromatography will be used to separate these desired materials from undesired products if the amount is found to be significant. RT-qPCR will be used to evaluate the expression levels of our gene of interest and Western blots will be used to evaluate protein levels. If the production of desired materials is insignificant, alterations to the vector systems will be performed and genome sequencing may be performed.

Analytical Methods

Genetic Modifications

- Antibiotic selection
- Electrophoresis
- PCR
- RT-qPCR
- Western blots
- Sequencing

- Thin layer chromatography – identify of interest materials
- Liquid chromatography – separate materials into fractions
- Gas chromatography – quantify composition of products

Acknowledgments and Future Work

Acknowledgements

- NSF EPSCoR RII Track-1 #1849213, Kentucky Advanced Manufacturing Partnership for Enhanced Robotics and Structures
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Future Work

- Advance the production of the *ggb* mutant among other necessary materials.
- Increase production to industrial size.
- Utilize these current systems to develop other materials.