Research Experience for Undergraduates Program

Genetic Engineering for Biofuel and Chemical Production

A reversal of the β-oxidation cycle for the production of industrial chemicals

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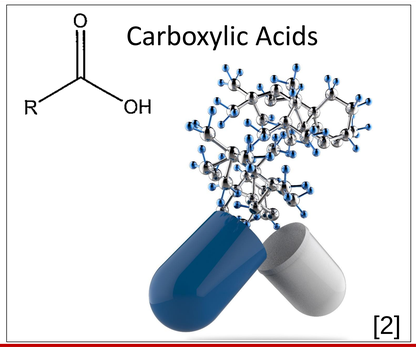
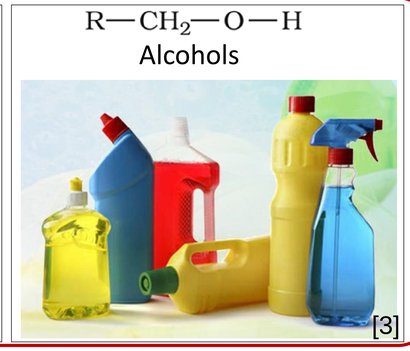
Introduction: Biosynthesis is the production of chemicals using living organisms. The engineering of biological systems has garnered attention recently due to the increased demand for sustainable conversion and production of biofuels and other industrial chemicals. The biological pathway of interest is a multi-turn reverse β-oxidation cycle. Our hypothesis is that by selecting for different genes the reverse β-oxidation cycle will efficiently produce fatty alcohols with minimal side products.


E-coli Fermentor [1]

Implications: Utilizing biosynthesis, bioprocesses can be engineered to minimize side products and cut out energy wasting steps. This is a step forward in the development of sustainable renewable fuels that can begin to displace our reliance on non-renewable petrochemicals.



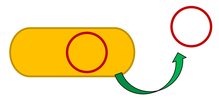
[2]



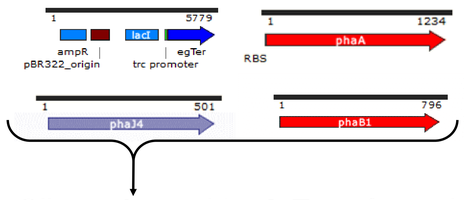
[3]

Methods:

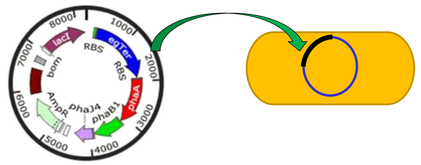
1. Plasmid/Genomic DNA Preparation


pTRC99a-egTER
or PSMB6-paPhaJ4

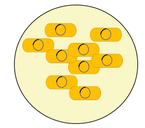
2. Selection of Gene Inserts



3. Gibson Assembly **4.** Transformation

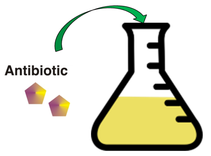


5. Colony PCR

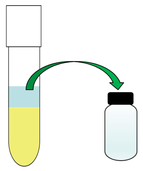


6. Overnight Culturing

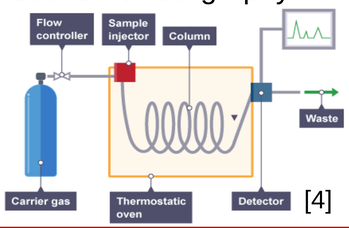
7. Production Culturing



8. Chemical Extraction



9. Gas Chromatography



[4]

Theory: The reverse β-oxidation cycle adds two carbons to the chain with each turn.

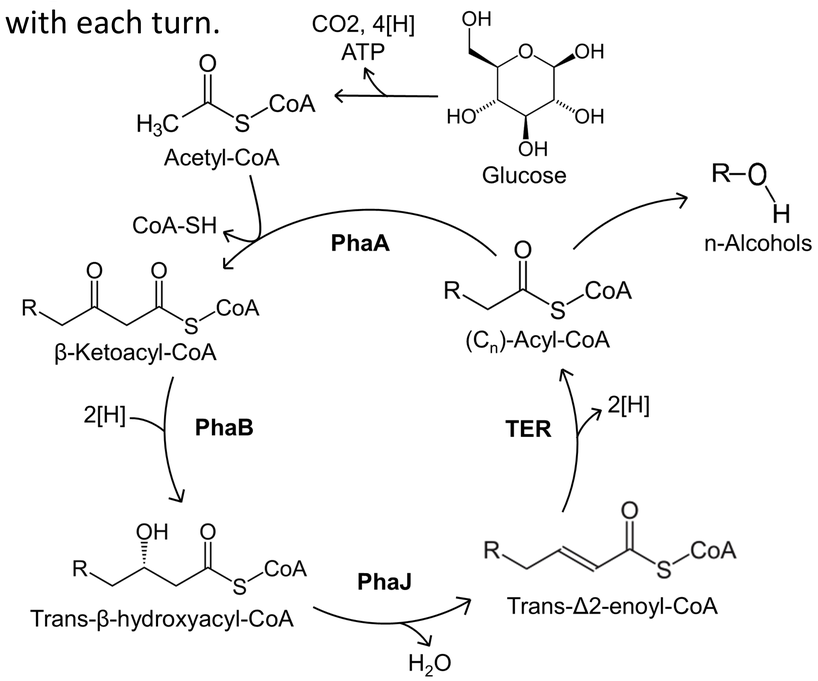


Figure 1: A functional reverse β-oxidation cycle with an alcohol product

Discussion: There are many benefits to a modular reverse β-oxidation cycle including:

- easy transferal to many different host organisms
- ability to fine-tune the cycle for specific products
- optimizable efficiency by minimizing cell maintenance needs and maximizing kinetics [5]

To optimize our process, we substituted the genes shown and are working with four genera of TER. There are many other termination pathways that can be selected for using other genes. For example, the Gonzales group successfully manufactured carboxylic acids [5]. In order to manufacture alcohols, we are inserting the PhaABJ genes into *E. coli*. These genes encode the corresponding steps in Figure 1. Alongside this, we are testing four genera (egTER, ppTER, tdTER, and vhTER) for efficiency of conversion.

Future Research: In the future, we look to test our strains for efficiency and specificity of end products. We will use these results to determine the best genetic sequences for a variety of target products, starting with alcohols. After experimentally determining this, we can do an industrial scale up.

References:

[1] BIOMM Technology, "1 - *E. coli* fermentation," (7/24) [www.biomm.com]
[2] Phantom Plastics, "Biodegradable polymer," (7/20) [www.phantomplastics.com]
[3] Harel Mac Exports, "Soaps and Detergents," (7/20) [www.harelmacexports.com]
[4] The BBC, "GCSE Bitesize: Gas Chromatography," (7/24) [www.bbc.co.uk/schools/gcsebitesize/]
[5] Clomburg, James M., Vick, Jacob E., Blankschien, Matthew D., Rodríguez-Moyá, María, Gonzalez, Ramon (2012). "A synthetic biology approach to engineer a functional reversal of the β-oxidation cycle." ACS Synthetic Biology 541-554.

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