In-space Biomanufacturing Using CO2 with *Methylobacterium extorquens*

Tristan Caro[1]|
Jamie Stankiewiz[1]|
Asif Rahman[3]|
John Hogan[3]|
Jonathan Galazka[2]|

The Potential of CO2

Reducing CO_2 concentrations onboard the ISS and future spacecraft is mission-critical. Astronauts report symptoms of CO_2 poisoning. Venting of CO_2 represents a loss of carbon for utilization as biomass & energy source.

Carbon Dioxide is an untapped carbon source

The Martian atmosphere is 96% CO_2

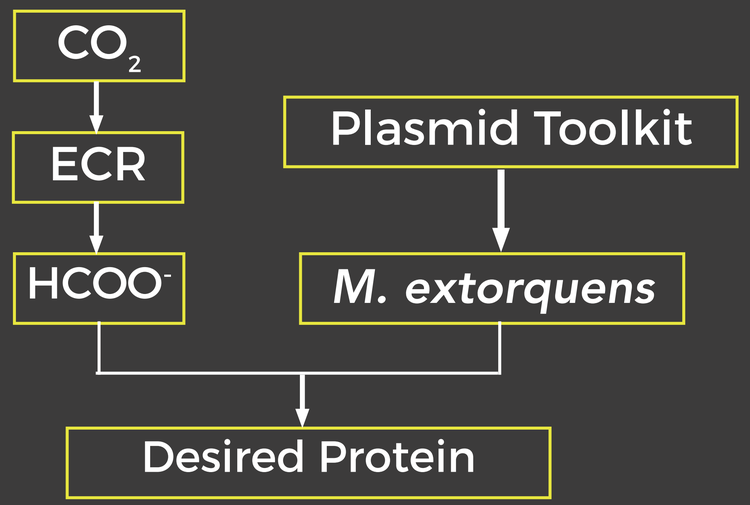
An astronaut produces 1kg of CO2/24hrs.

Our Proposal

Demonstrate use of an electrochemical reactor (ECR) to convert CO_2 into formate (HCOO-).

Engineer *M. extorquens*, a formatotroph, to produce desired compounds through a modular plasmid system.

Investigate the formate metabolism of two strains, AM1 and PA1, to determine optimal formate concentration.



Plasmid Assembly

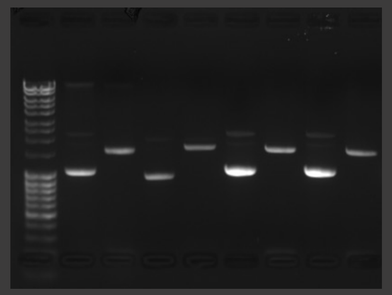
Hierarchical Assembly

Plasmid parts are domesticated into part plasmids for long-term storage and quick amplification (E. coli K12).

Part-plasmids are ligated into cassettes using Golden Gate assembly to ensure correct sequence.

Cassette plasmids can be assembled into a multi-gene plasmid with a second round of Golden Gate assembly. This vector will contain elements native to the host chassis (M. extorquens).

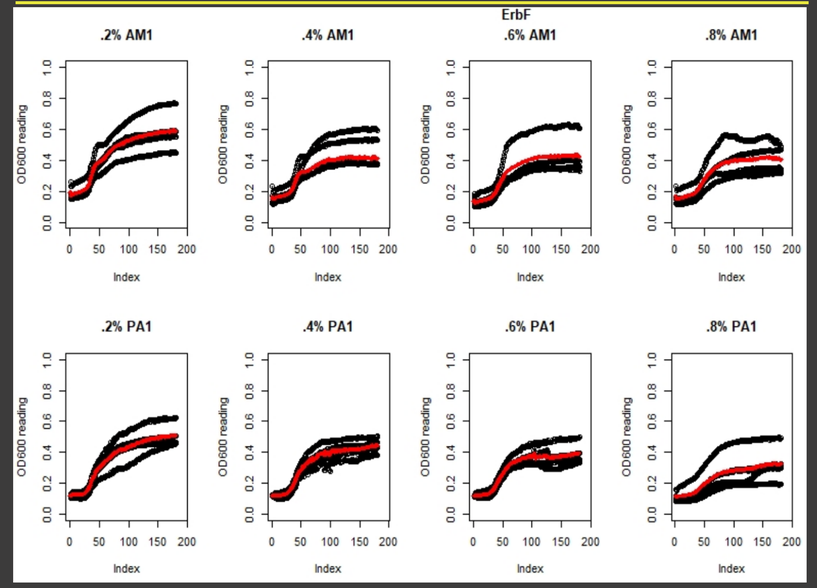
Assembly Progress



Alternating bands of circular plasmid (runs further on gel) and linearized digest.
1: NEB DNA Ladder
2/3: Terminator 2
4/5: Promoter pFumC
6/7: Promoter pMxaF

Carbonic Anhydrase: Sequence Verified
Promoter pMxaF: Sequence Verified
Promoter pFumC: Sequence Verified
Terminator MexTerm2: Incorrect Sequence
Native Back

Metabolism Results



Highest growth rate is observed at 0.2% formate. At high concentration, formate becomes toxic to the cells. No statistically significant difference between growth rates for AM1 and PA1 for all concentrations except 1%.

Future Directions

In situ resource utilization is critical for NASA's planned lunar and interplanetary human missions.

We plan on expanding the suite of target proteins in our plasmid library. Future studies are needed to optimize the growth and competence of M. extorquens.

Acknowledgements

We'd like to thank the NASA Ames SLSTP Program, NASA Space Biology Program, NASA Ames Space BioSciences Division, and our mentors Dr. Jon Galazka, Dr John Hogan, and Dr. Asif Rahman.

1. University of California, Berkeley; Department of Molecular & Cell Biology. 2. NASA Ames Research Center, Space Biosciences Division (Code SCR) 3. NASA Ames Research Center, Bioengineering Branch (Code SCB)