Designing an Archaea-Bacteria Co-Culture Terpene Production Platform for a Microbial Electrolysis Cell

Sean R. Carr[1], Jared Aldrige[2], Nicole Buan[2]
University of Nebraska-Lincoln, Lincoln Nebraska, [1]School of Biological Sciences, [2]Department of Biochemistry.

Abstract

Weaning humanity off of its dependence on non-renewable resources will require a multifaceted approach utilizing both conventional and novel methods. Recent decades have provided many advancements in methods for the production of alternative energy though limitations in energy storage and transportation remain major hurdles. Additionally, our dependence on non-renewable fossil fuels is not limited to energy production, as many products we use every day are derived from those sources and true sustainability will require us to find alternative means of production for those products. The goal of this project is to develop a means of producing renewable and storable biofuels as well as industrial commodities using electricity and CO2 via an Archaea-Bacteria co-culture in a microbial electrosynthesis cell. To accomplish goal we will be taking advantage of the ability for the acetogenic bacteria Clostridium ljungdahlii to grow electrosynthetically by using CO2 as the sole carbon source and an electric cathode as an electron sink. The acetate produced by the electro-acetogenic bacteria will support the growth of an engineered methanogenic archaea Methanosarcina acetivorans to produce methane for biofuels as well as a variety of industrially relevant products such as terpenes. Methanogens are ideal organisms for the microbial production of terpenes due to their isoprenoid lipid which are distinct from the fatty-acid lipids found in bacteria and eukaryotes. Additionally their lean central metabolism and low maintenance energy results in a high product:substrate conversion rate which can be exploited for more economically feasible products than in other microbial systems.

Why Use Methanogens to Produce Terpenes?

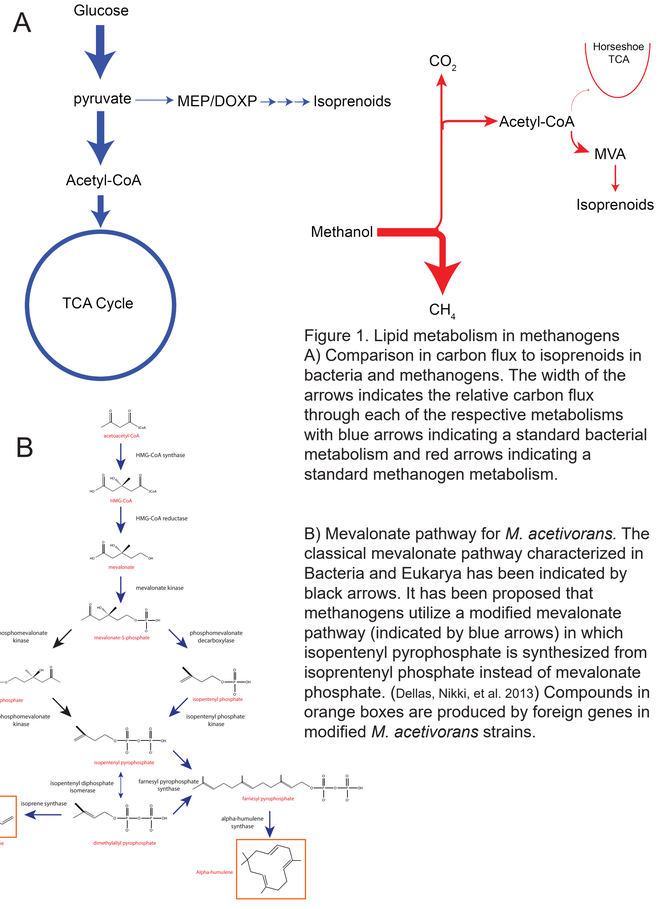
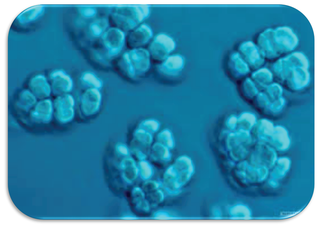


Figure 1. Lipid metabolism in methanogens
A) Comparison in carbon flux to isoprenoids in bacteria and methanogens. The width of the arrows indicates the relative carbon flux through each of the respective metabolisms with blue arrows indicating a standard bacterial metabolism and red arrows indicating a standard methanogen metabolism.

B) Mevalonate pathway for *M. acetivorans*. The classical mevalonate pathway characterized in Bacteria and Eukarya has been indicated by black arrows. It has been proposed that methanogens utilize a modified mevalonate pathway (indicated by blue arrows) in which isopentenyl pyrophosphate is synthesized from isoprentenyl phosphate instead of mevalonate phosphate. (Dellas, Nikki, et al. 2013) Compounds in orange boxes are produced by foreign genes in modified *M. acetivorans* strains.

Which Organisms Would be Utilized?

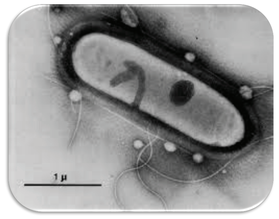
A



Methanosarcina acetivorans

*Methanogenic archaea
*Produces methane as byproduct of respiration
*Obligate anaerobe
*4.84 mbp genome
*Mesophilic
*Capable of autotrophic and heterotrophic growth on
methanol, acetate, methylamines, and/or H_2:CO_2
*Genetically tractable

B



Clostridium ljungdahlii

*Acetogenic bacterium
*Produces acetate as the end product of fermentation
*Obligate anaerobe
*4.63 mbp genome
*Mesophilic
*Capable of autotrophic and heterotrophic growth
on hexoses, pentoses, H_2:CO_2, and/or CO
*Genetically tractable

Methanogens as a Terpene Production Platform

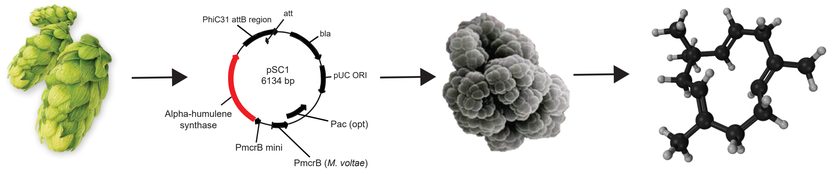
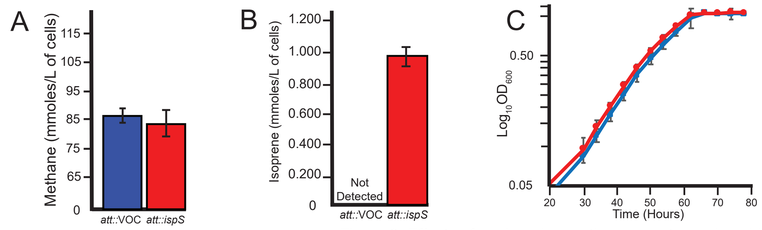


Figure 2. General overview of engineering a terpene producing methanogen.
A gene for converting an isoprenoid lipid precursor in methanogens is identified in an organism, in this case the alpha-humulene synthase gene from hops. The gene is codon optimized for expression in Methanosarcina species and is cloned into a plasmid containing an archaeal antibiotic resistance marker. The plasmid is transfected into M. acetivorans and selected for under antibiotic stress. Confirmation of gene insertion of attained by PCR and expression is confirmed via reverse transcription. The methanogens are cultivated and the terpenes are harvested from either the gaseous headspace or liquid medium depending on the volatility of the compound.



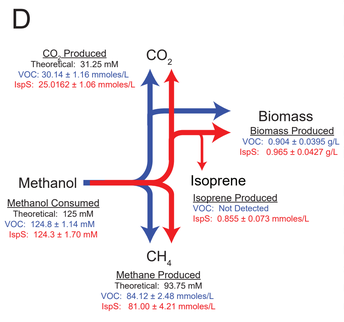


Figure 3. Effects of engineering methanogens to produce bioisoprene
A) Change in methane production in M. acetivorans engineered to produce bioisoprene. Methane quantification was achieved via GC-FID. The blue bars indicate methane production by a vector only control whereas the red bars indicate ispS+ strains.
B) Isoprene production from modified M. acetivorans strains. As expected the vector only control strain produced no detectable isoprene. The ispS+ strains produced nearly 1mM of isoprene per liter of cells.
C) Growth rates of engineered M. acetivorans strains. Growth rates of ispS+ and VOC strains were measured in HS+MeOH medium via absorbance at 600nm. There was no significant variance in growth rate between the two strains.
D) Mass balance of ispS+ and VOC strains of M. acetivorans. Methanol consumption as well as methane, CO2, and isoprene production was measured by GC-FID. Cultures used for this experiment were desiccated and measured for dry weight. It was found that the carbon utilized for isoprene production was not diverted from biomass but rather CO2 production.

How Does a Microbial Elecrosynthesis Cell Work?

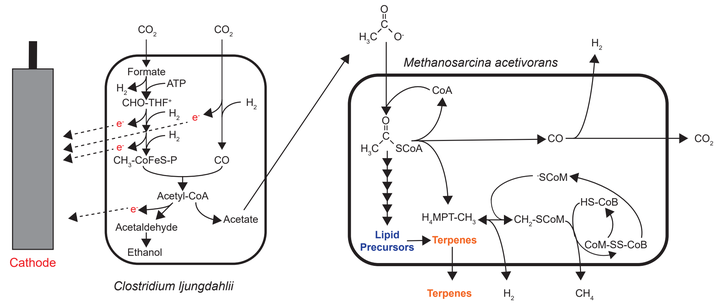


Figure 4.
Carbon and electron schematic through a microbial elecrosynthesis cell. The Clostridium species uptakes carbon dioxide from the environment and fixes it into cellular biomass and acetate through the Wood-Ljungdahl pathway. Rather than utilizing traditional cofactors to uptake the electrons produced by the acetogenesis, the electrons are shuttled to the cathode within the microbial elecrosynthesis cell allowing for redox balancing. The acetate produced by the electro-acetogenesis is subsequently consumed by the engineered methanogen partner in the co-culture and through acetoclastic methanogenesis methane and terpenes are produced.

Can *M. acetivorans* and *C. ljungdahlii* Be Cultivated in The Same Media?

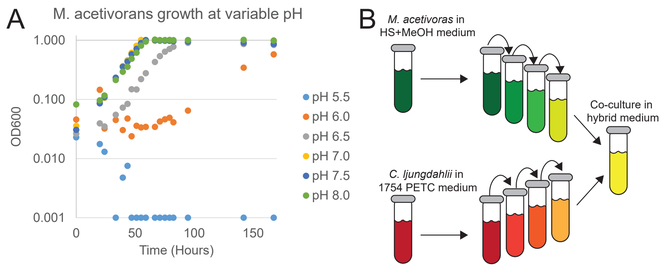


Figure 5.
A) Growth of Methanosarcina acetivorans in HS+MeOH medium at variable pH. M. acetivorans was cultured in a high salt growth media supplemented with MeOH and growth was measured at via absorbance at 600nm. It was found that M. acetivorans grows optimally between pH 6.5-8.0, growth was detected in media at pH 6.0. According to the literature Clostridium ljungdahlii grows optimilally at pH 6.0 though growth can be achieved between pH 4.0-7.0.
B) Flowchart of adapting M. acetivorans and C. ljungdahlii for growth in a mutually compatible medium. A hybrid media recipe for both M. acetivorans and C. ljungdahlii was designed which should support both organisms. Due to differences in nutrient abundance, solute concentrations, and pH, the strains must gradually be adapted to the new media over generations.

Future Directions

- **Engineer *M. acetivorans* to produce alpha-humulene**
- **Adapt *M. acetivorans* and *C. ljungdahlii* to a mutually compatible medium**
- **Quantify growth and respiration of both *M. acetivorans* and *C. ljungdahlii* the MEC**
- **Optimize growth and terpene production conditions MEC**

References

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