Engineering of Methane Metabolism in *Pichia pastoris* through Methane Monooxygenase Expression

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### Background

Utilization of available resources is important to minimize the need for costly resupply from Earth. Currently, the oxygen containing Sabatier system on the ISS reacts CO2 and H2 to form H2O and CH4. The water is recycled back into the ISS system, but the methane is vented into space as waste. One potential use for this methane is as a carbon substrate for a biological production platform such as the methylotrophic yeast, P. pastoris. P. pastoris is a well-established synthetic biology platform and its native methanol metabolism is one enzymatic step away from metabolizing methane. In methanotrophic bacteria that step is carried out by methane monooxygenases (MMOs), which oxidize methane to methanol. In this project, we have attempted to engineer methane metabolism into P. pastoris by expressing a bacterial MMO system.

**Sabatier produces methane**
- The ISS Sabatier system produces methane and water from CO2 and H2.
- Water is recycled but CH4 is vented to space.
- CH4 could feed heterotrophic microbes for in-space bio-manufacturing.

**Natural methanotrophs have limitations**
- Natural methanotrophic (consume methane) bacteria exist and are being developed as microbial factories.
- They utilize Methane Monooxygenases to hydroxylate methane to methanol.
- These microbes have limited engineering tools available and innovation is relatively slow.

**Pichia pastoris as a synthetic methanotrophic yeast**
- *P. pastoris* is well-established methylotrophic (consumes methanol) yeast
- Used to produce Trypsin, murine TNF-α, and FDA approved drugs Ribulose and Jatrecs.
- Addition of Methane Monooxygenase should allow growth on methane.

CO2 → Sabatier → CH4 → MMO → Pina → Product

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### Conclusions

*P. pastoris* is capable of expressing MMOH, but further testing needs to be done to confirm expression and functionality. While completing this testing we are also moving forward with engineering expression of other proteins in the MMO system, with the goal of ultimately growing engineered *P. pastoris* on a methane substrate for functional testing.

### References

