Development of a Methanotroph Chassis: Genetic engineering of *Methylomicrobium album* BG8 for production of value-added products

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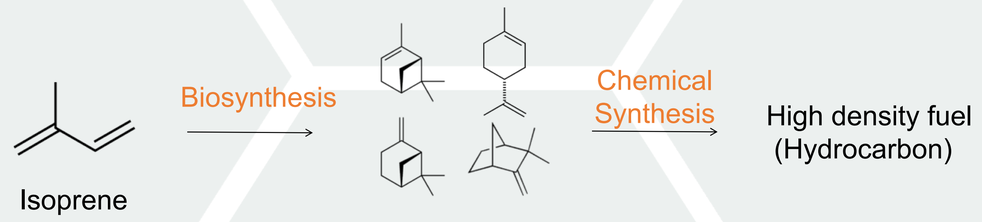
Methanotrophy: Solution for C1 waste

- Methanotrophs consume single carbon (C1) compounds, such as methane and methanol, for energetic needs and biomass
- C1 compounds are produced as waste by industries such as oil and gas, forestry, agriculture and waste treatment.
- *Methylomicrobium album* BG8 is a rapidly-growing, robust strain whose genetic content and physiology are well defined, making it a candidate for industrialization
- Initial efforts have focused on isoprene production, due to its potential as a precursor of various industrially relevant compounds such as a biojet fuel.

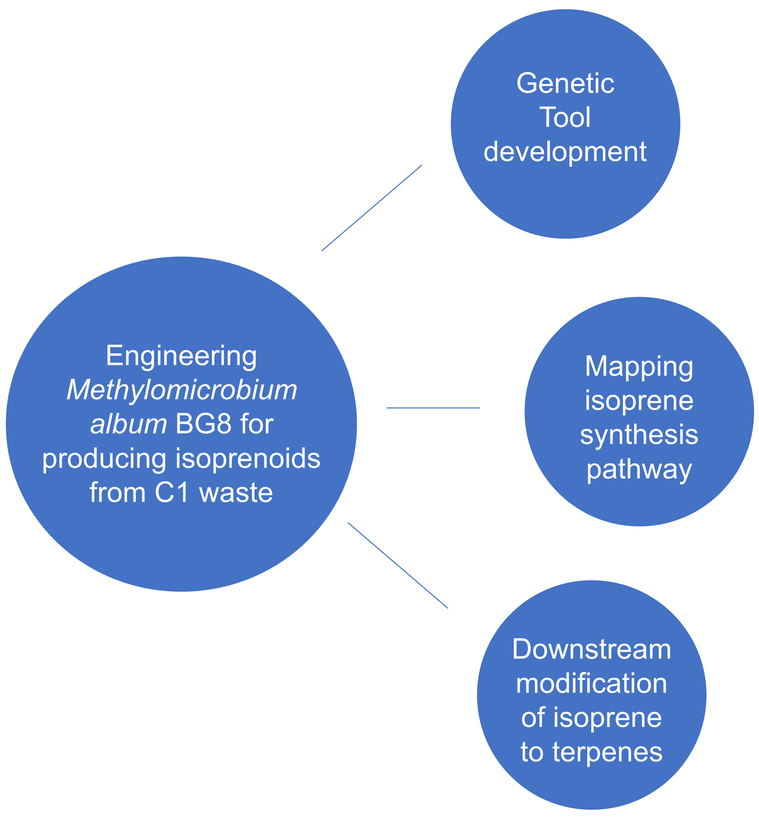
Figure 1: Methanotroph under SEM
J.C. Lara, University of Washington

Isoprene and Bio-industry



- Isoprene is of industrial interest as a precursor of biofuel, chemotherapy agents, and other products
- Terpenes made from isoprene can be synthesized into biofuels with increased energy density as compared to ethanol, fatty acid esters, or biodiesel
- These many uses of isoprene define it as a platform chemical, a compound that can be chemically built off of to make many other valuable compounds

Objective



Preliminary Results

Genetic tool development

- Two plasmids, pCM433kanT and pCAH01, are being investigated for unmarked allelic exchange and extra-chromosomal expression systems, respectively.
- Attempts at electroporation in *M.album* BG8 have so far been unsuccessful.
- Conjugation via *Escherichia coli* strain S17-1 λpir has shown success in initial experiments

Isoprene synthesis pathway

- Based on the genomics of *M.album* BG8, it is determined that isoprene is synthesized via 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP/DOXP) pathway made up of seven enzymes.
- Quantitative Polymerase Chain Reaction analysis of the MEP/DOXP pathway is currently ongoing

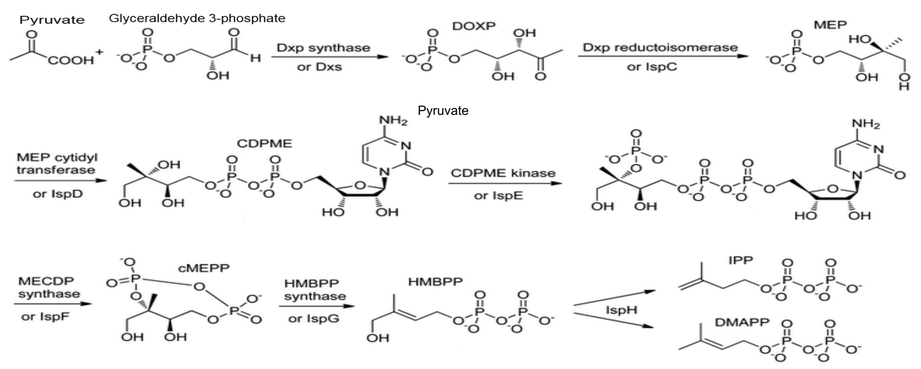


Figure 2: MEP/DOXP pathway.
Hale et al, Med. Chem. Commun.,2012

FUTURE DIRECTIONS

- Attempts to establish electroporation protocol for genetic transformation have been insofar unsuccessful; this may suggest the presence of a stringent Restriction-Modification system in *M.album* BG8. Identification and deletion of this system will better attenuate BG8 to further genetic manipulation
- Based on the expression profile of the genes of the DOXP pathway, isoprene production can then be optimized by targeting bottle-necks along the metabolic pathway
- Among terpenes synthesized from isoprene, a mixture of α-pinene, camphene and limonene has been reported to be highly efficient for chemical synthesis of high density fuel. Synthases for these terpenes can be introduced in *M.album* BG8 using the aforementioned genetic tools being developed.

PARTNERS

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FES PROJECT OVERVIEW

T01-P03 Bioconversion of Single--Carbon Effluents into Biofuels and Biofuel Precursors

The aim of this project is to develop a platform technology for the bioconversion of C1 compounds resulting from forestry activities (fermentation, thermal processing, anaerobic digestion) into biofuels (alcohols, lipids) and biofuel precursors (e.g. isoprenoids). This platform will be integrated in the greater context of biomass conversion by, for example, using by-product streams from other bioconversion activities (e.g. anaerobic digestion and pyrolysis) as feedstock.

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