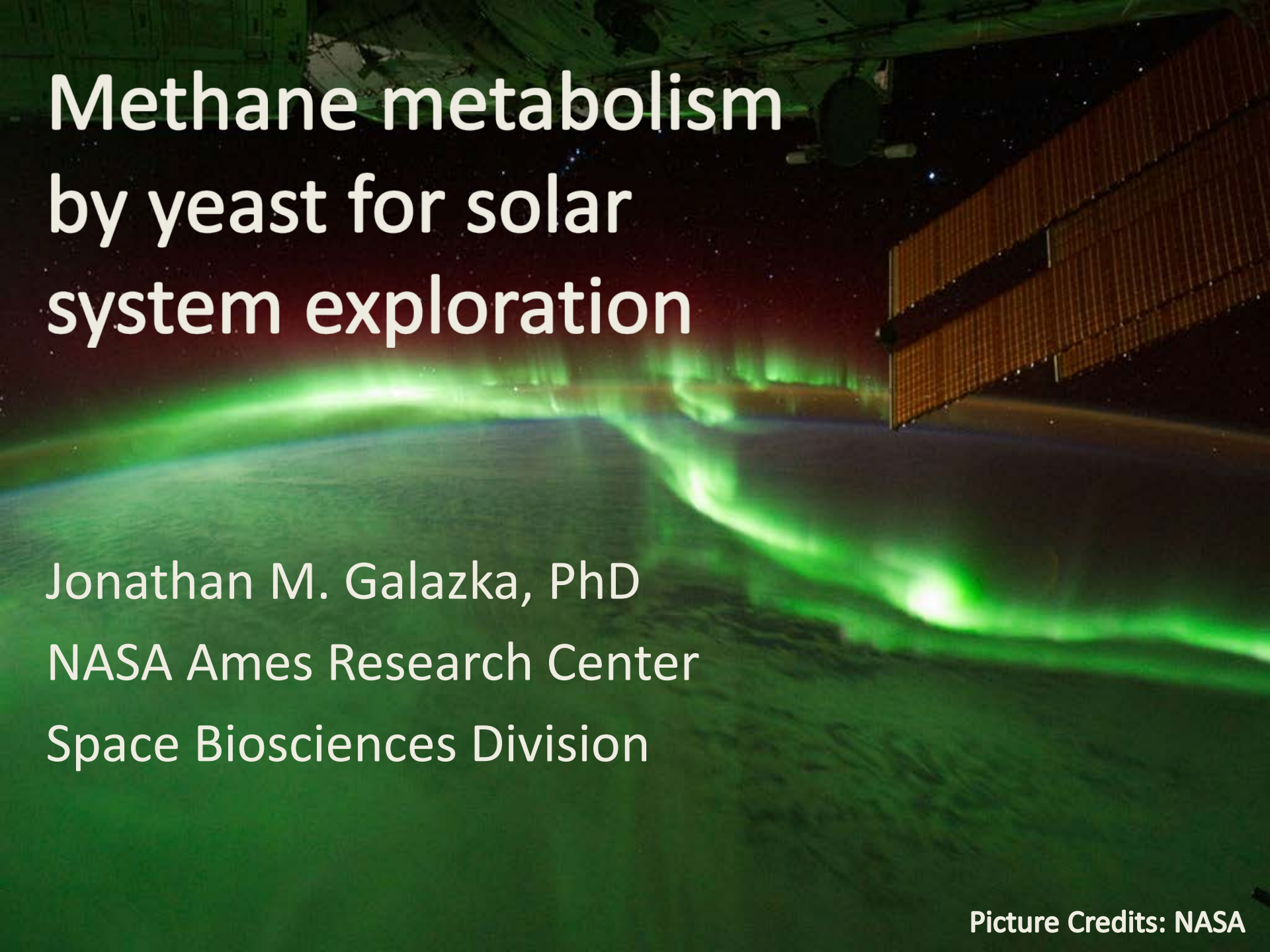


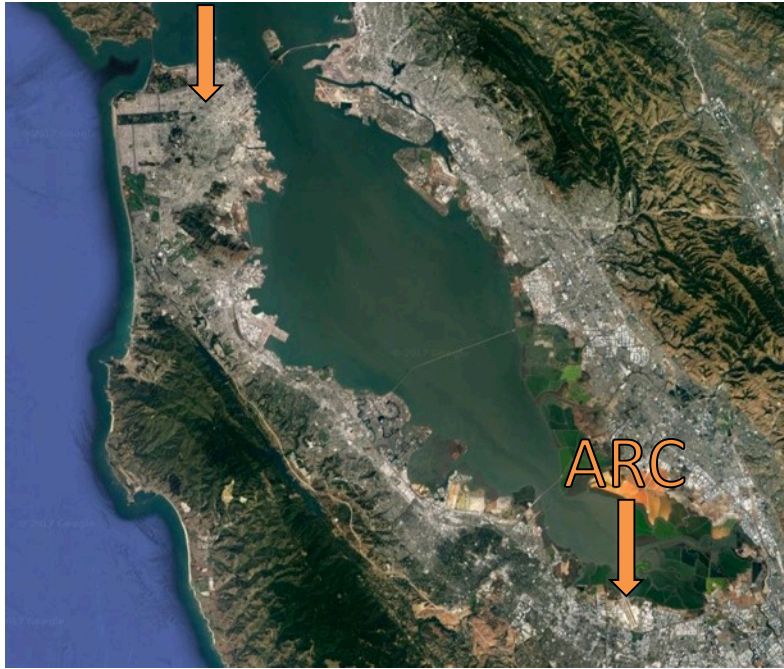
Methane metabolism by yeast for solar system exploration



Jonathan M. Galazka, PhD
NASA Ames Research Center
Space Biosciences Division

NASA Ames Research Center

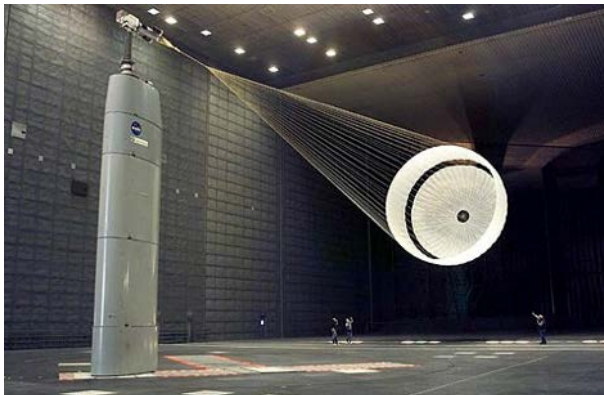
ACS



41 min. drive without traffic :)



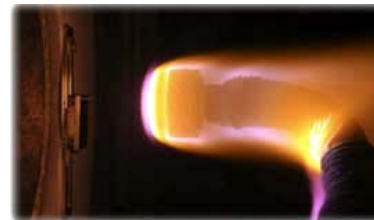
NASA Ames Research Center



NASA Full Scale Aerodynamics Complex



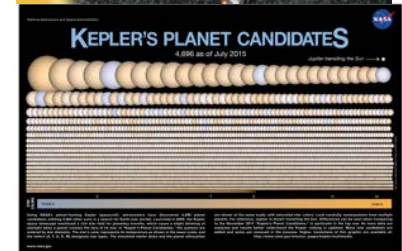
Quantum Computer



ARC Jet



Supercomputer

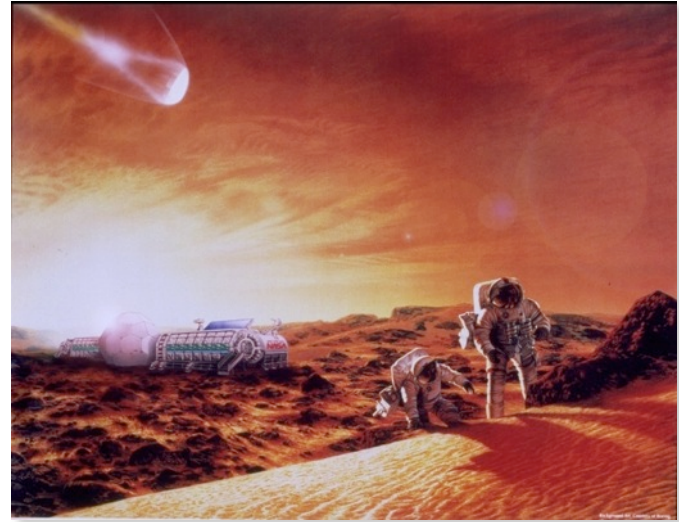


Kepler Project

Future Missions Need a Different Approach



Credits: NASA



Credits: NASA

- | | | |
|--|---|--|
| • Short crew duration | → | • Extended crew durations |
| • Frequent resupply of food, water, O ₂ , medical supplies, replacement parts | → | • Infrequent or no resupply of food, water, O ₂ , medical supplies, replacement parts |
| • Emergency return to Earth | → | • No emergency return to Earth |
| • No ET planetary protection requirements | → | • Possibly strict planetary protection requirements |

Potential of Biotechnology in Space

Biological systems are:

- Scalable
- Programmable
- Precise (pure isomers)
- The only route of production in some cases (protein therapeutics)
- Low T° and pressure
- Regenerable

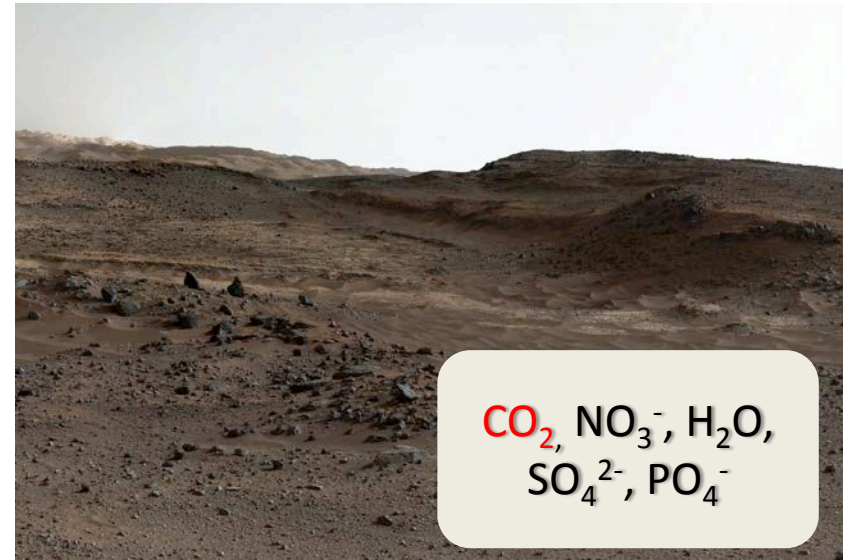


Credits: NASA

Barriers to Biotechnology in Space

Space technologies must be...

- Robust
- Simple to operate
- Stable during storage
- Compatible with available resources and infrastructure



Mount Sharp, Mars. Credits: NASA.

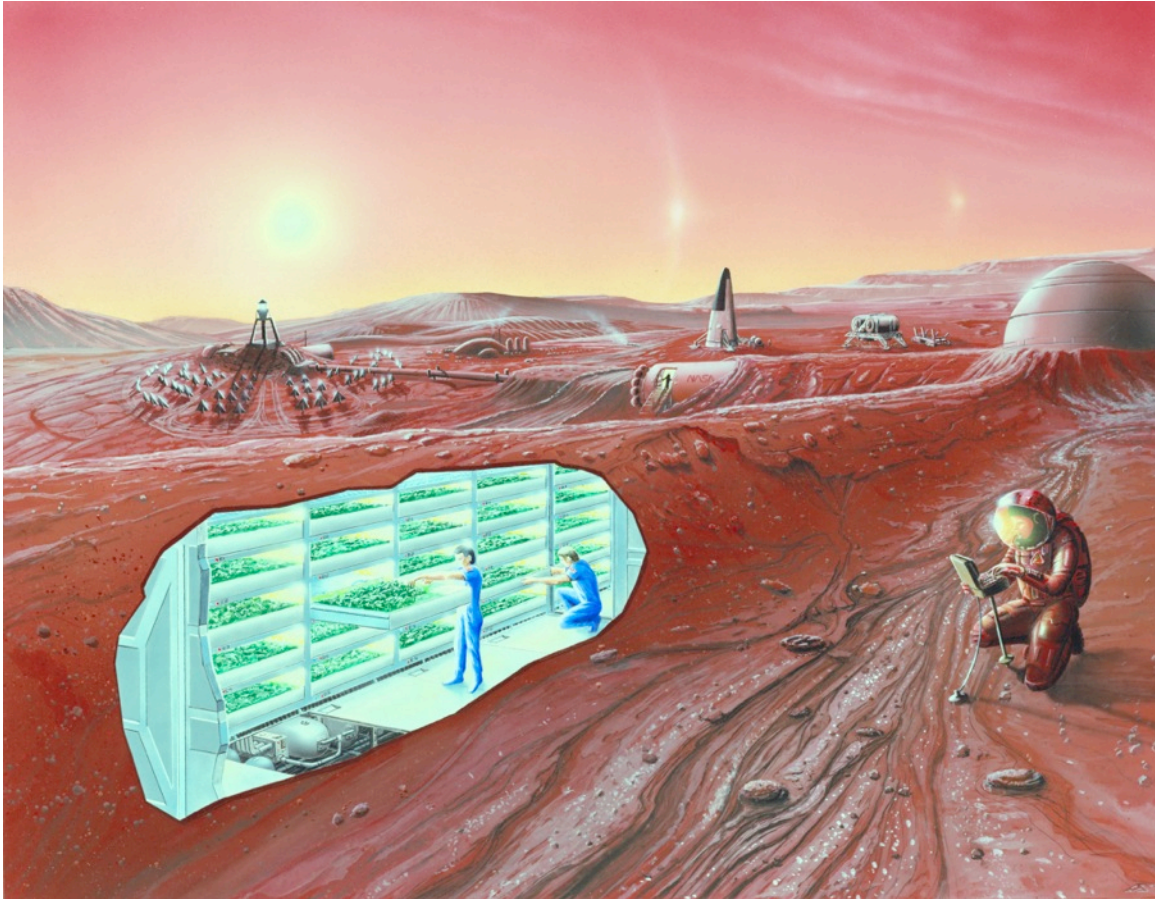
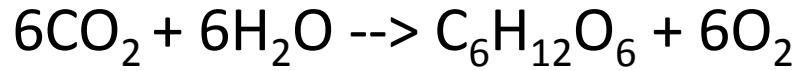
We will have this

YPD:

Not this

0.3% yeast extract
1% peptone
1% glucose

Photoautotrophs

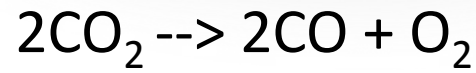
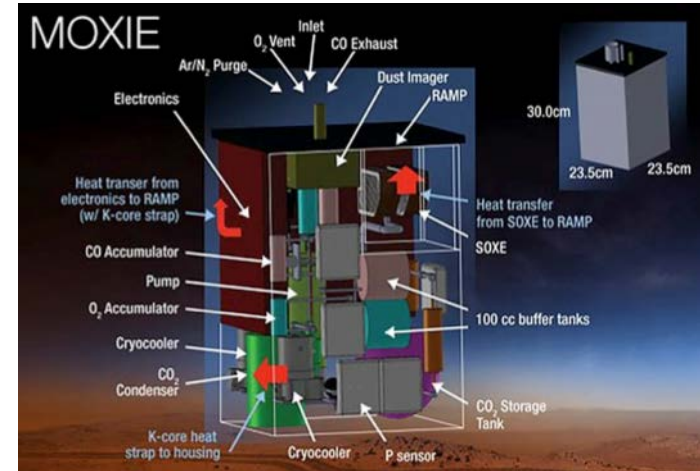
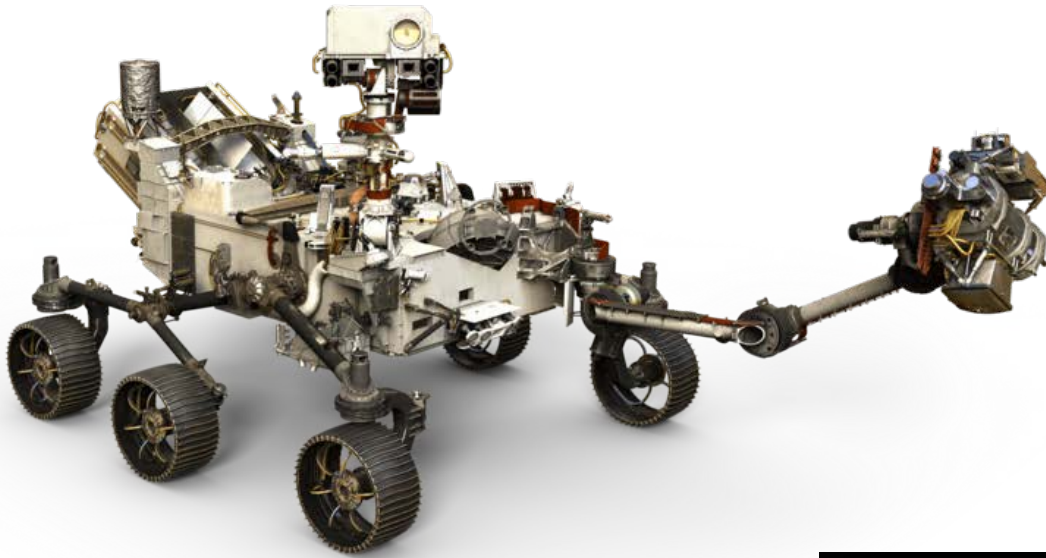


Credits: NASA

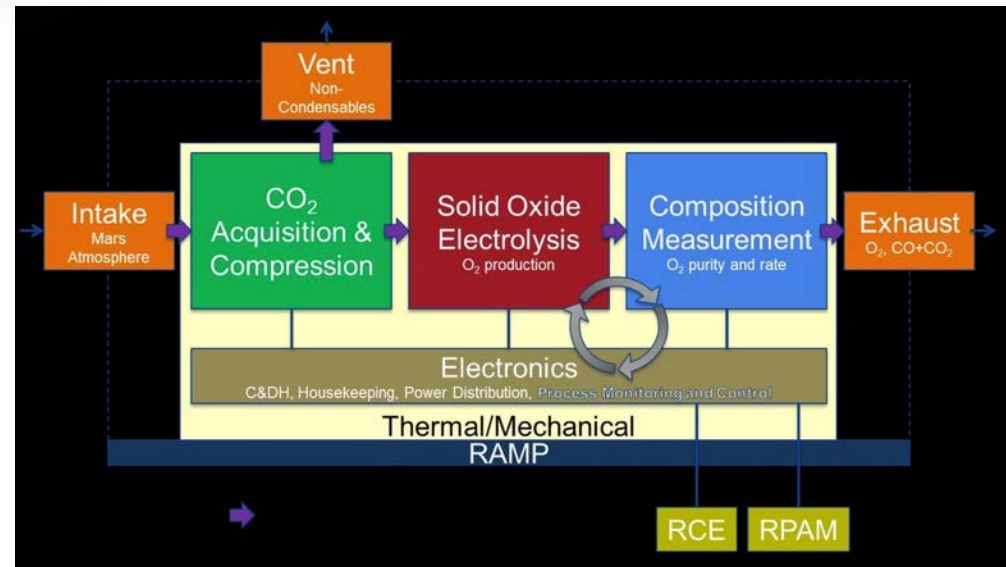
But...

- Usually have slower growth rates than heterotrophs
- Require photobioreactors

MOXIE: Mars Oxygen ISRU Experiment



Clostridium spp.



Picture Credits: NASA

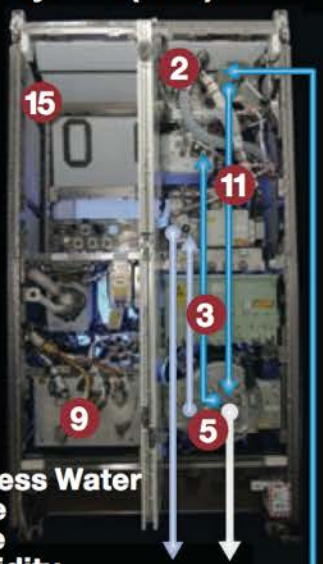
Environmental Control and Life Support System

U.S. Regenerative Environmental Control and Life Support System (ECLSS)

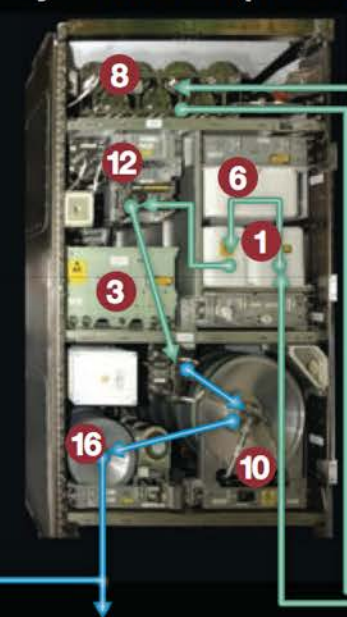
- | | |
|---------------------------|--|
| 1 Catalytic Reactor | 12 Reactor Health Sensor |
| 2 Deionizer Beds | 13 Storage Tanks |
| 3 Digital Controller | 14 Urine Processor Pumps |
| 4 Distillation Assembly | 15 CO ₂ Reduction System (Sabatier) |
| 5 Electrolysis Cell Stack | 16 Water Processor Delivery Pump |
| 6 Gas Separator | 17 Water Processor Pump & Separator |
| 7 Multifiltration Beds | 18 Water Processor Wastewater Tank |
| 8 Particulate Filter | |
| 9 Power Supply | |
| 10 Product Water Tank | |
| 11 Pumps & Valves | |

- | | | | | | |
|--|---|-----------------------------|---|---|---------------------|
|  | = | Oxygen |  | = | Process Water |
|  | = | Hydrogen (vented overboard) |  | = | Urine |
|  | = | Potable Water |  | = | Brine |
| | | |  | = | Humidity Condensate |

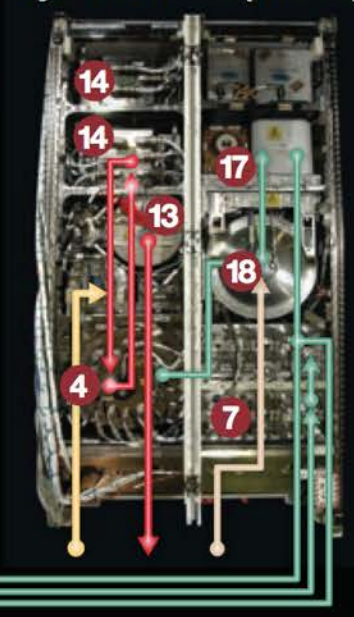
Oxygen Generation System (OGS) Rack



Water Recovery System Rack 1 (WRS-1)



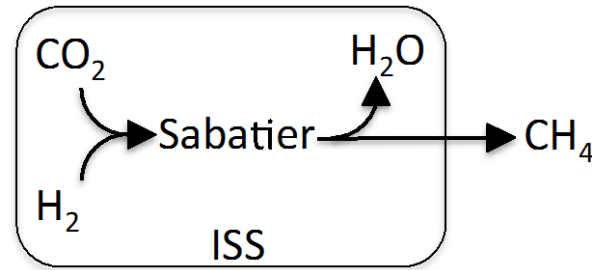
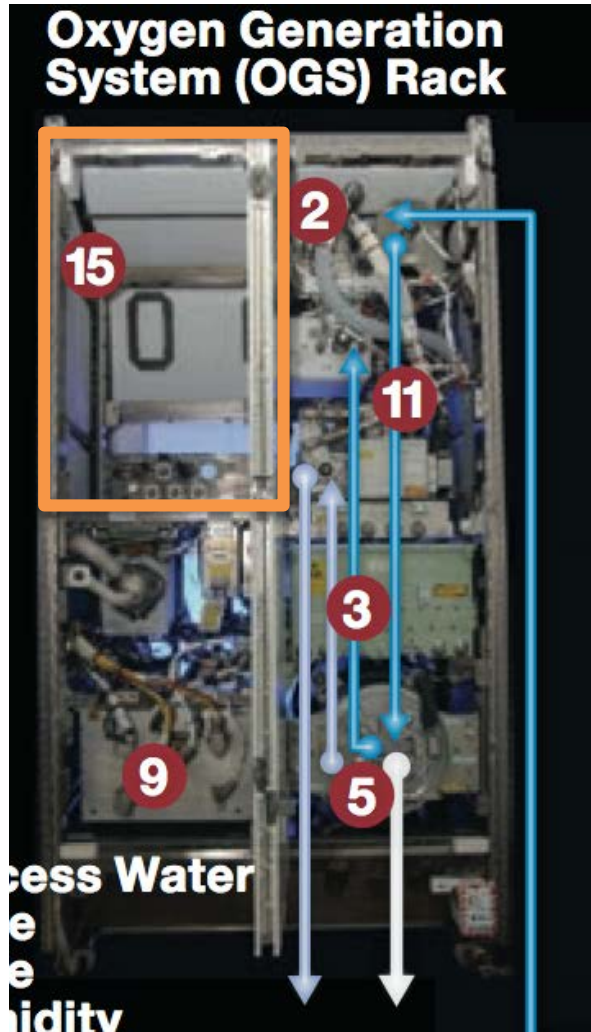
Water Recovery System Rack 2 (WRS-2)



Credits: NASA

"Reference Guide to the International Space Station"

Methane production by Sabatier



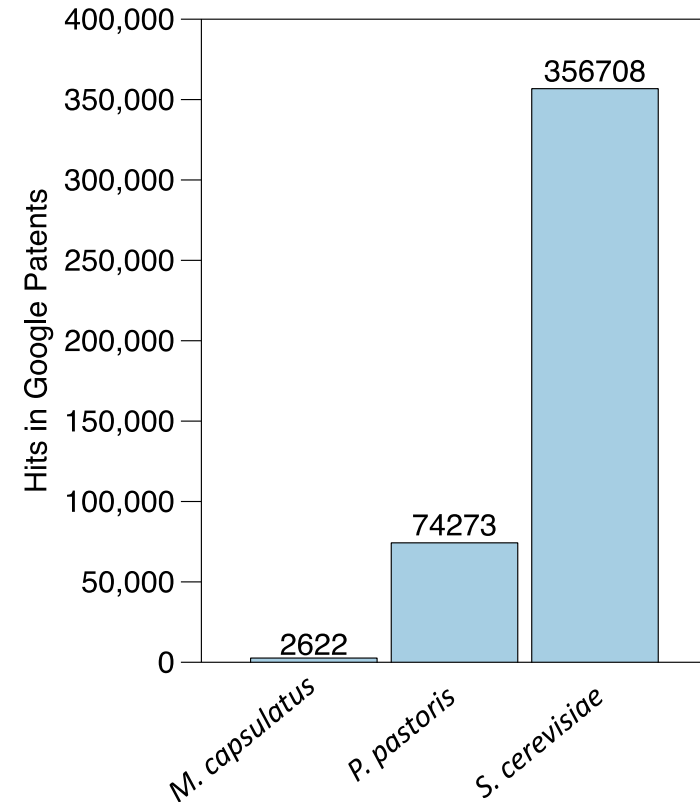
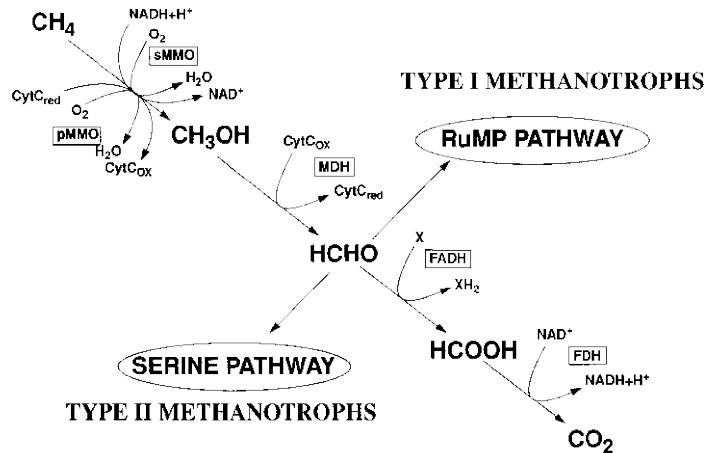
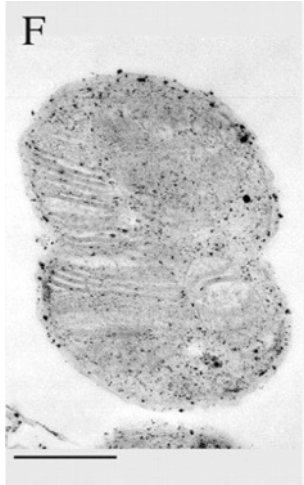
- Reacts CO₂ and H₂ to form CH₄ and H₂O
- H₂O is recycled
- CH₄ vented to space
- Operational since 2010



Astronaut Doug Wheelock
installing Sabatier system on ISS

Why Not Bacterial Methanotrophs?

M. capsulatus



J. Bacteriol. October 2003
vol. 185 no. 19 5755-
5764

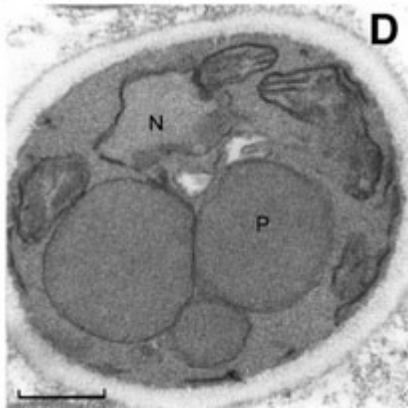
Microbiol. Mol. Biol. Rev. June
1996 vol. 60 no. 2 439-471

- NASA STTR to Mango Materials (Small Technology Transfer Innovation Research)

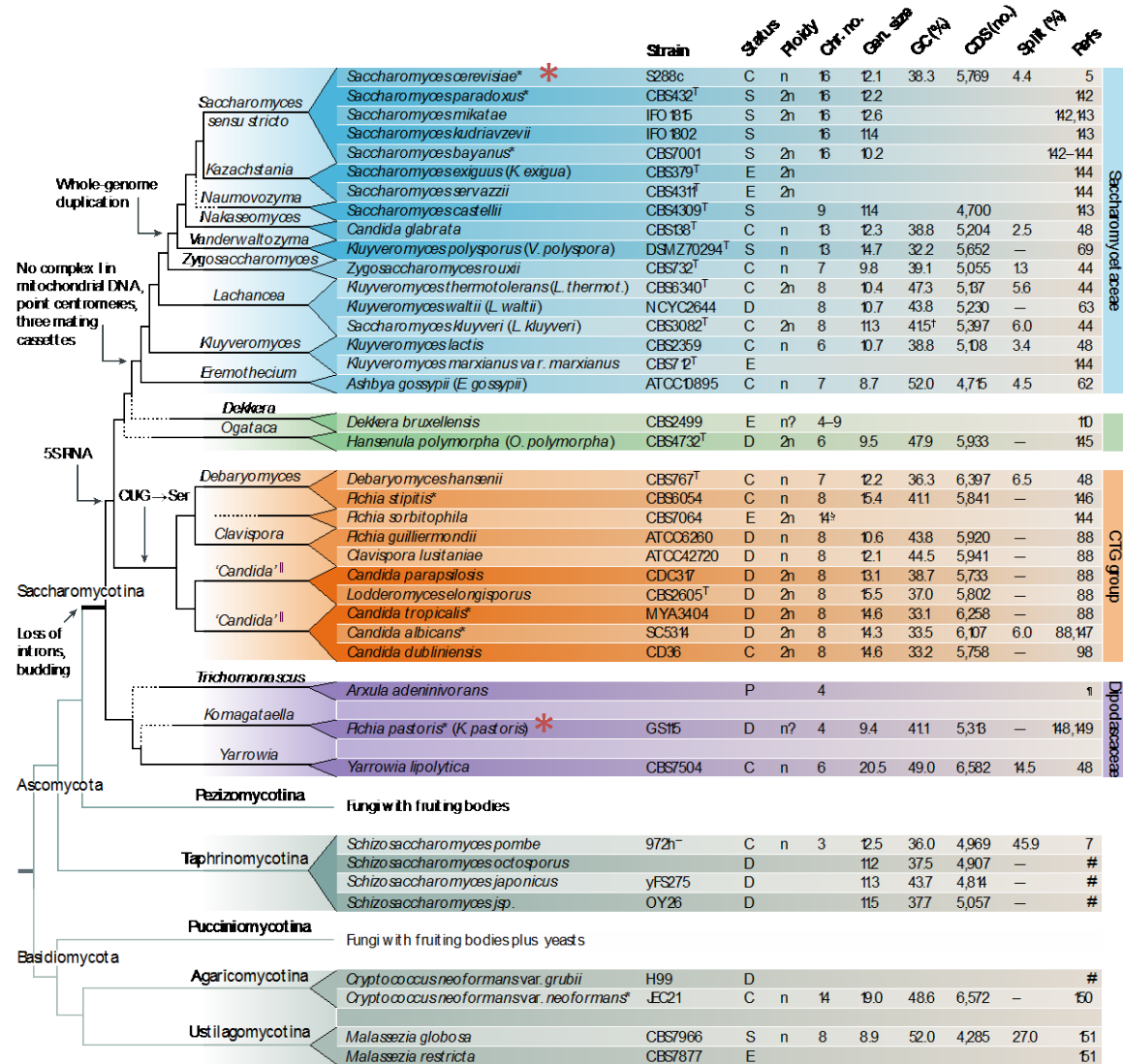
- “A Novel, Membrane-Based Bioreactor Design to Enable a Closed-Loop System on Earth and Beyond”

- “...a membrane bioreactor system to produce a biopolymer from methane gas...will enable bacterial growth and biopolymer production to occur in microgravity environments...”

Pichia pastoris (*Komagataella phaffii*)



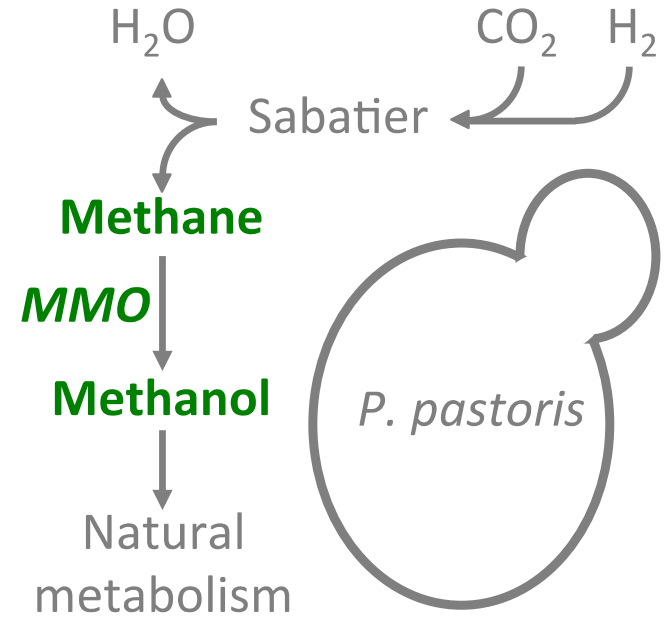
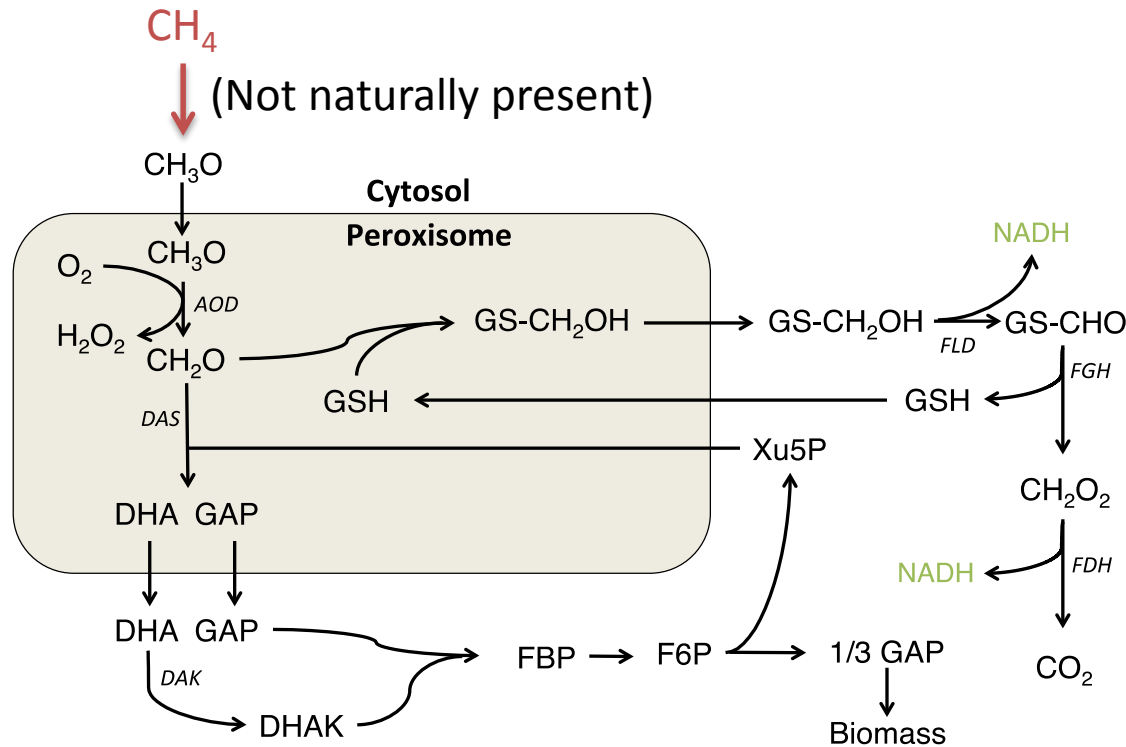
Ida J. van der Klei et al. EMBO J. 1998;17:3608-3618



Dujon, 2010, Nature Reviews Genetics

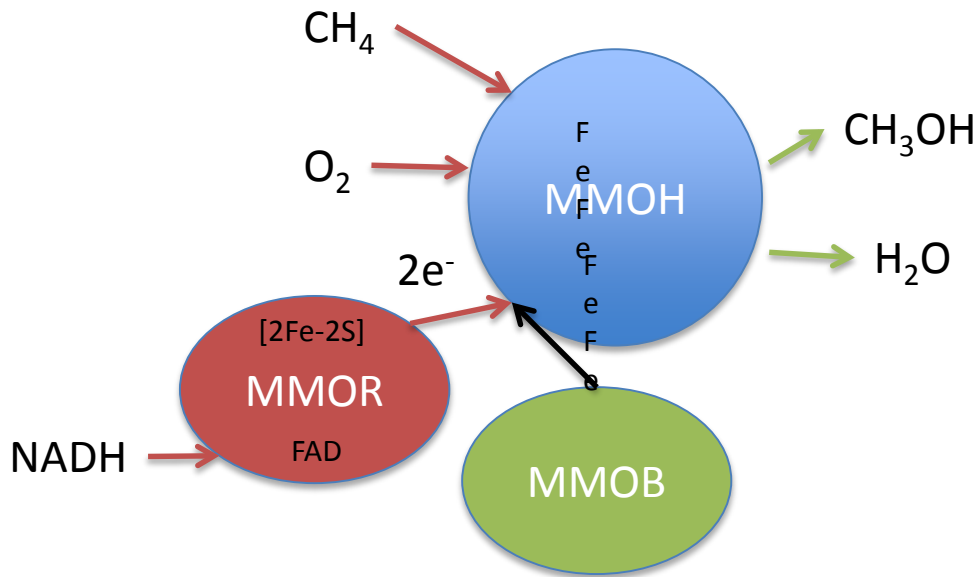
- Methylophilic yeast
- Widely used as a protein production host
- Capable of producing reduced and glycosylated proteins
- Grows to very high cell density (optical densities up to 630, Dr. Julia Cino, New Brunswick Scientific)

Porting methane metabolism to *P. pastoris*



Methanol (CH₃O) is oxidized to formaldehyde (CH₂O) by alcohol oxidase (AOD). Formaldehyde can either be oxidized to CO₂ through the successive action of formaldehyde dehydrogenase (FLD), S-formylglutathione hydrolase (FGH), and formate dehydrogenase (FDH), or appended to xylulose-5-phosphate and assimilated into biomass through a pathway involving dihydroxyacetone synthase (DAS) and dihydroxyacetone kinase (DAK).

Porting Soluble Methane Monooxygenase to *Pichia*



1. Hydroxylase: MMOH

1. alpha
2. beta
3. gamma

Oxidizes methane hydroxylates methane to methanol

2. Reductase: MMOR

Oxidizes NADH and transfers electrons to MMOH

1. Regulatory: MMOB

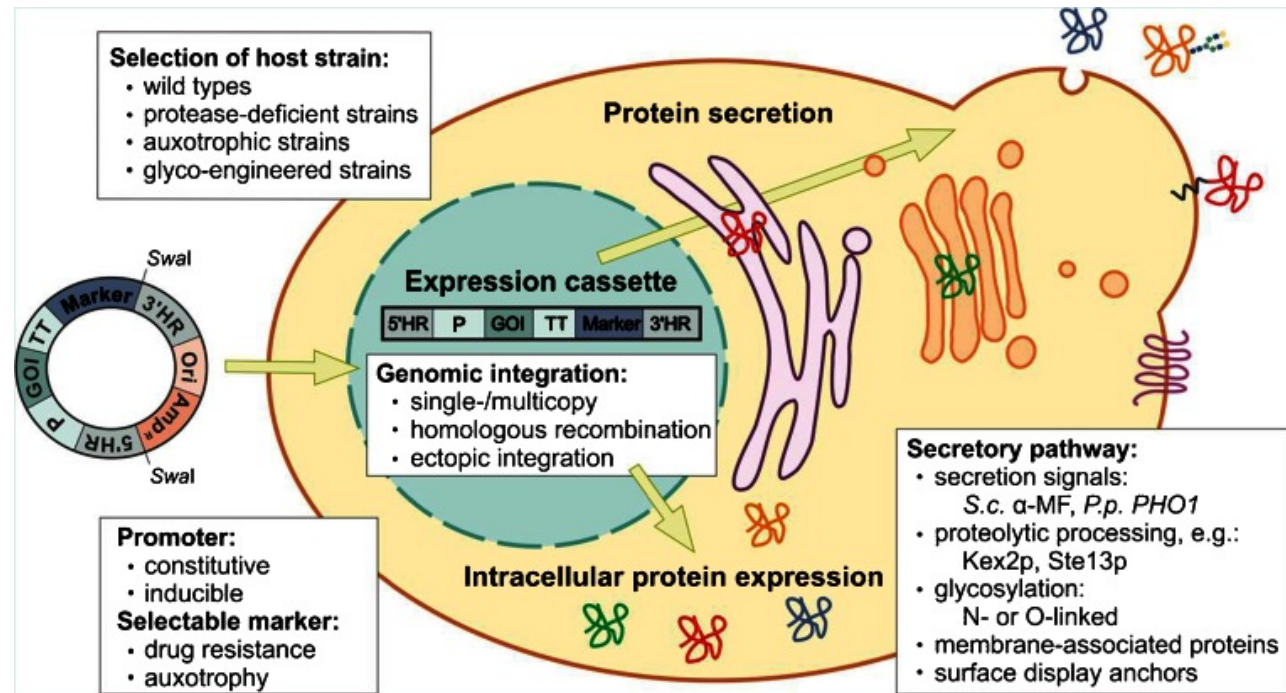
Binds to same site as MMOR. May help drive cycle.

2. Assembly chaperone: MMOG

Could help in assembly of MMOH

Developing *Pichia* tools

- *Pichia* tools are limited compared to *S. cerevisiae*
- Most engineers rely on genomic integration of DNA constructs using a limited number of selectable markers
- Plasmid systems have only recently been developed



Ahmad et al. 2014. Appl Microbiol Biotechnol. 2014; 98(12): 5301–5317.

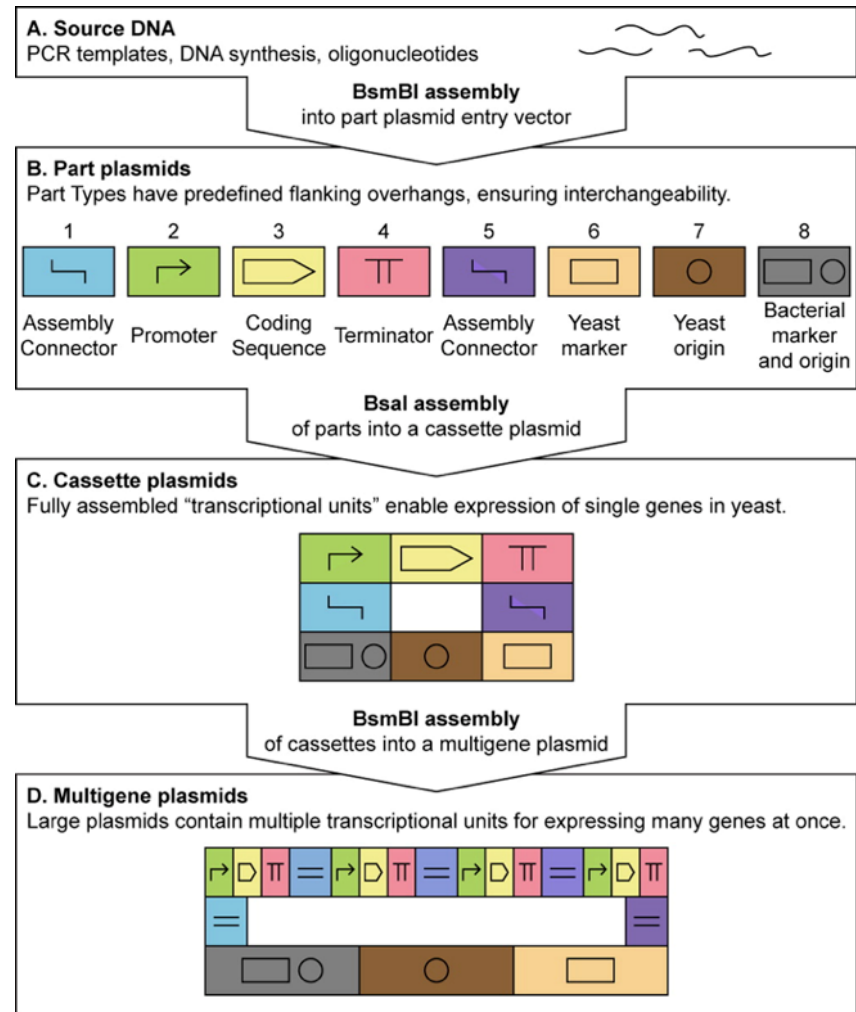
Developing *Pichia* tools

Pichia tools are limited compared to *S. cerevisiae*

Plasmid systems have only recently been developed

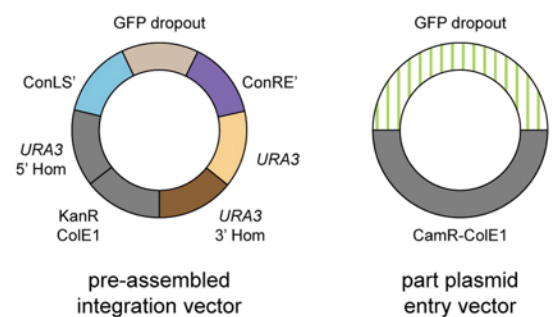
John Dueber at Berkeley has developed a “*S. cerevisiae* Toolkit”

This could be adapted for use in *Pichia*



Lee et al. 2015. ACS Synth Biol.

Lee et al. 2015.
ACS Synth Biol.

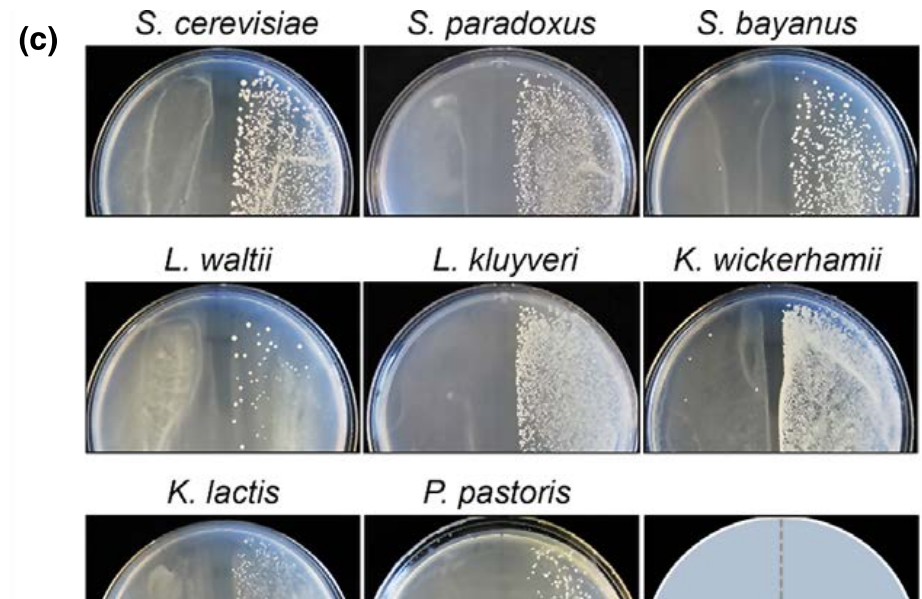
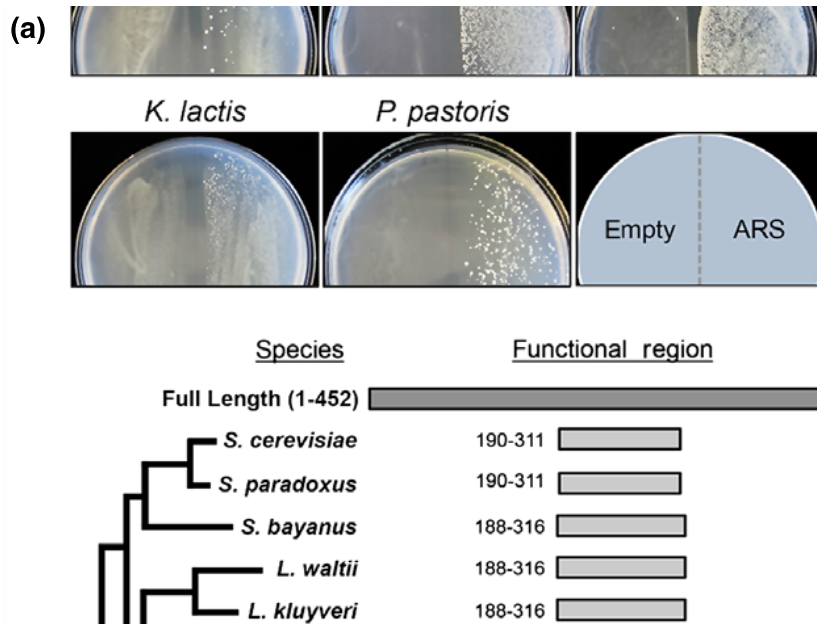


Origin of replication: panARS

An autonomously replicating sequence for use in a wide range of budding yeasts

Ivan Liachko & Maitreya J. Dunham

Department of Genome Sciences, University of Washington, Seattle, WA, USA

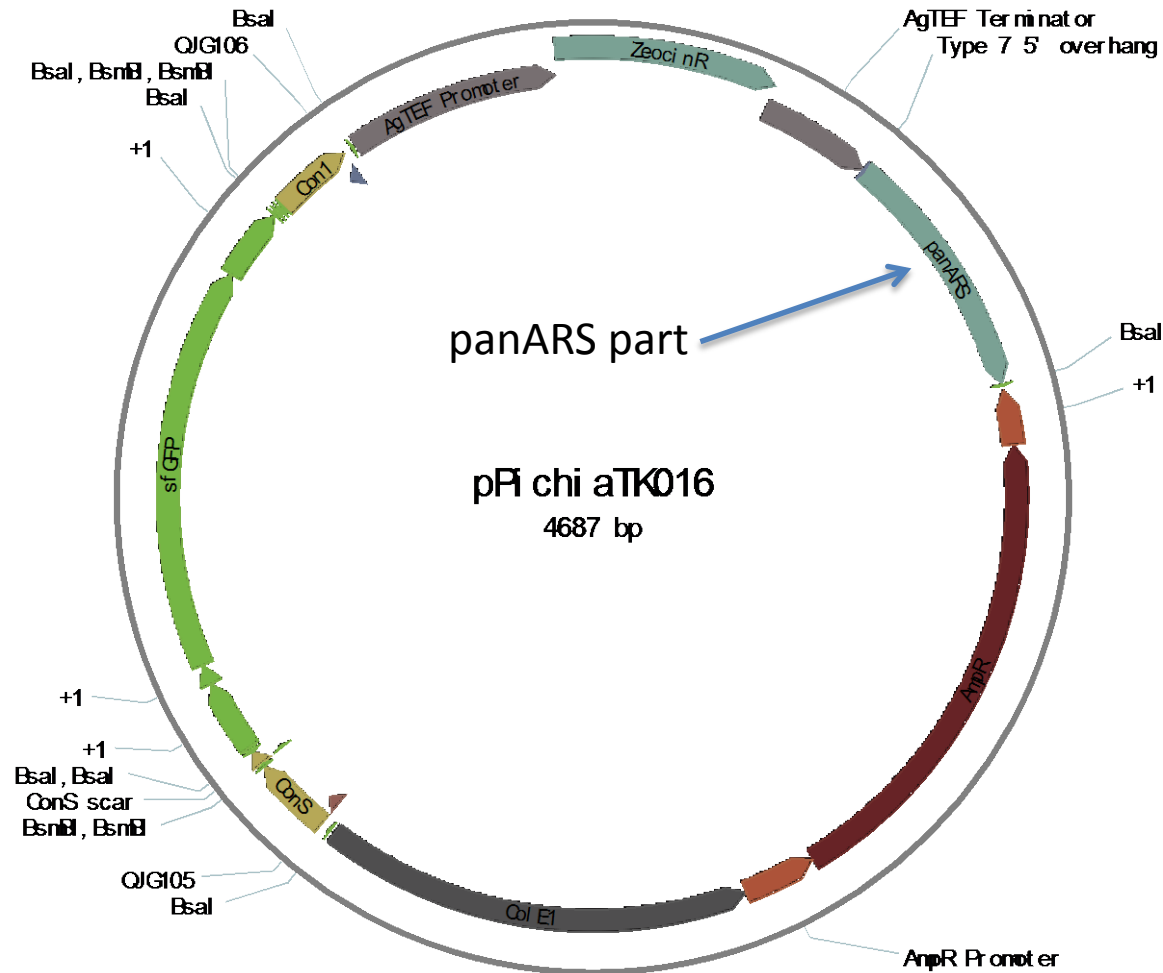


Plasmid system for *Pichia*

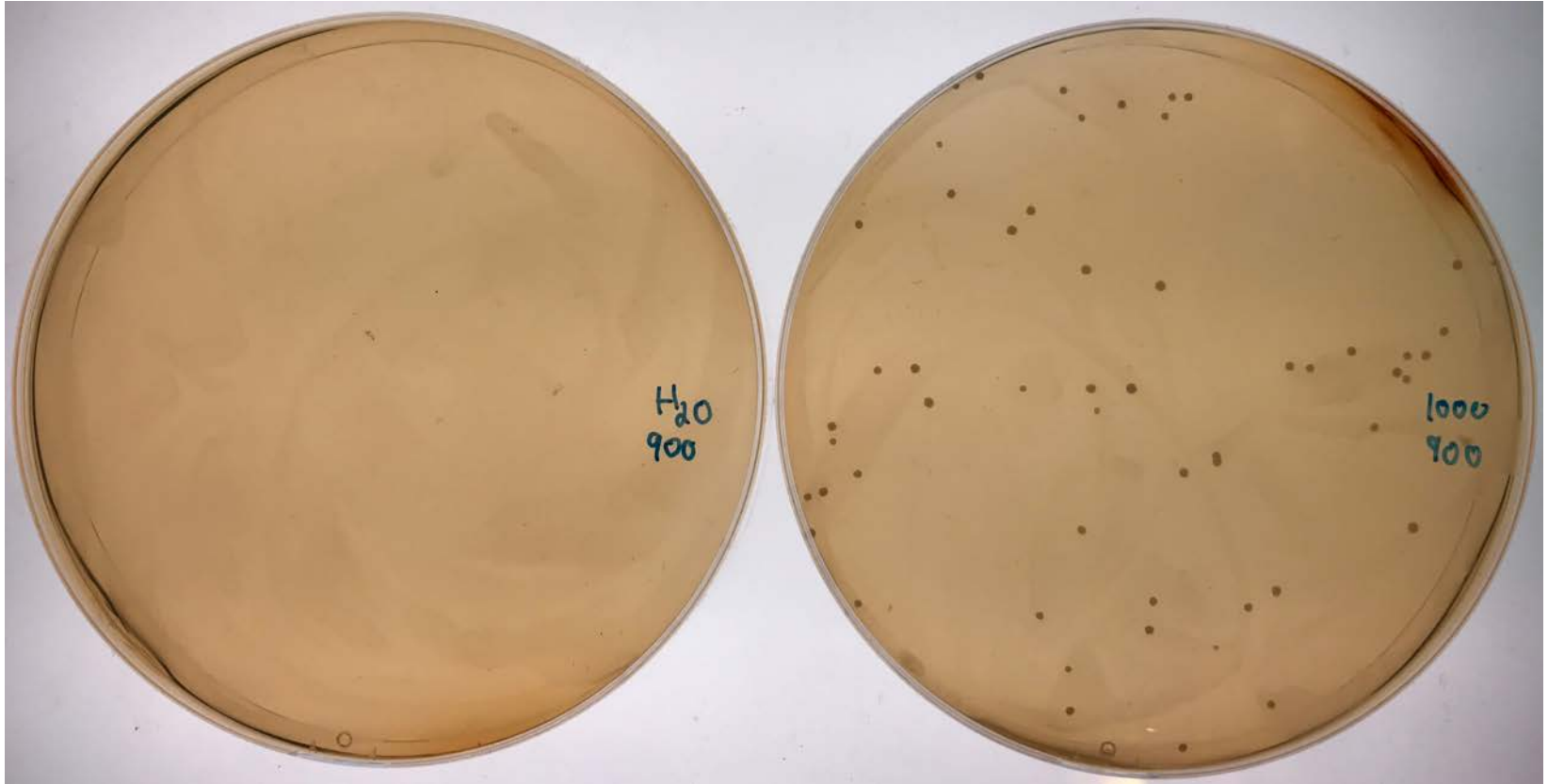
Adapted plasmid system from John Dueber by incorporating recently identified “panARS” sequence to allow replication of plasmid in *Pichia*.

Most other parts should be reusable.

Constructed empty vector that should impart resistance to Zeocin.



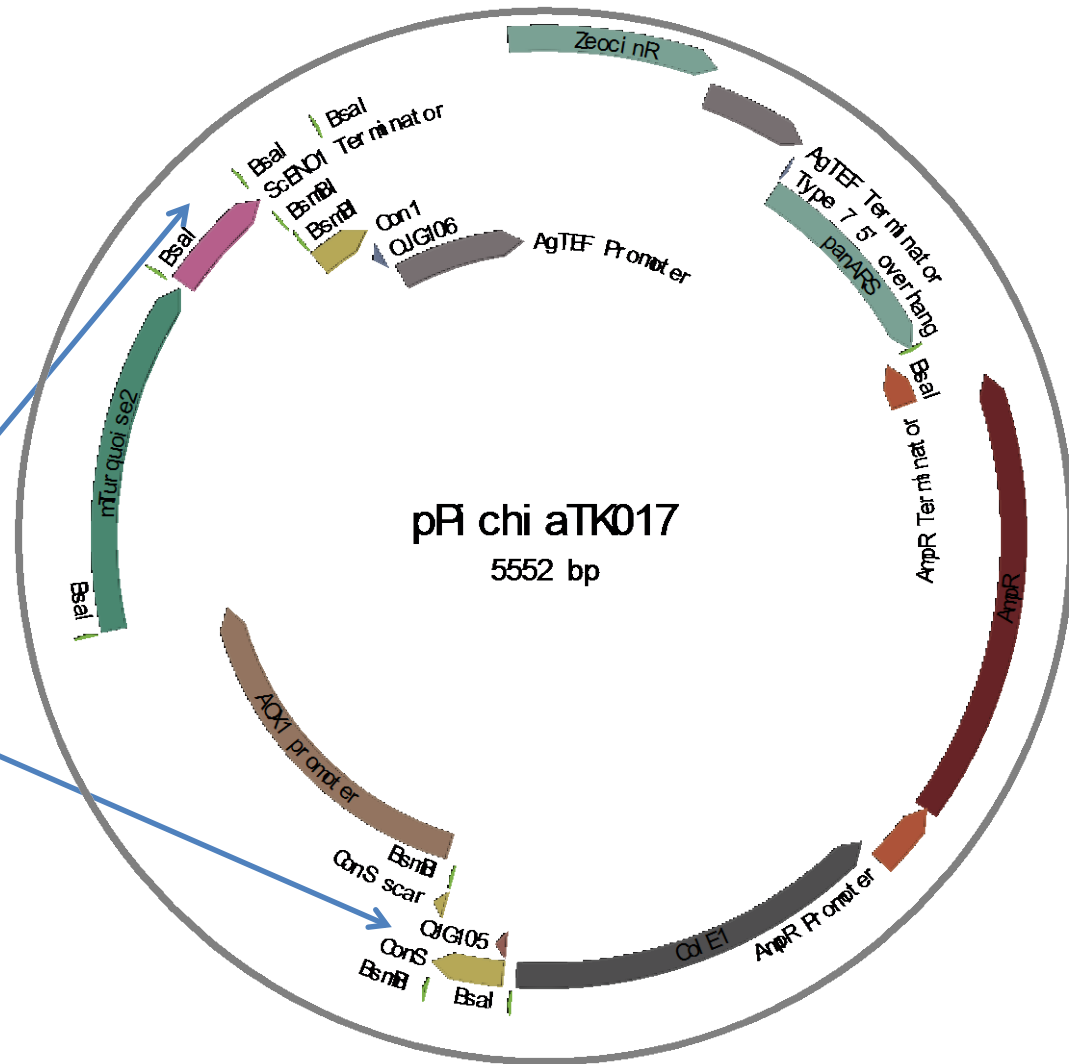
Plasmid system for *Pichia*



Water

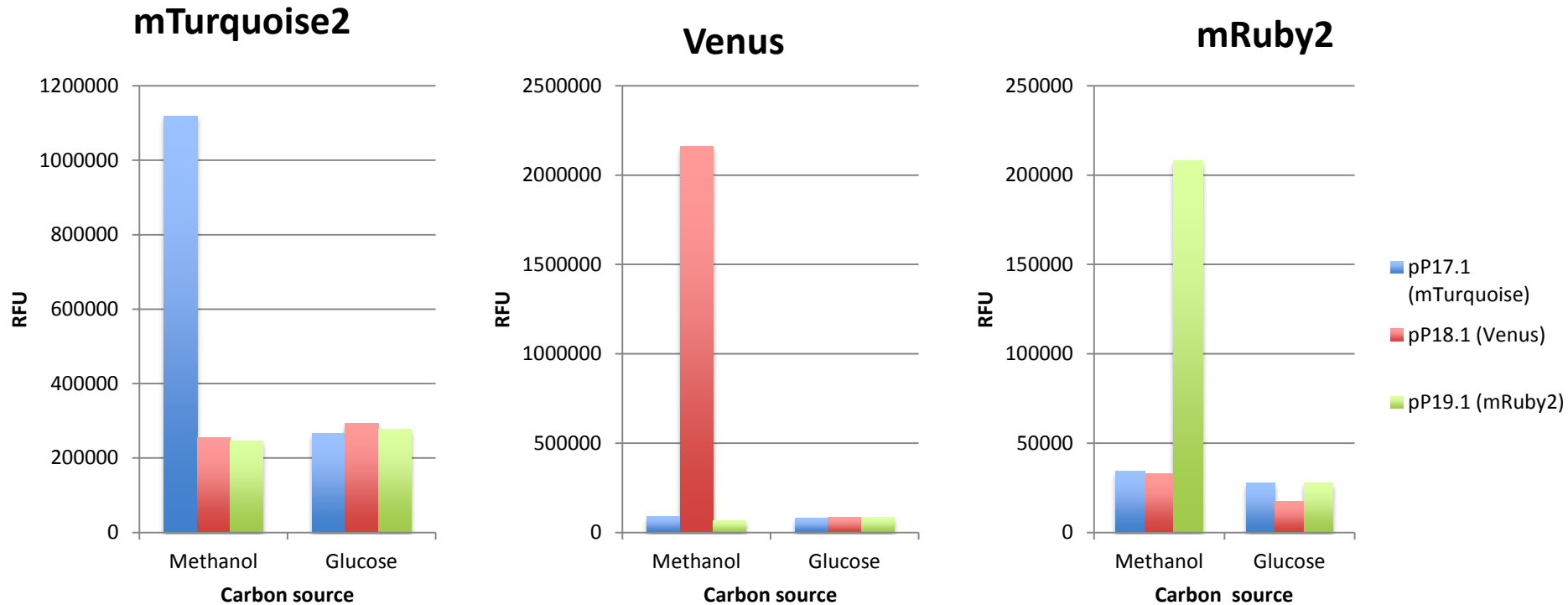
1 μ g pTK16

Plasmid demonstration in *P. pastoris*

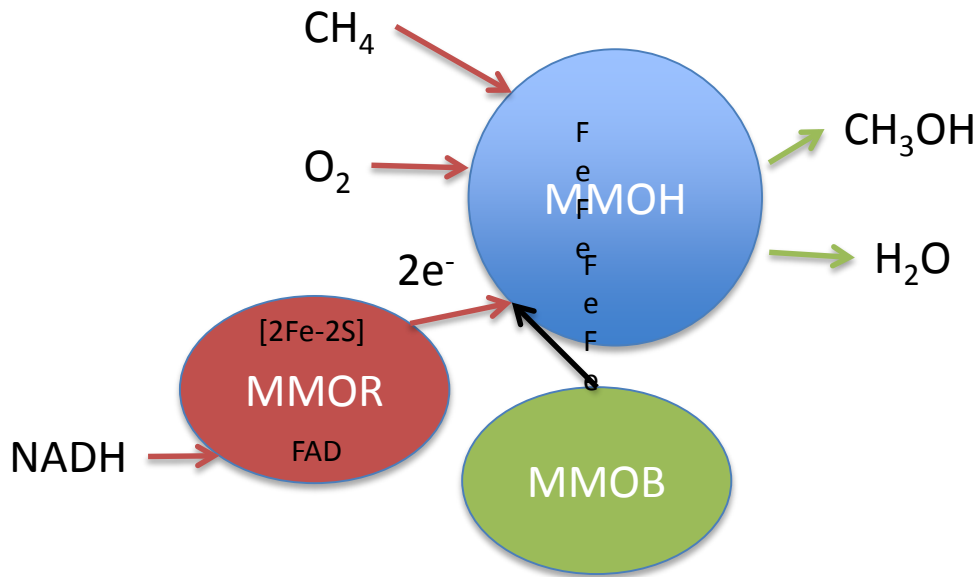


pPichia17 – AOX1p-mTurquoise-ScENO1
pPichia18 – AOX1p-Venus-ScENO1
pPichia19 – AOX1p-mRuby-ScENO1

Plasmid demonstration in *P. pastoris*



Porting Soluble Methane Monooxygenase to *Pichia*



1. Hydroxylase: MMOH

1. alpha
2. beta
3. gamma

Oxidizes methane hydroxylates methane to methanol

2. Reductase: MMOR

Oxidizes NADH and transfers electrons to MMOH

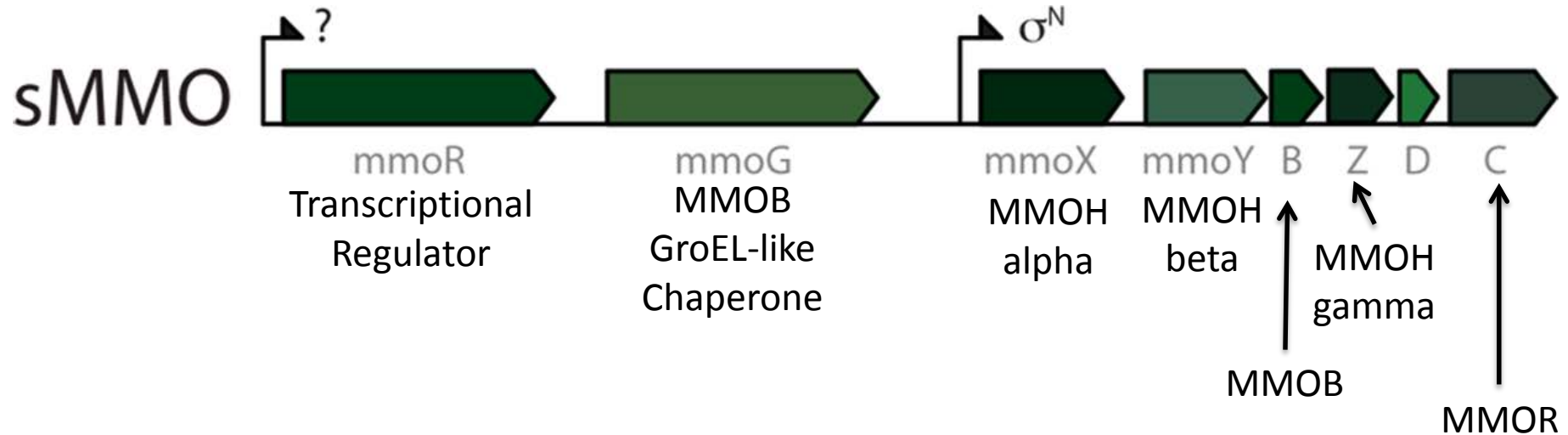
1. Regulatory: MMOB

Binds to same site as MMOR. May help drive cycle.

2. Assembly chaperone: MMOG

Could help in assembly of MMOH

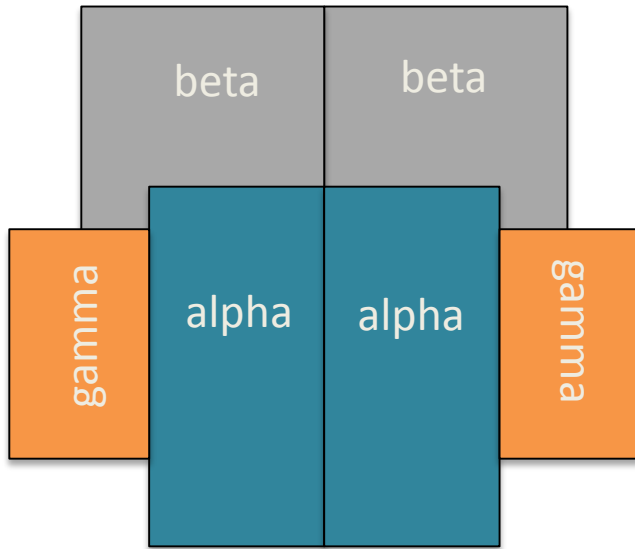
MMO components are expressed as an operon



- Most studies are sMMO from *Methylococcus capsulatus* (Bath) or *Methylosinus trichosporium* OB3b
- MMO from *M. trichosporium* OB3b has a higher turnover number (3.5 vs. $0.2 - 1.0 \text{ s}^{-1}$)

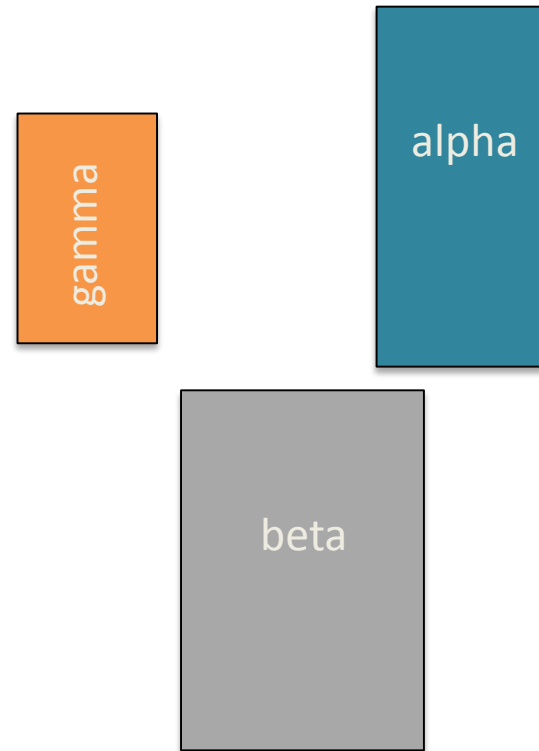
Proper assembly of MMOH is challenging

Assembled Complex



Stable. Active complexes are readily purified from *M. capsulatus*.

Pre-assembled Components



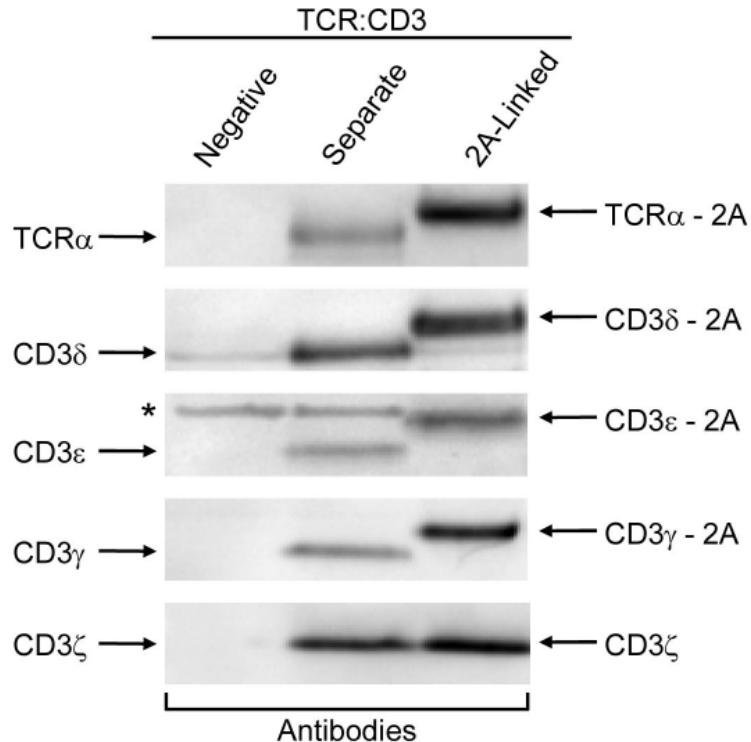
Unknown stability in *Pichia*. Assumed to be unstable.

Type 2a peptides balance component stoichiometry

VKQTLNFCLLKLAGDVESNPGP



Ribosome skips this peptide bond



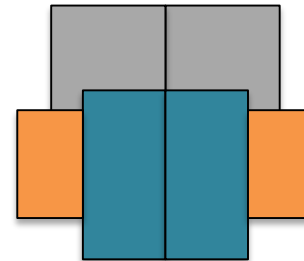
Szymczak AL *et al. Nat Biotechnol*
2004; **22**: 589–594; Epub 2004, April
4.

MMOH

Alpha---2a---Beta---2a---Gamma

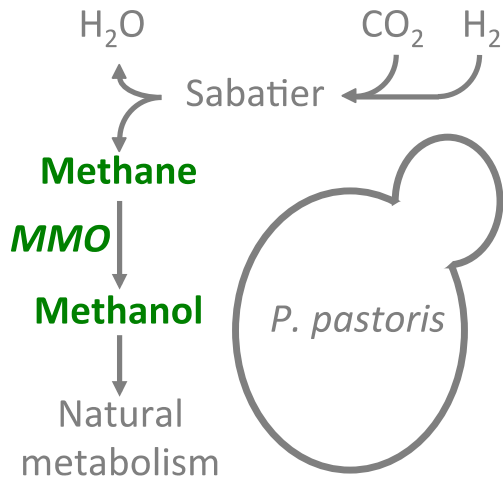
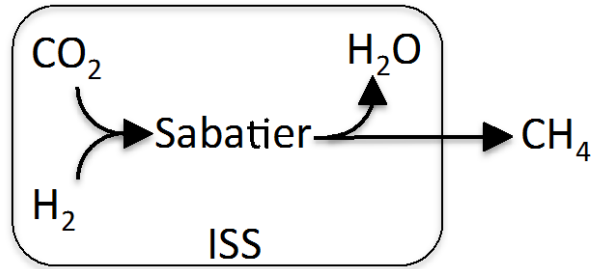
↓ ↓ ↓

Alpha-2a Beta-2a Gamma



Stoichiometric production of complex components

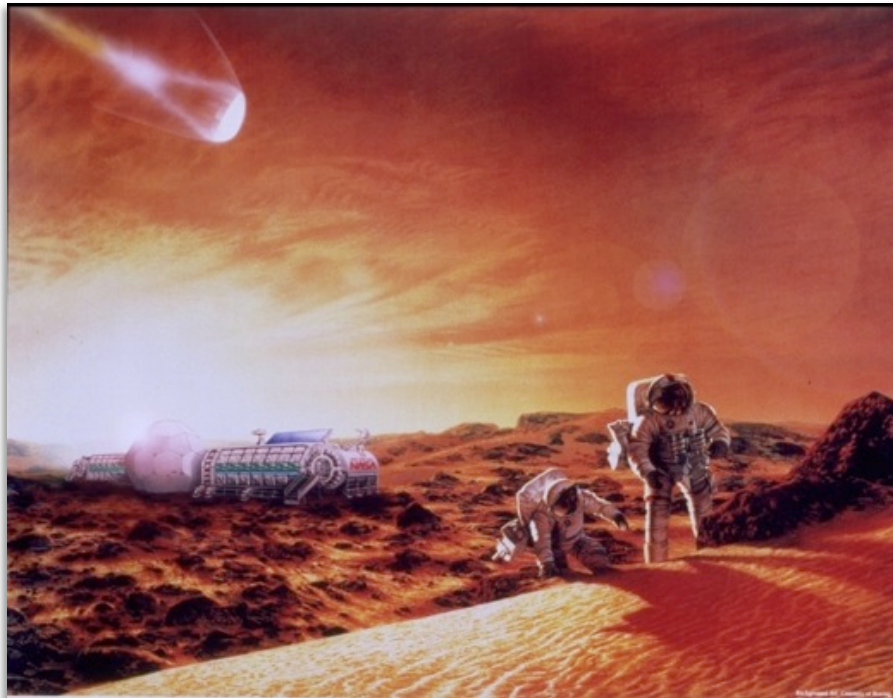
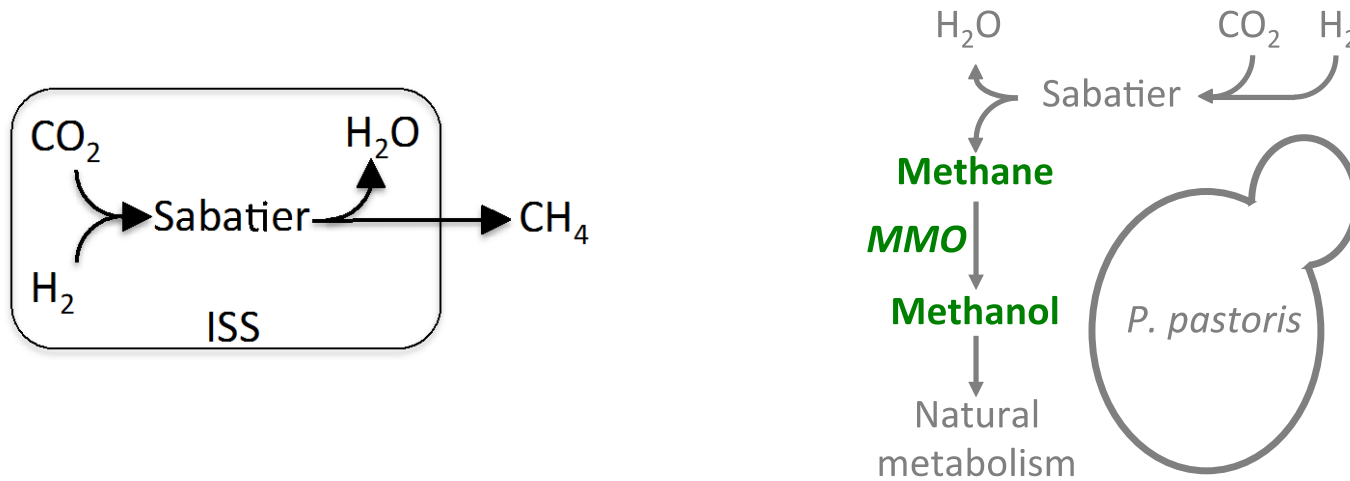
Porting sMMMO to *Pichia*: Current status



Status

	Constructed	Tested
Pichia tools		
MMOH alpha		
MMOH beta		
MMOH alpha		
Assembly		
MMOR		
MMOB		
^{13}C GC/MS		
MMOG		

Potential of Biotechnology in Space



Credits: NASA

Acknowledgements

Dr. John A. Hogan (NASA)

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NASA Ames Team Members

Dr. Michael Dougherty (Wyle Labs)

Natalie Ball (Wyle Labs)

Dr. Hiromi Kagawa (SETI)



Dr. John Hogan

WASHU Collaborators

Fuzhong Zhang

Chris Bowen

Cameron Sargent

Sarah Rommelfanger

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NASA Space Technology Mission Directorate Center Innovation Fund

NASA Advanced Exploration Systems

NASA Space Biology Project