"Two Birds, One Stone"

Reduction of HMBPP by the Iron-Sulfur Protein (IspH) for Isoprene Synthesis

Valders SMART Team: Rebecca Ansorge, Angela Brandl, Grace Ebert, Elizabeth Evans, Theresa Evenson, Breanna Glaeser, Phoenix Kaufmann, Zack Leschke, Mitchel Meissen, Paige Neumeyer, Alexis Patynski, Ian Schmidt, & Christopher Singer

Teacher: Mr. Joseph Kinscher   Mentor: Dr. Eric Singsaas, Professor of Biology, University of Wisconsin- Stevens Point & Research Director, Wisconsin Institute for Sustainable Technology

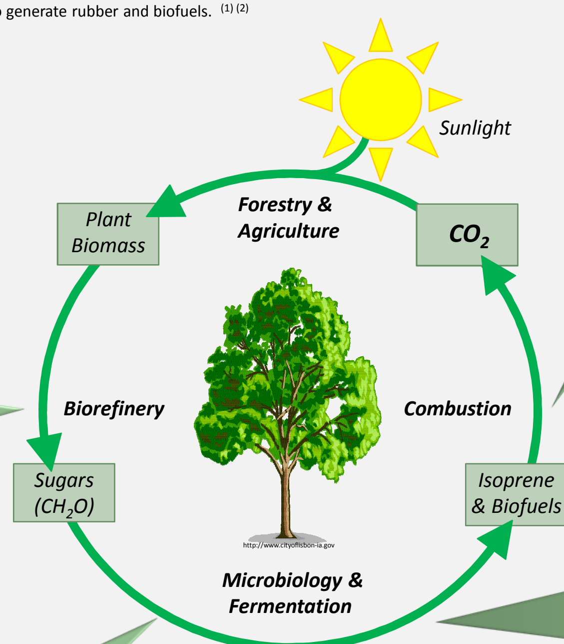
Abstract: A global natural rubber shortage may exceed one million tons by 2020, according to the International Rubber Study Group. While global demand is increasing for rubber, a fungus *Microcyclus ulei*, is killing rubber trees in South America and reducing supply of rubber globally. This has resulted in new monoculture plantations being developed in Southeast Asia where rainforests once existed. In order to satisfy demand for rubber in the future, renewable isoprene, a hydrocarbon produced by the nonmevalonate pathway used by bacteria, may be polymerized and used to create natural rubber and biofuels. Renewable isoprene production requires the iron-sulfur protein IspH, which reduces the substrate HMBPP (1-hydroxy-2-methylbut-2-enyl-4-diphosphate) to produce IPP (isopentyl diphosphate) and DMAPP (dimethylallyl diphosphate), the two precursors in the nonmevalonate pathway. The Valders SMART Team used 3D printing technology to model IspH. The substrate HMBPP enters the active site, and the rotation of HMBPP's hydroxymethyl group allows a complex to form with the Fe_4-S_4 cluster. The complex is further stabilized by the IspH amino acid Thr167 and HMBPP hydrogen bonding with Glu126. Electron transfer from the Fe_4-S_4 cluster and the protonation of HMBPP by Glu126, results in IPP or DMAPP in an approximate ratio of 6:1 at equilibrium. Industrial production of isoprene would be favored by shifting the IPP/DMAPP equilibrium towards DMAPP, as DMAPP is directly catalyzed into isoprene. By understanding how DMAPP production may be increased, the mass production of isoprene could be made more efficient to generate rubber and biofuels. [1] [2]

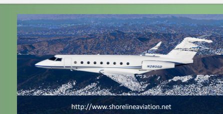
V A L D E R S

S M A R T

T E A M

Introduction: In Wisconsin, an average of one paper mill has been decommissioned each year since 2006, resulting in a loss of 300 to 600 jobs per mill. Overall, hundreds of millions of dollars are lost from the Wisconsin economy. If some paper mills shift their production focus to biorefinery capabilities, pulp could be fermented by engineered bacteria in order to create renewable isoprene. Isoprene (C_5H_8) is a hydrocarbon building block for many oil based products that could be used to produce rubber, jet fuel, and other value added products. This provides an opportunity to address two problems with one solution like hitting two birds with one stone. [3]





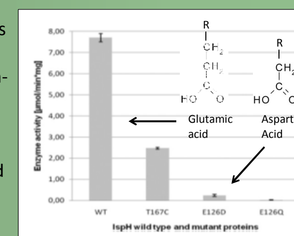
V. Bio-products: The combustion of bio-fuels produces and releases CO_2 into the atmosphere to repeat the cycle. Isoprene can be made into jet fuel, rubber, and value added products.

Future Directions:
A better understanding of how IspH produces IPP and DMAPP would lead to a more efficient production of renewable isoprene by biorefineries. The industrial production of isoprene may save jobs at paper mills that have recently become idle in states like Wisconsin. Production of renewable isoprene could also slow deforestation in regions such as Southeast Asia where rainforests are cut down to establish rubber tree plantations.

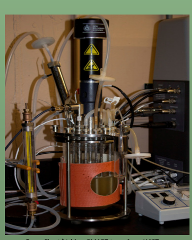
I. Biorefinery: By using biorefineries to produce biofuels, rubber, and other value added products from isoprene, the dependence on energy from fossil fuels will decrease. Idle or underused paper mills can provide the infrastructure for biorefinery capabilities. Biorefineries can use renewable resources, such as wood, pulp mill waste, and other cellulosic feed stocks to create renewable isoprene.

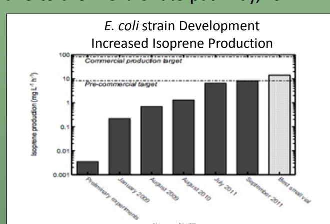
IV. Threonine 167 and glutamic acid 126 are important to the activity of the IspH enzyme

Wild-type IspH enzyme activity is high as shown to the right. Threonine 167 stabilizes the iron-sulfur complex for HMBPP reduction. Glutamic acid 126 hydrogen bonds with HMBPP, stabilizes it in the active site, and donates a proton to HMBPP. When Threonine 167 and Glutamic acid 126 are mutated, enzyme activity for the reduction of HMBPP is decreased drastically. Glutamic acid and Aspartic acid have a structural difference of only one carbon atom and yet, this mutation decreases enzyme activity greatly. [1]



II. Biorefining Research: WIST (Wisconsin Institute for Sustainable Technology) researchers have demonstrated a fermentation system using transformed *E. coli*. These engineered microbes are used to increase the production of isoprene by the MEP (2-C-methyl-D-erythritol 4-phosphate) pathway. Also known as the non-mevalonate pathway, the MEP pathway is found in plants, some parasitic protozoa, and bacteria. The pathway is used by these organisms, as an alternative to the mevalonate pathway, for isoprenoid biosynthesis. [1]





The graph shows how the production of isoprene increased due to genetic engineering of *E. coli*.

III. Non-Mevalonate (MEP) Pathway & Renewable Isoprene

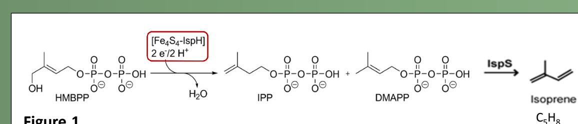


Figure 1

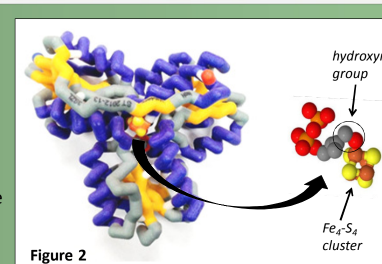


Figure 2

The rotation of HMBPP's hydroxy-methyl group allows a complex to form with the Fe_4-S_4 (iron-sulfur) cluster in the IspH active site.

Renewable isoprene would be produced by the non-mevalonate pathway. The final steps of this pathway are shown in **figure 1**. IspH is an enzyme **(fig. 2)** of the non-mevalonate pathway and is crucial to catalyzing the production of IPP and DMAPP. Electron transfer from the Fe_4-S_4 (iron-sulfur) cluster **(fig. 1 & fig. 2)** and the protonation of HMBPP results: Fe_4-S_4 + HMBPP → IPP (6) + DMAPP (1). Industrial production of renewable isoprene would be favored by shifting the IPP/DMAPP equilibrium towards DMAPP, as DMAPP is directly catalyzed into isoprene. [1]

References:

(1) Span, I. et al. Crystal Structures of Mutant IspH Proteins Reveal a Rotation of the Substrate's Hydroxymethyl Group during Catalysis. Journal of Molecular Biology, November 2011
(2) Mann, Charles C. Addicted to Rubber. Science Magazine, vol. 325, July 2009
(3) Schmid, John. Wausau Paper to sell three mills, including two in state. Milwaukee – Wisconsin Journal Sentinel, January 2013

The SMART Team Program (Students Modeling A Research Topic) is funded by a grant from NIH-SEPA 1R25OD010505-01 from NIH-CTSA UL1RR031973.