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

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Background

In space environments such as the ISS or Mars, utilization of available resources is important to minimize the need for costly resupply from Earth. Traditionally used carbon sources such as glucose or large hydrocarbons are scarce in these environments, but CO_2 is abundant. Currently the oxygen reclaiming Sabatier system on the ISS reacts CO_2 and H_2 to form H_2O and CH_4. 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, *Pichia 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.

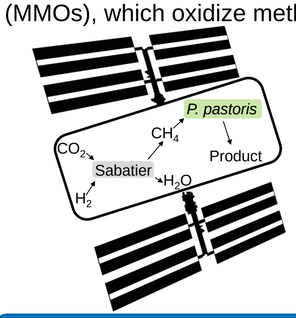

Figure 1. Schematic for the proposed biosynthesis pathway. The Sabatier reaction produces methane that can be utilized as a carbon source by engineered *P. pastoris* to make a protein product.

Methods

The MMO system includes one protein complex - MMOH, a hydroxylase - and three other proteins: MMOR, a reductase, MMOB, a regulatory protein, and MMOG, a chaperone protein. Engineering *P. pastoris* to express the MMO system should allow *in situ* conversion of methane into methanol that can then be utilized by its natural metabolic pathways. Genes encoding the MMO proteins were inserted into *P. pastoris* using a plasmid based system. Plasmids were assembled from components based on a plasmid toolkit designed for S. cerevisiae. Parts were built to extend this toolkit including promoters, terminators and the MMO genes

Figure 2. How a plasmid based expression system works

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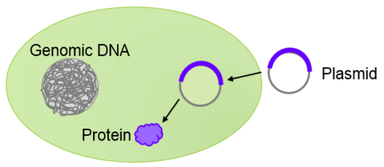


Results

Promoter Testing

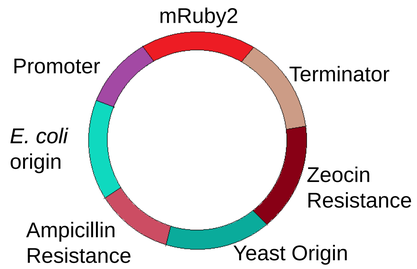


Figure 3. Plasmid design for testing newly designed promoter sequences. Promoters were put upstream of a gene encoding red fluorescent protein (mRuby2) and the plasmids were expressed in *P. pastoris.*

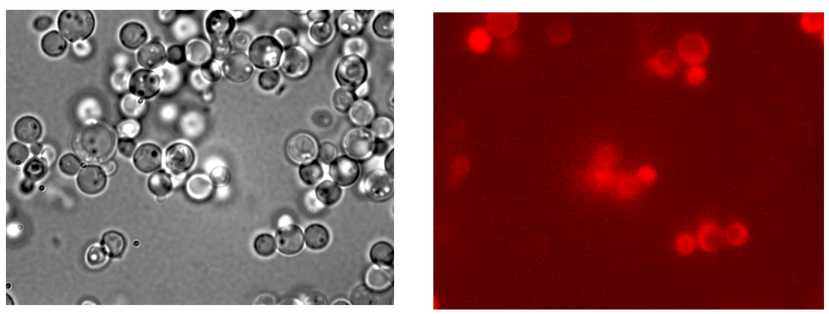


Figure 4. *P. pastoris* expressing a plasmid encoding mRuby2 under the TEF2 promoter. Microscopy. 100x oil objective. Left: bright field. Right: fluorescence colored red.

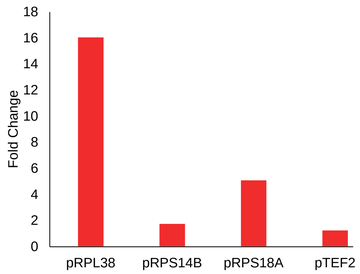


Figure 5. Fluorescence of *P. pastoris* expressing RFP under different promoters. Fluorescence shown as fold increase relative to *P. pastoris* expressing no plasmid.

MMOH Expression

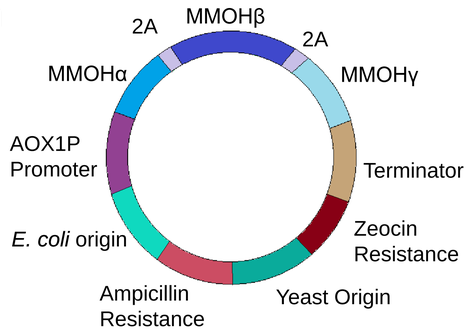
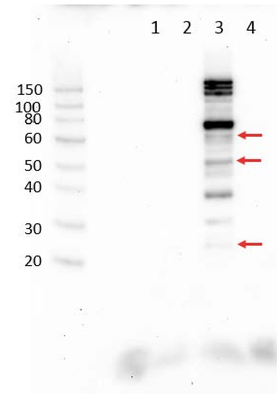


Figure 6. Plasmid design for testing MMOH expression Genes encoding the three subunits of the MMOH were interspaced with 2A skipping sequences to allow three peptides to be made from a single transcript.



1 – PPTK057 (control) Glucose
2 – PPTK057 (control) Methanol
3- PPTK053 (MMOH) Methanol
4 – PPTK053 (MMOH) Glucose

Figure 7. Western blot with markers indicating MMOHα (63.4kDa) and MMOHβ (48.4kDa) and MMOHγ (21.0kDa). Bands corresponding to MMOH subunits were found only in the lane from *P. pastoris* expressing the MMOH plasmid grown on methanol as expected for a gene expressed under a methanol induced promoter.

Conclusion

Engineering *P. pastoris* to metabolize methane offers one way to utilize currently wasted methane. To engineer *P. pastoris* we have created new engineering tools including promoters to work in *P. pastoris* and shown that they are functional based on their ability to drive expression of RFP. Preliminary data suggests that *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

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Acknowledgements

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