Factors governing a synthetic microbial mutualism

DOE Early Career Program Award
DE-SC0008131
2012 - 2018

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Overview

1. Development of a synthetic H_2-producing bacterial coculture

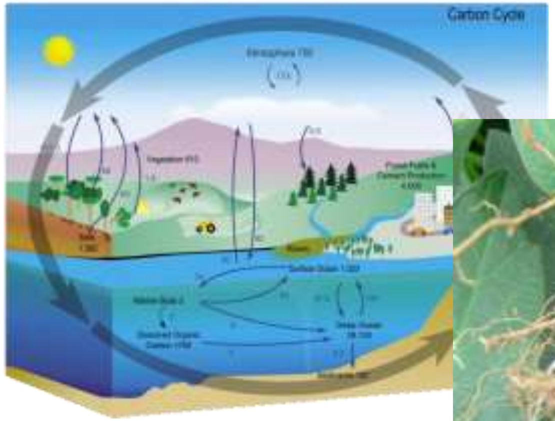
2. Discovery highlights from the coculture system

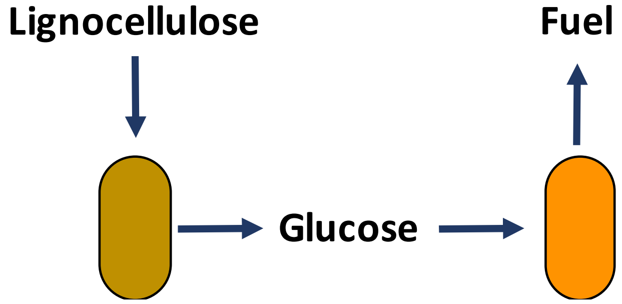
3. Unanticipated projects stemming from the coculture system

4. Other impacts of the award on my research program and the people involved in it

Microbial cross-feeding is important

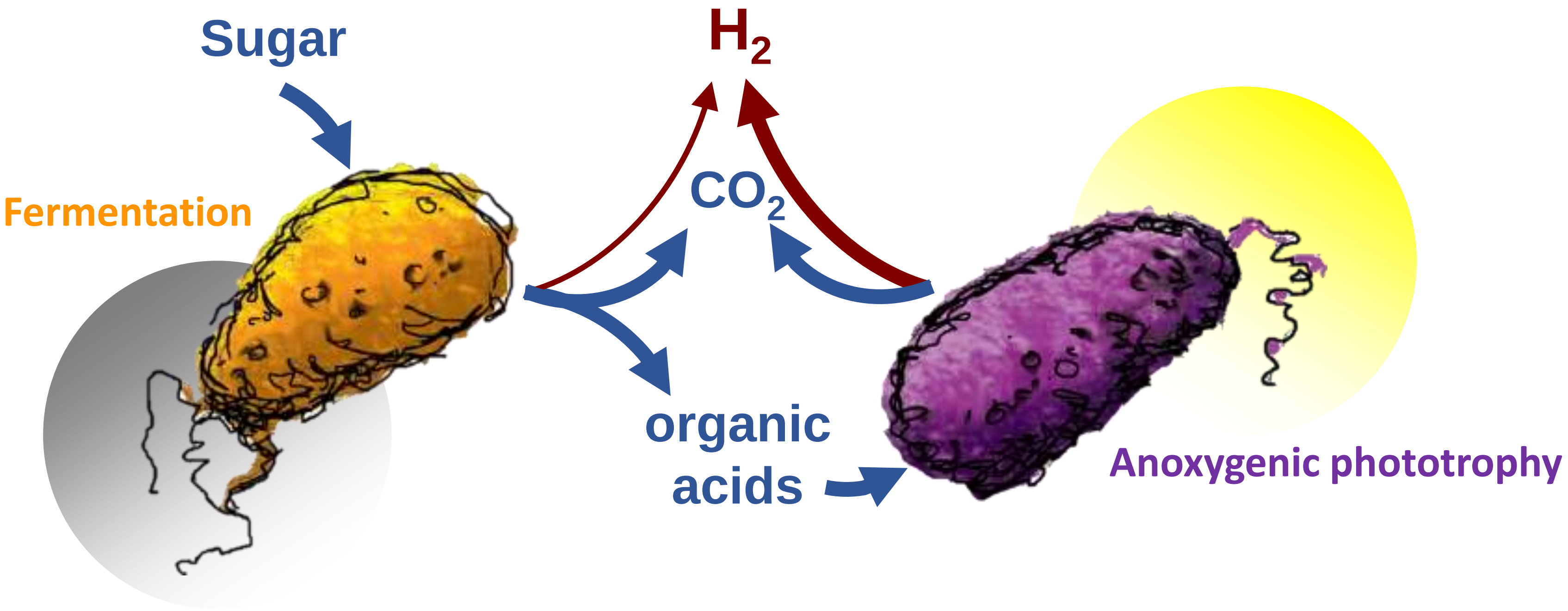
- Global elemental cycles
- Agriculture
- Biotechnology





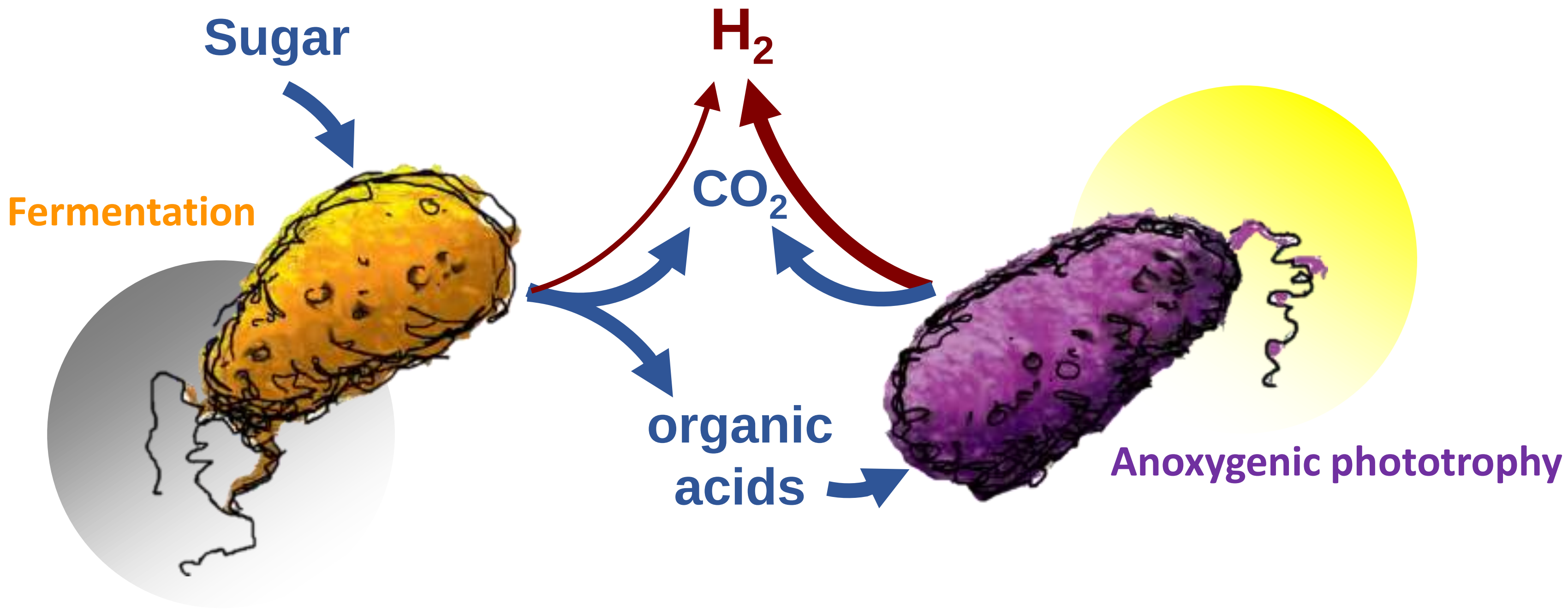
- **Understanding the factors that govern cross-feeding interactions has implications for our environment, health, and industries**

Cross-feeding of fermentation products



- The unidirectional excretion of organic acids can lead to cross-feeding
- Resembles an anaerobic food-web

Traditional cocultures lacked stable coexistence and reproducibility



- Fast-growing fermentative bacteria paired with slow-growing phototrophs
- Establishing stable synthetic communities is rarely trivial

First example: Odom et al. 1983 *Appl. Environ. Microbiol*

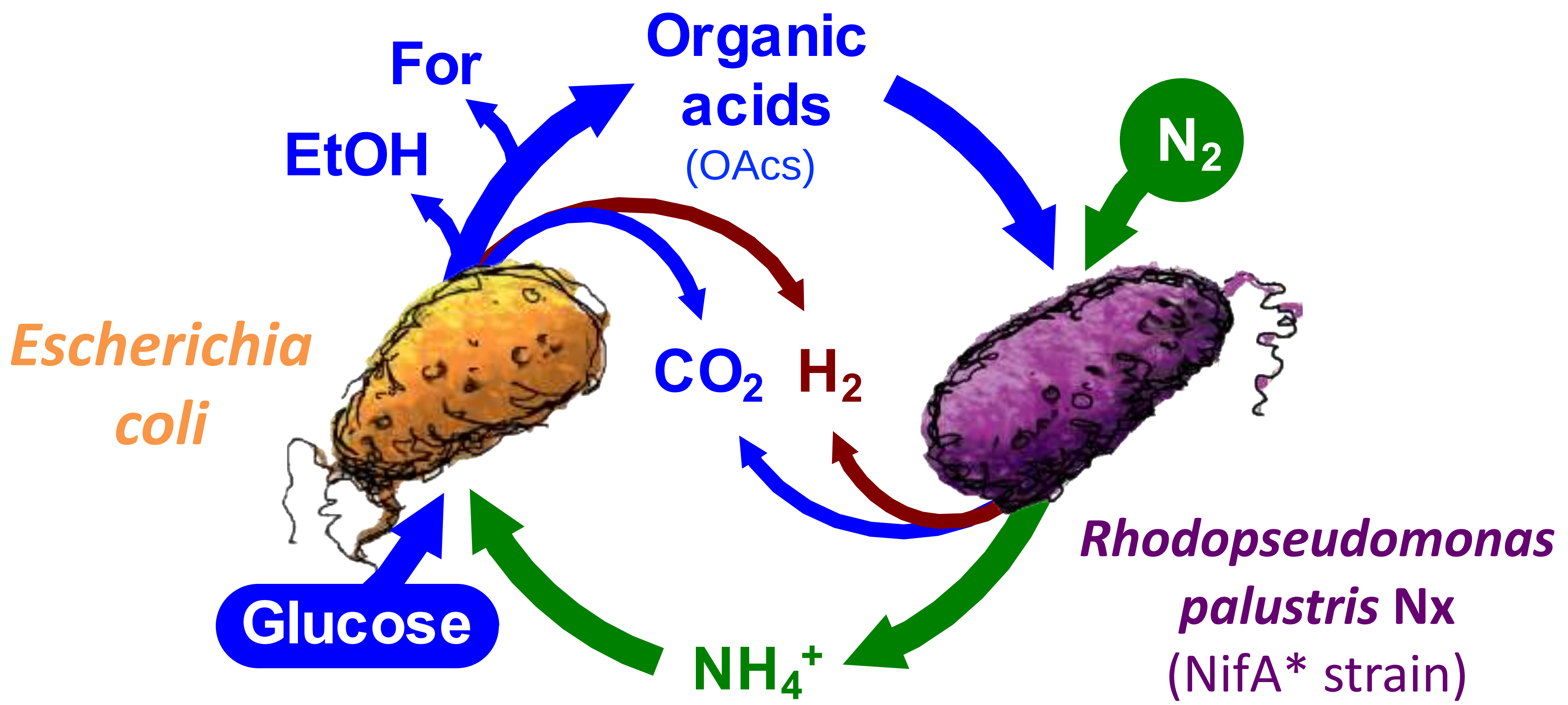
Building a practical coculture to study cross-feeding

Ryan Fritts

Dr. Breah LaSarre

Dr. Ali McCully

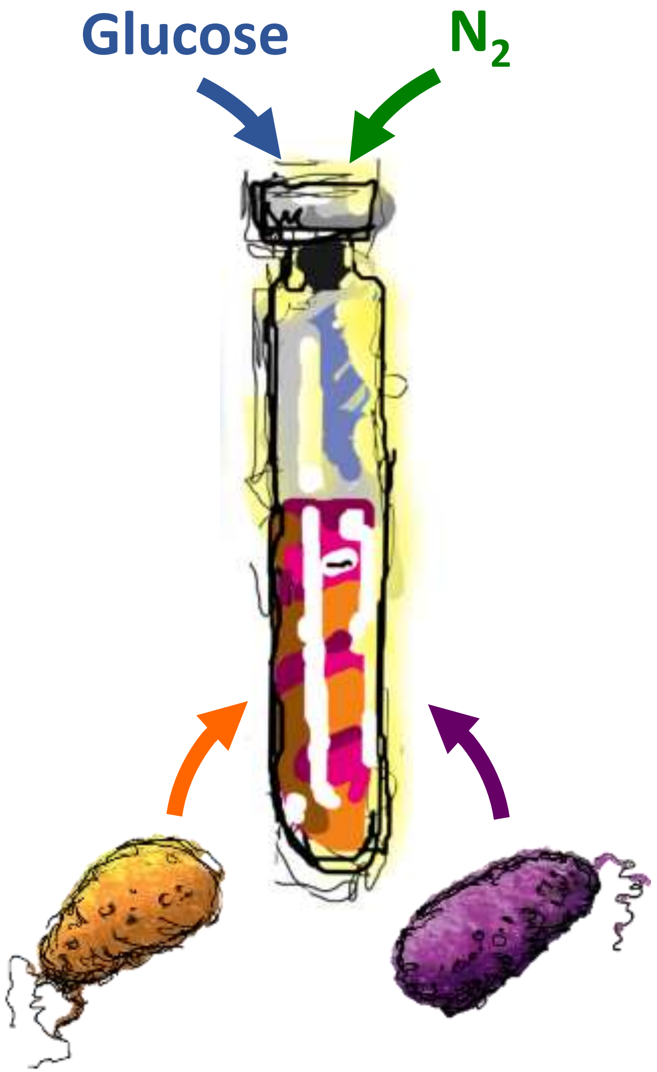
Reciprocal cross-feeding of ammonium could lead to a stable mutualism

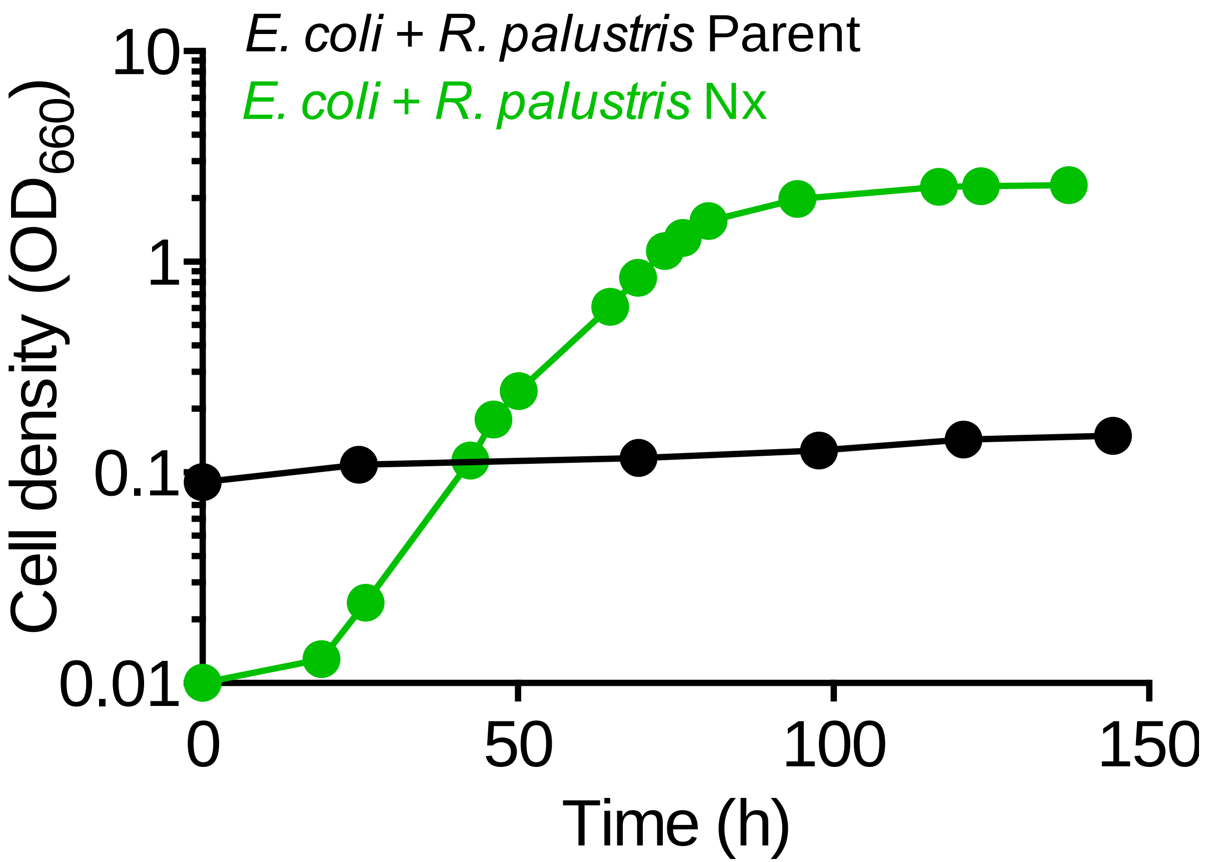


LaSarre et al. 2017. ISME J
McCully et al. 2017. Env. Microbiol

McCully et al. 2017. mBio
McCully et al. 2018. Appl. Environ. Microbiol.

R. palustris Nx mutation results in cross-feeding of ammonium to *E. coli*

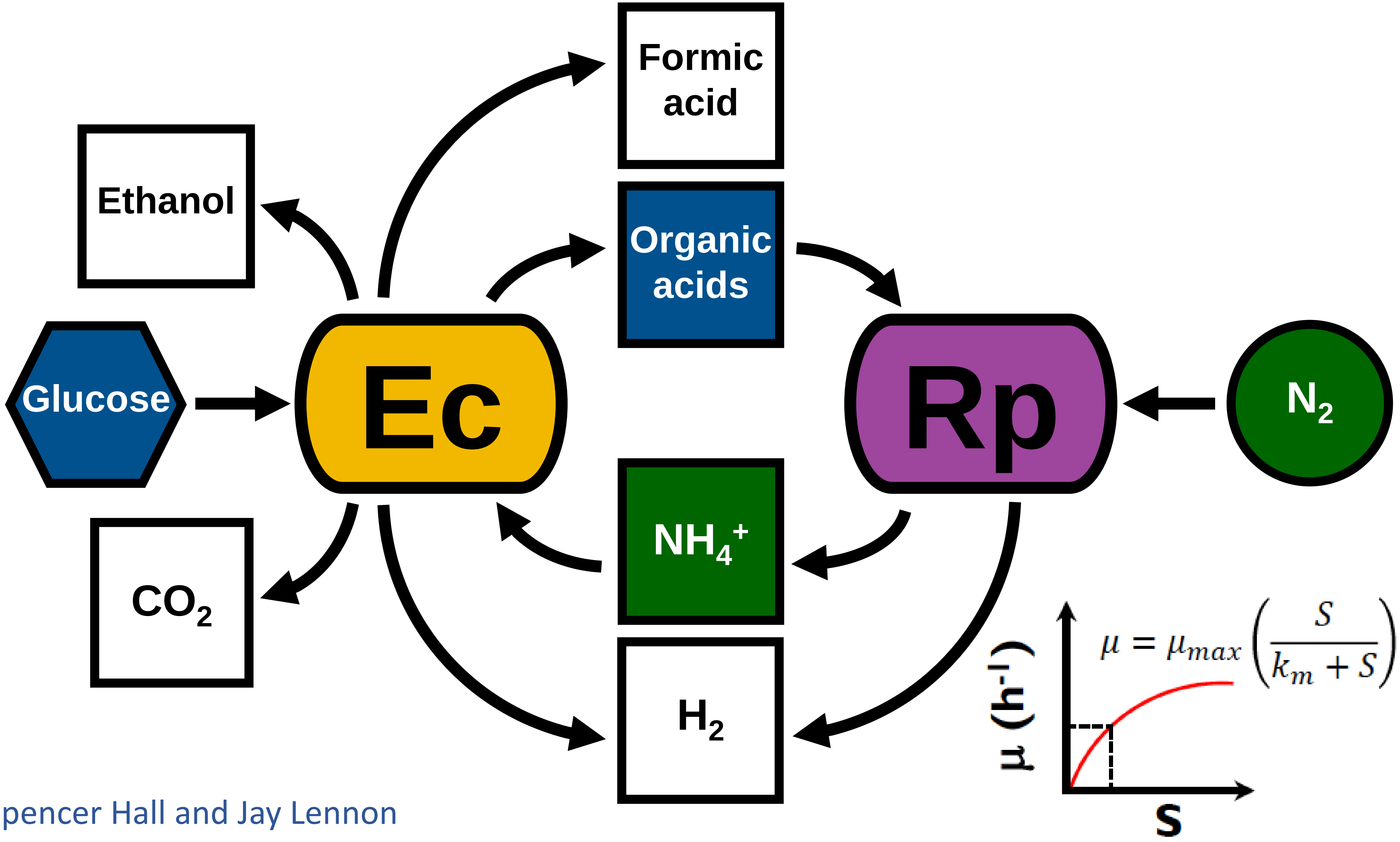




Coculture growth in phosphate buffered minimal medium

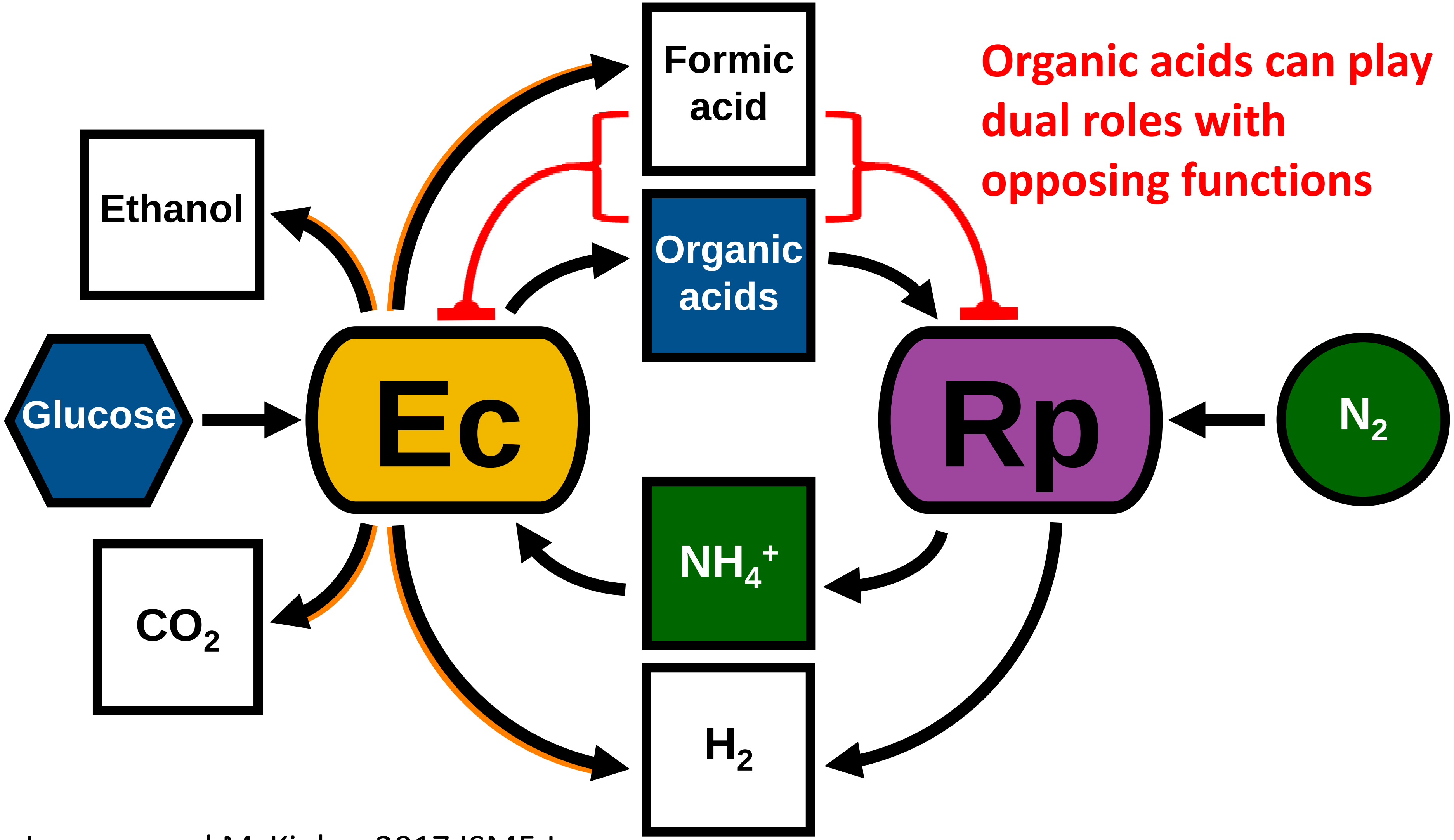
- Cocultures move to equilibrium from starting ratios spanning >6-orders of magnitude

A kinetic model for simulating coculture trends



With help from Spencer Hall and Jay Lennon

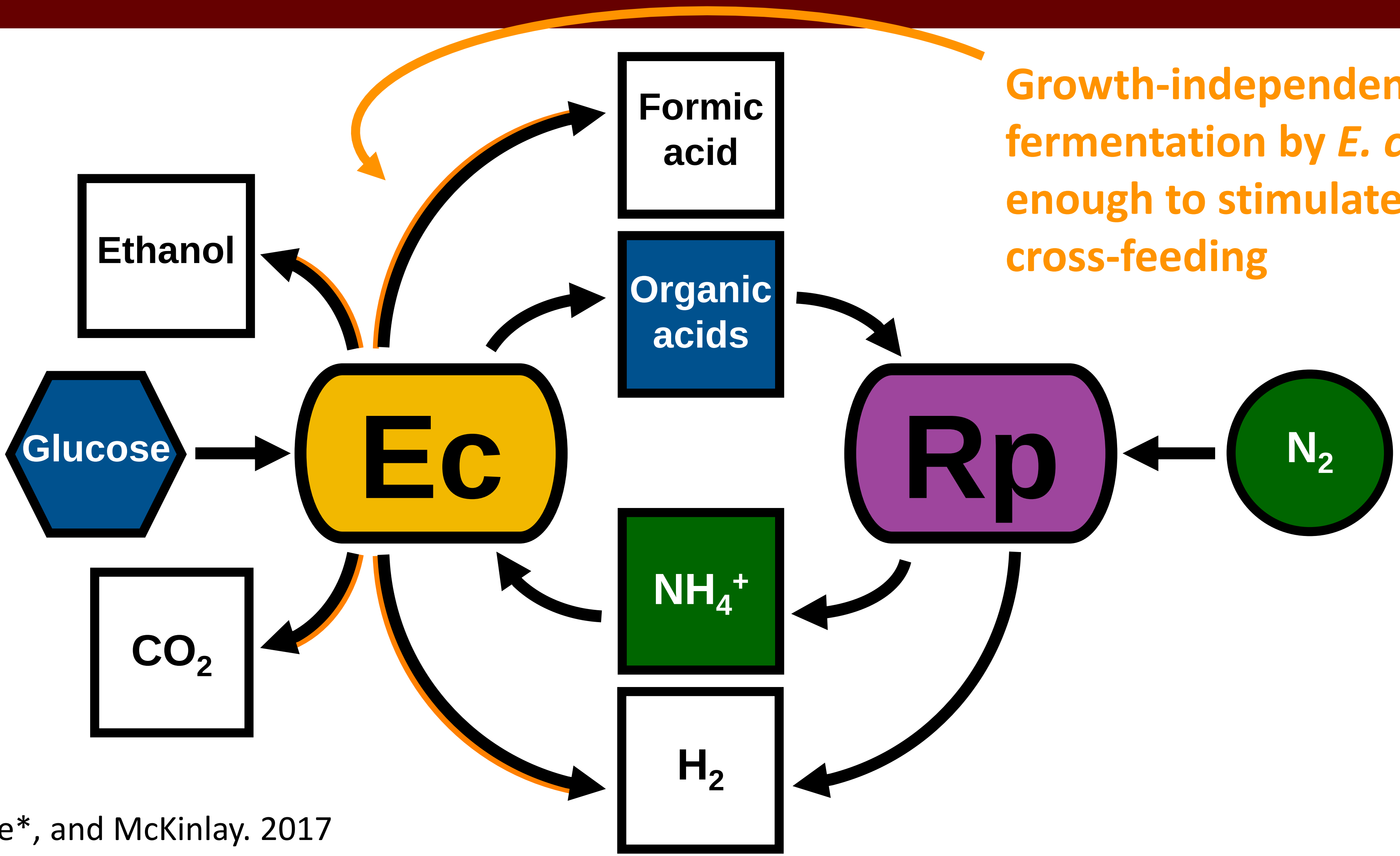
Accumulation of organic acids can inhibit growth



Organic acids can play dual roles with opposing functions

LaSarre, McCully, Lennon, and McKinlay. 2017 ISME J

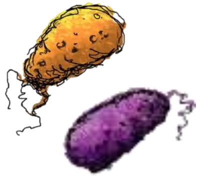
Growth-independent cross-feeding can maintain a mutualism through periods of starvation

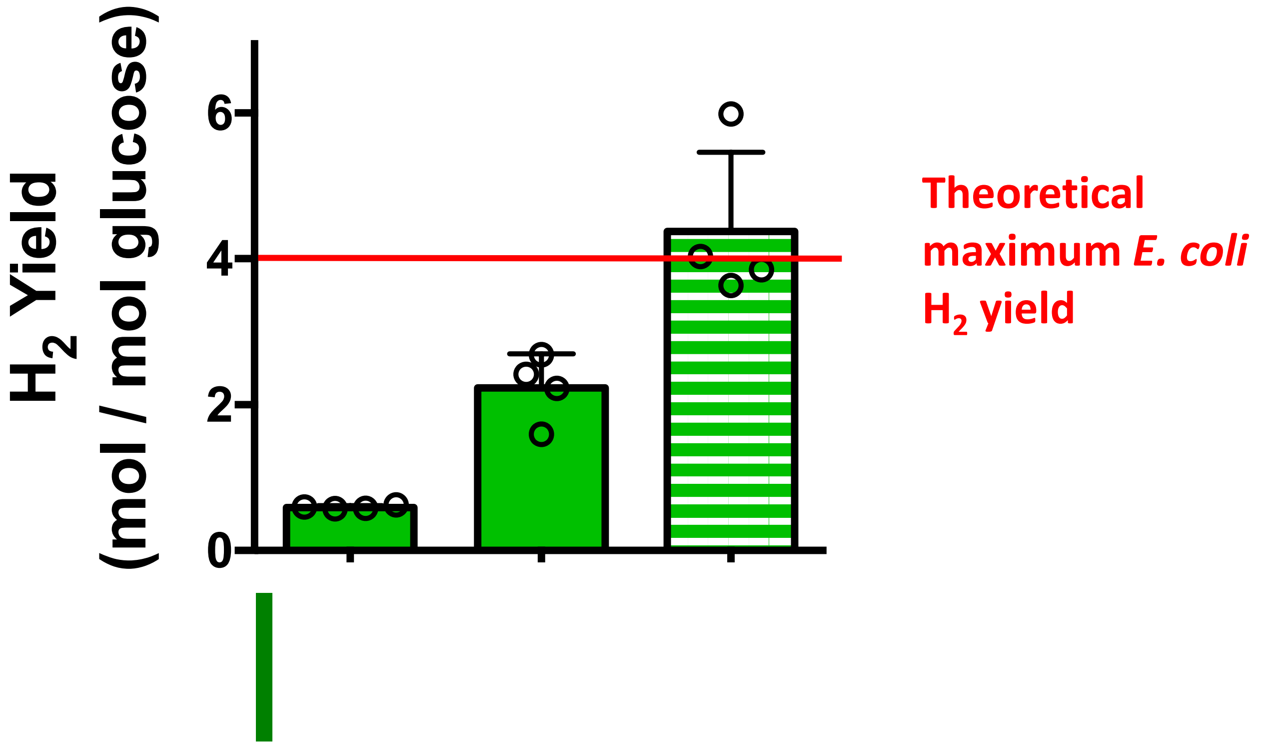


Growth-independent fermentation by *E. coli* is enough to stimulate reciprocal cross-feeding

McCully*, LaSarre*, and McKinlay. 2017 Environ Microbiol *equal contribution

Nitrogen-deprived cocultures also have high a H_2 yield





- H_2 yields can exceed the theoretical maximum for a fermentation

Competition during cross-feeding

Competition for communally valuable cross-fed metabolites

McKinlay Lab

Dr. Ali McCully

Dr. Breah LaSarre

Jennifer Gliessman

Jeffrey Mazny

Drummond Lab

Dr. Evgeny Pilipenko

Prof. Allan Drummond

Lynch Lab

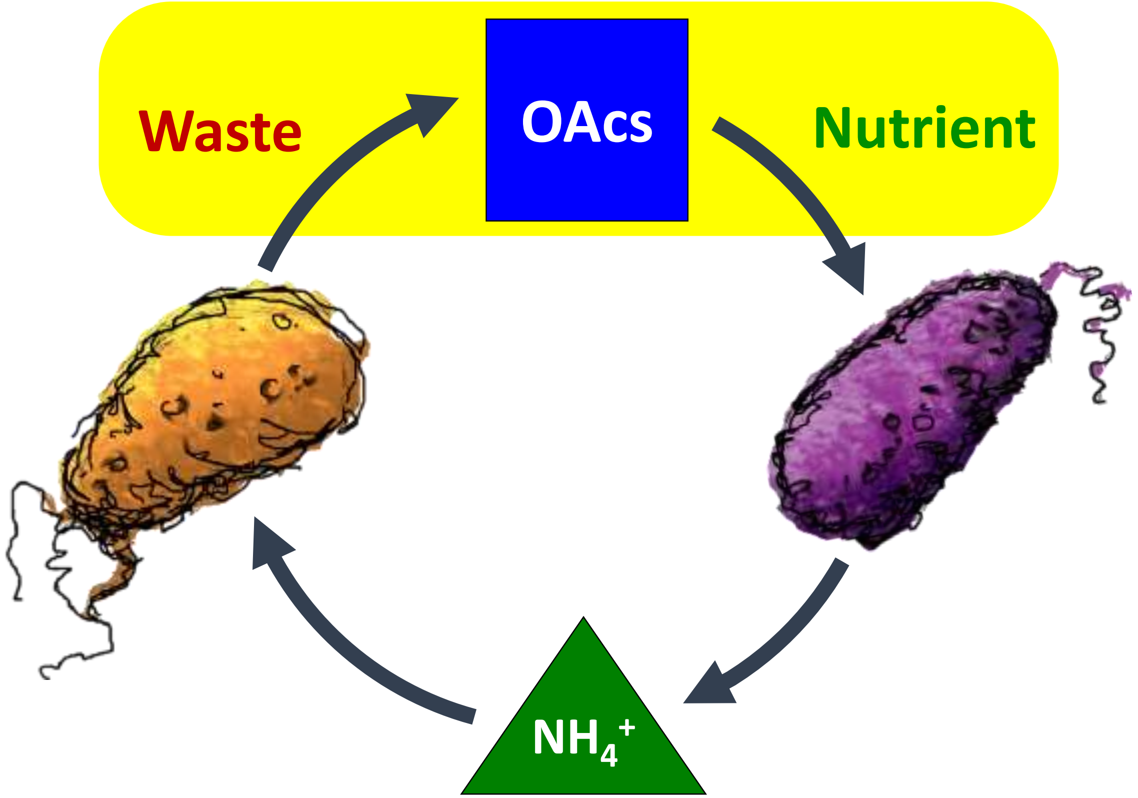
Dr. Megan Behringer

Prof. Mike Lynch

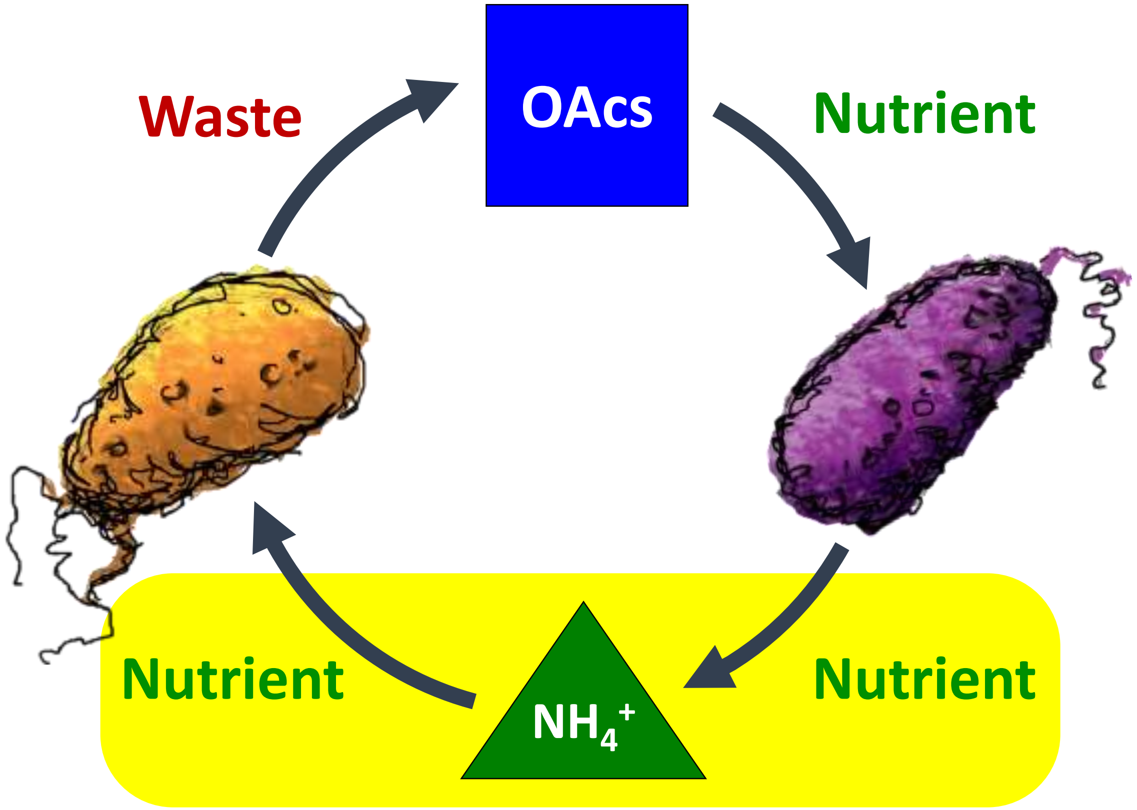
McCully, LaSarre, and McKinlay. 2017. mBio

McCully, Behringer, Gliessman, Pilipenko, Mazny, Lynch, Drummond, and McKinlay. 2018. Appl. Environ. Microbiol.

Some cross-fed metabolites are of value to only one partner

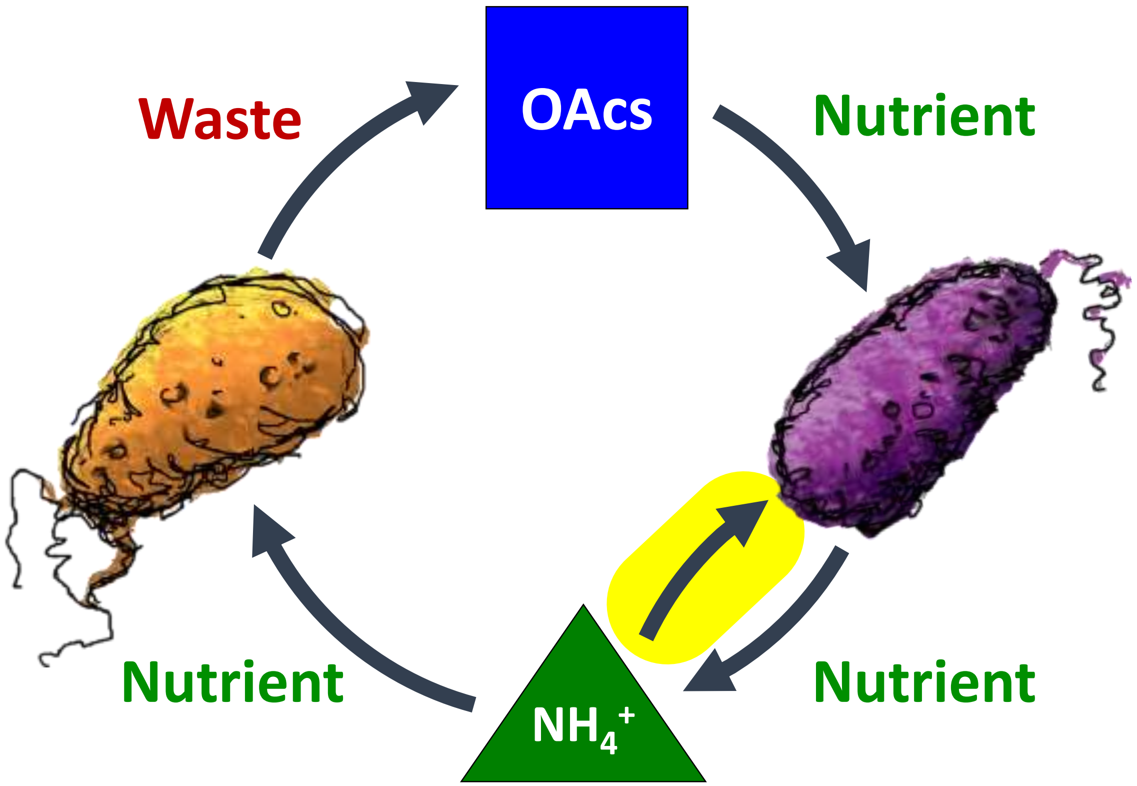


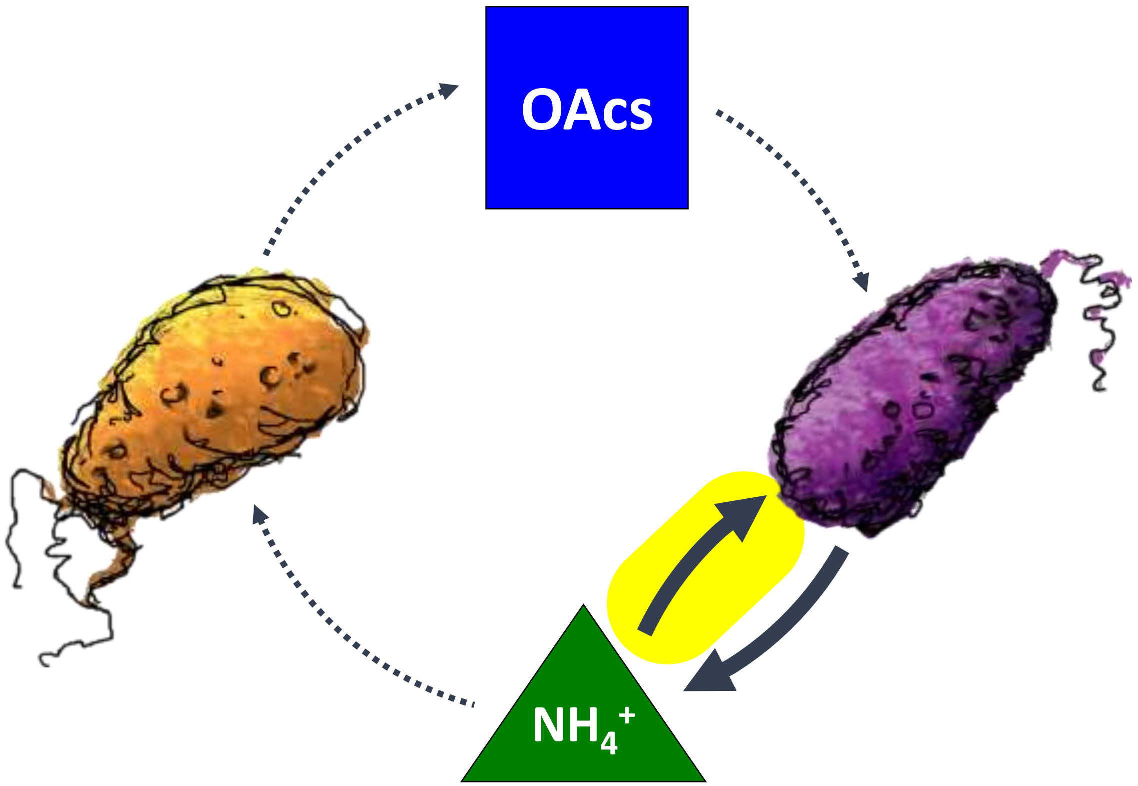
Other cross-fed metabolites are of value to both partners



- Other microbial examples
  - Nitrogen transfer from N_2-fixing bacteria to other bacteria, fungi, and plants
  - Transfer of vitamin B_{12} between bacteria and abundant marine algae

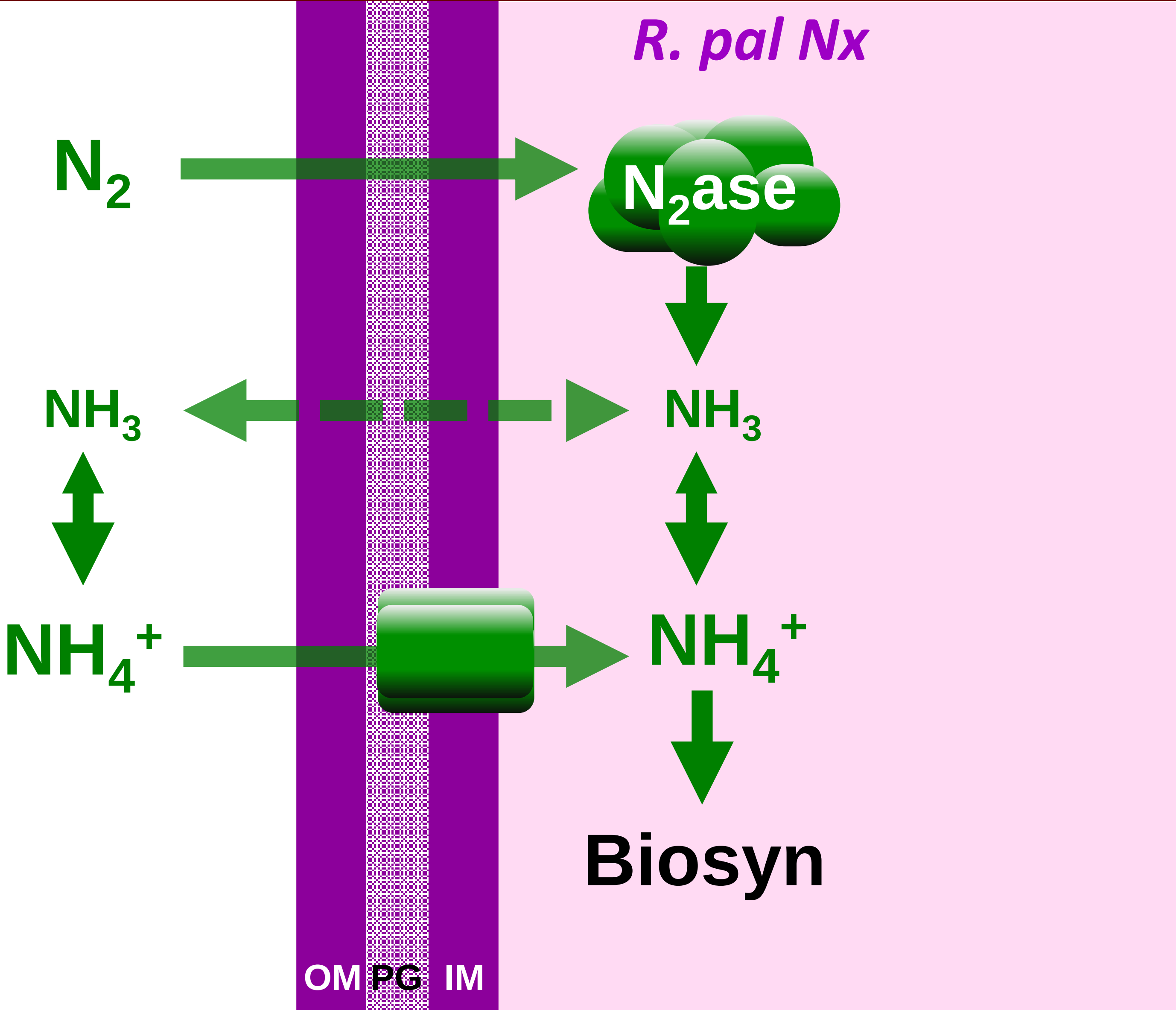
Do mutualistic partners compete for communally-valuable cross-fed nutrients?



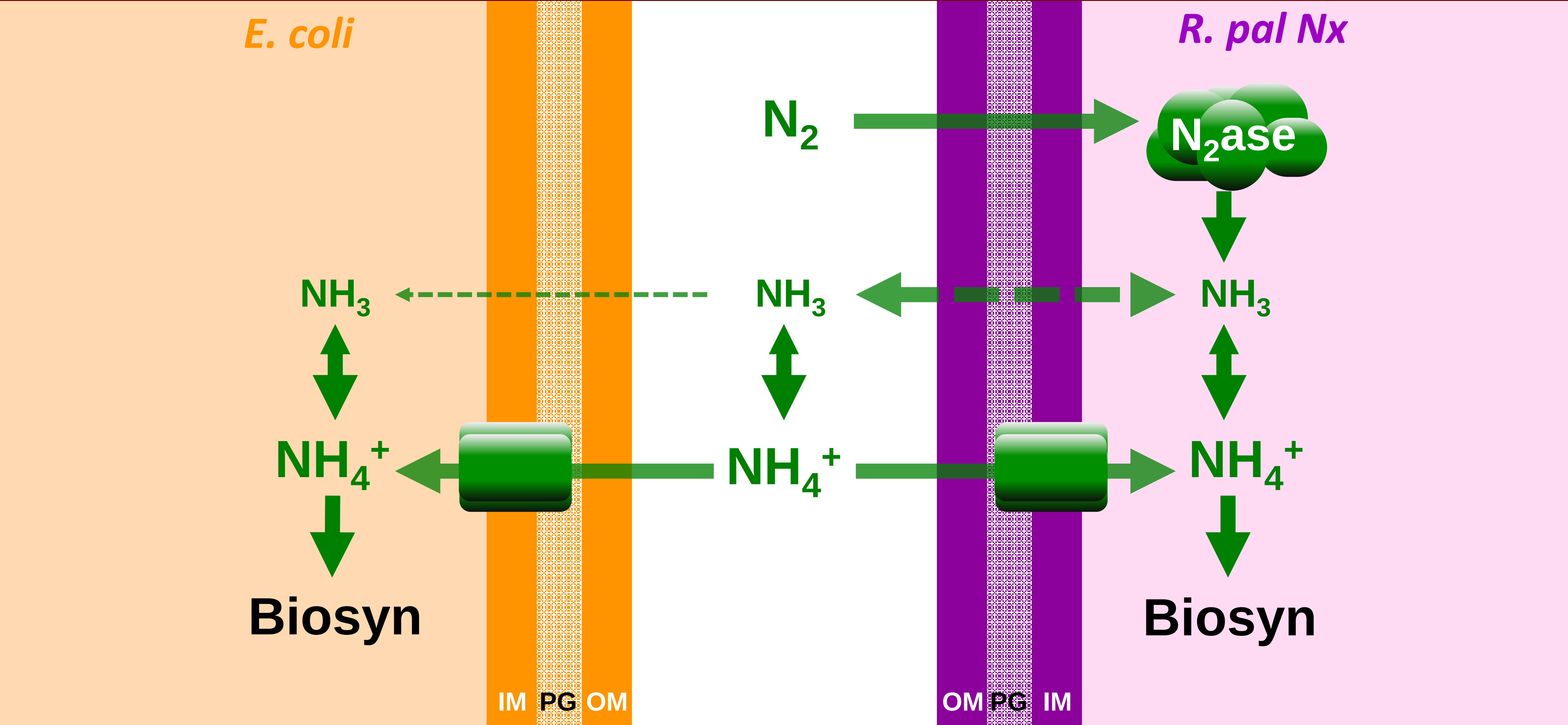


- Model predicts that competition for NH_4^+ must be biased in favor of *E. coli* or the mutualism will collapse

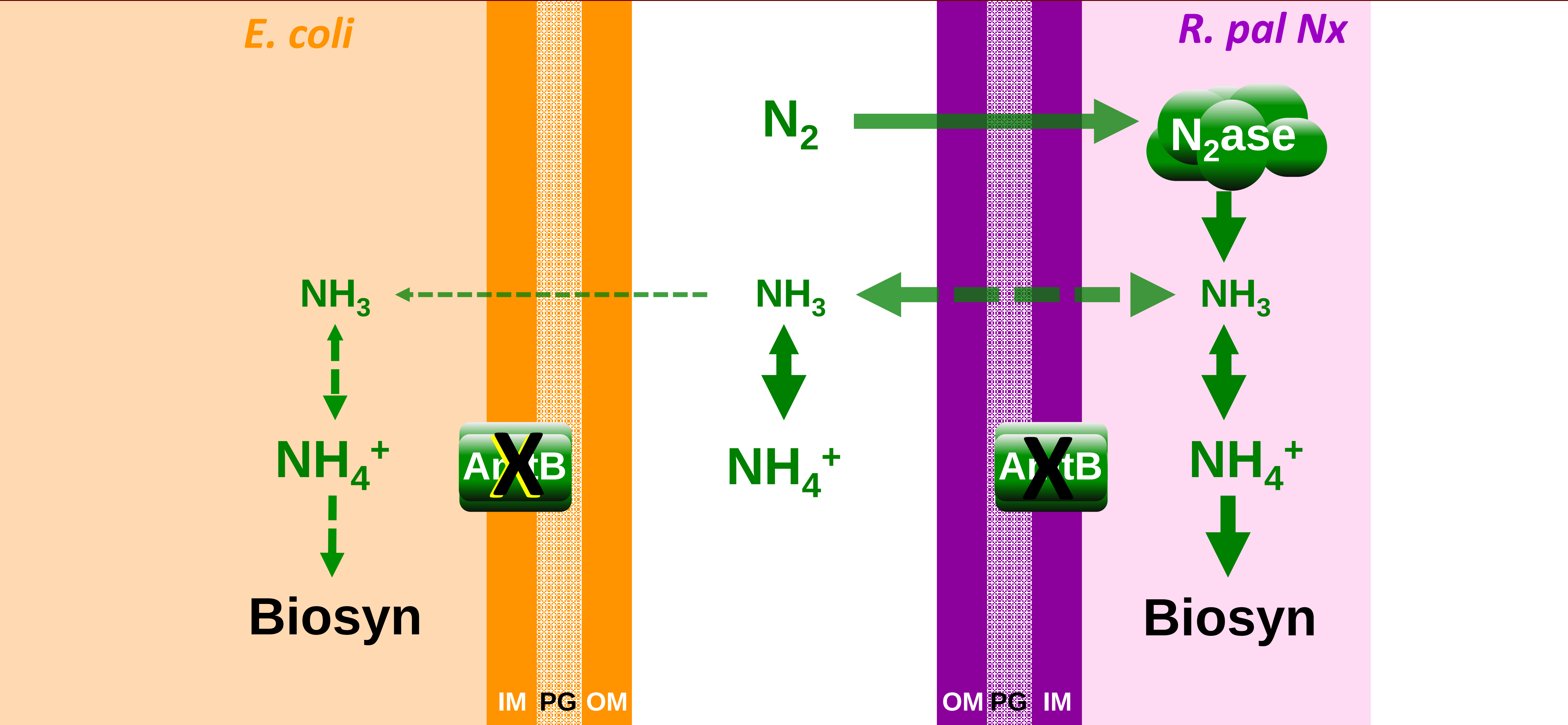
How is competition for ammonium occurring?



Competition is likely occurring at the level of AmtB NH_4^+ transporters



Coculture collapses whenever *E. coli* is lacking AmtB



E. coli AmtB is upregulated in coculture

Fold-changes for WT *E. coli* transcripts and proteins in coculture vs monoculture

RNA-seq			Proteomics		
Gene	**Description**	**Fold change**	**Gene**	**Description**	**Fold change**
rutACDEFG	Nitrogen scavenging from pyrimidines	157	argT	Lys/Arg/Orn binding protein	11
nac	Nitrogen assimilation control	97	ddpA	D-ala dipeptide permease	6
ddpX	D-ala dipeptidase	76	bfr	Bacterioferritin	5
csgB	Curli	64	gss	Glutathionylspermidine synthetase/amidase	4
argT	Lys/Arg/Orn binding protein	61	potF	Putrescine-binding periplasmic protein	4
patA	Putrescine aminotransferase	59	modA	Molybdate-binding periplasmic protein	4
glnK	Nitrogen regulation	37	gabD	Succinate-semialdehyde dehydrogenase	4
amtB	**NH_4^+ Transporter**	**24**	**amtB**	**NH_4^+ Transporter**	**4**

Most of the upregulated genes are controlled by NtrC, the master transcriptional activator of the nitrogen starvation response

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amtB	**NH_4^+ Transporter**	**24**	**amtB**	**NH_4^+ Transporter**	**4**

- Deleting *ntrC* can also lead to a collapse of the mutualism

Mutualism favors the scavenger

Emergence of a nascent mutualism

McKinlay Lab

Ryan Fritts

Dr. Jordan Bird

Joint Genome Institute

JGI
JOINT GENOME INSTITUTE
DEPARTMENT OF ENERGY

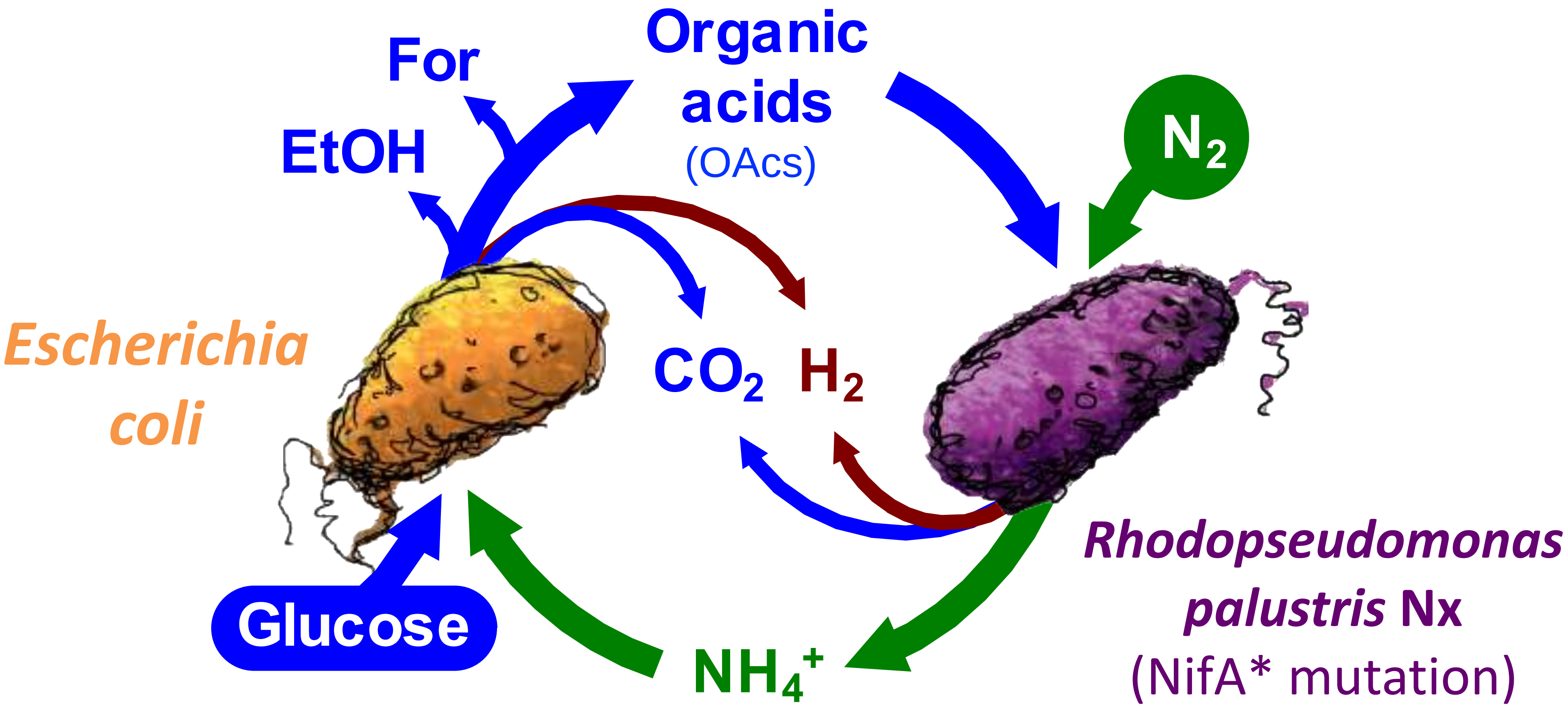
Community Science Program

Lynch Lab

Dr. Megan Behringer

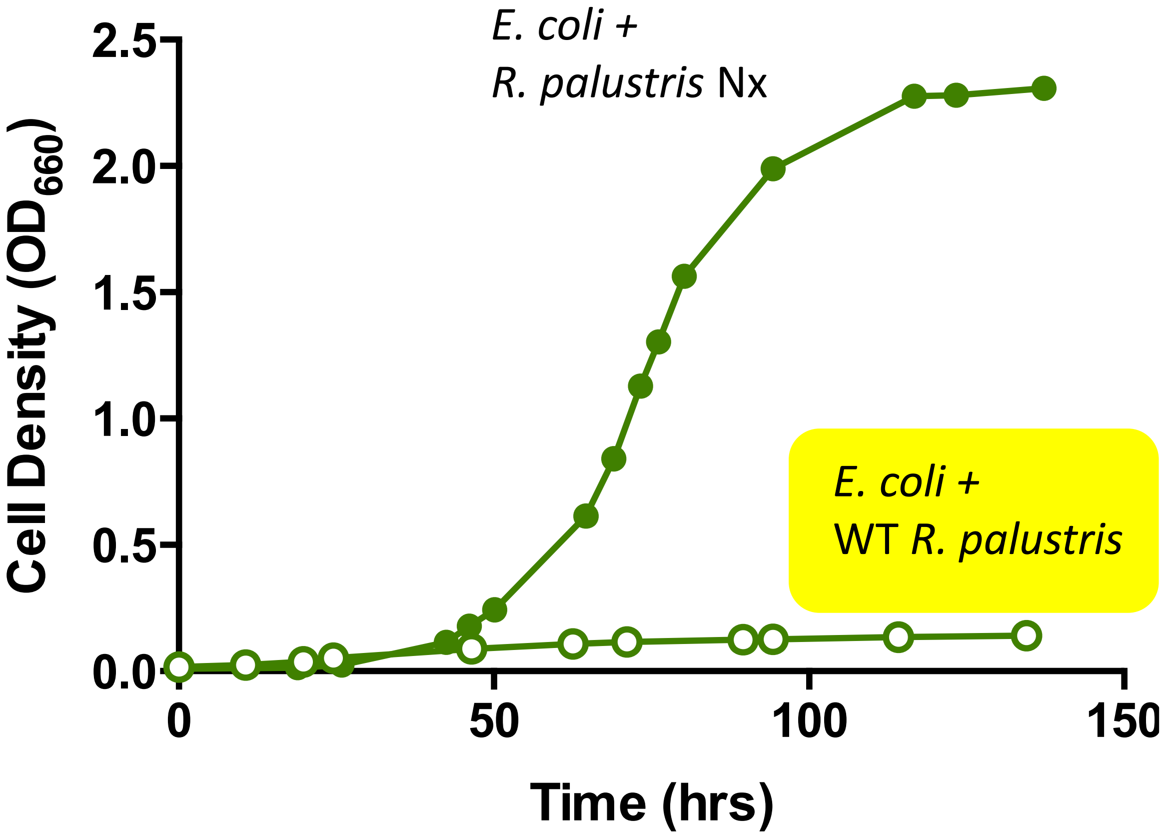
Prof. Mike Lynch

A carefully engineered mutualism



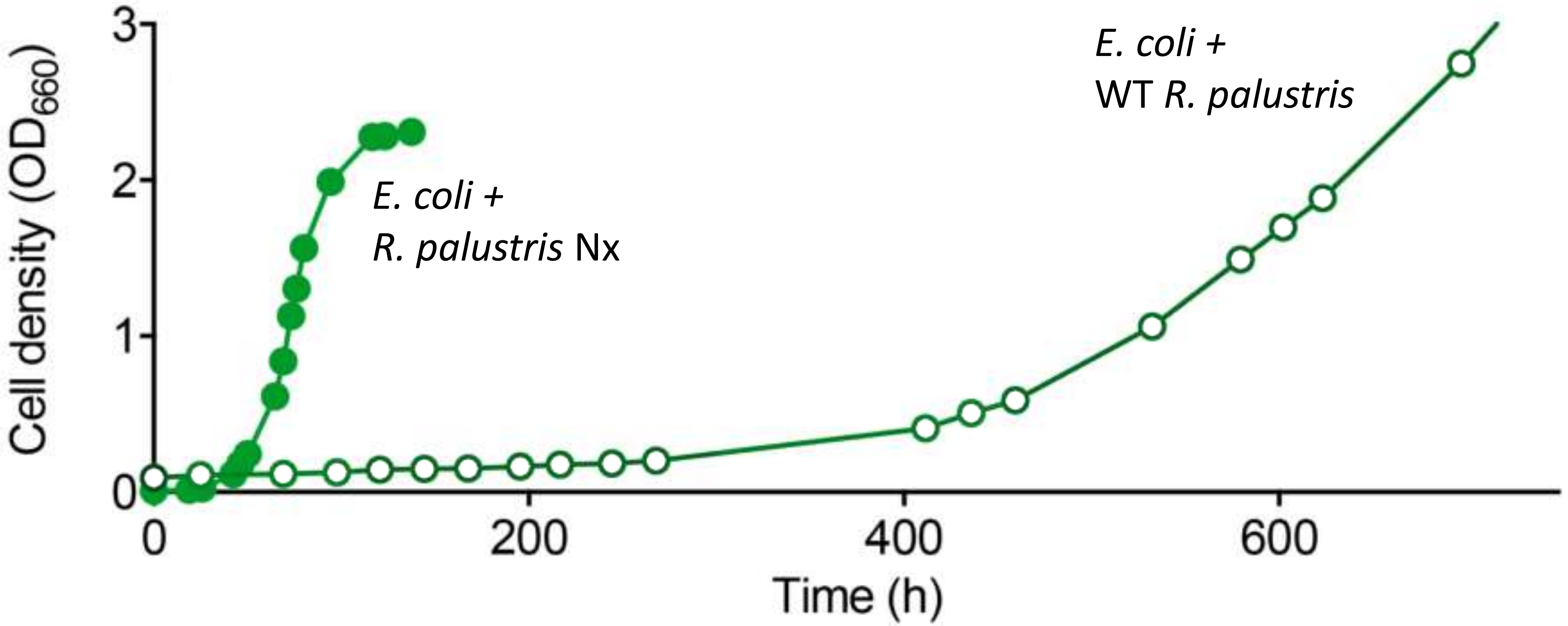
Could such a mutualism arise naturally?

Wildtype *R. palustris* does not support coculture growth in the short term



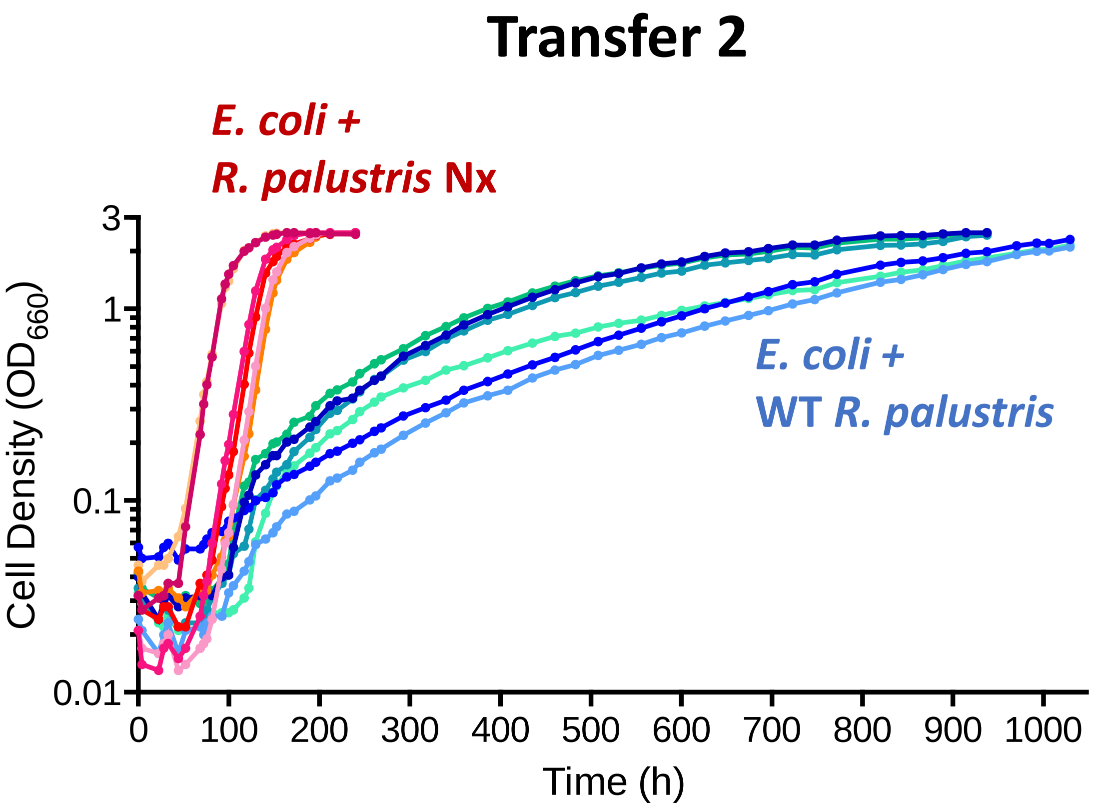
- Cocultures with wildtype *R. palustris* can be used to enrich for spontaneous mutants that support coculture growth

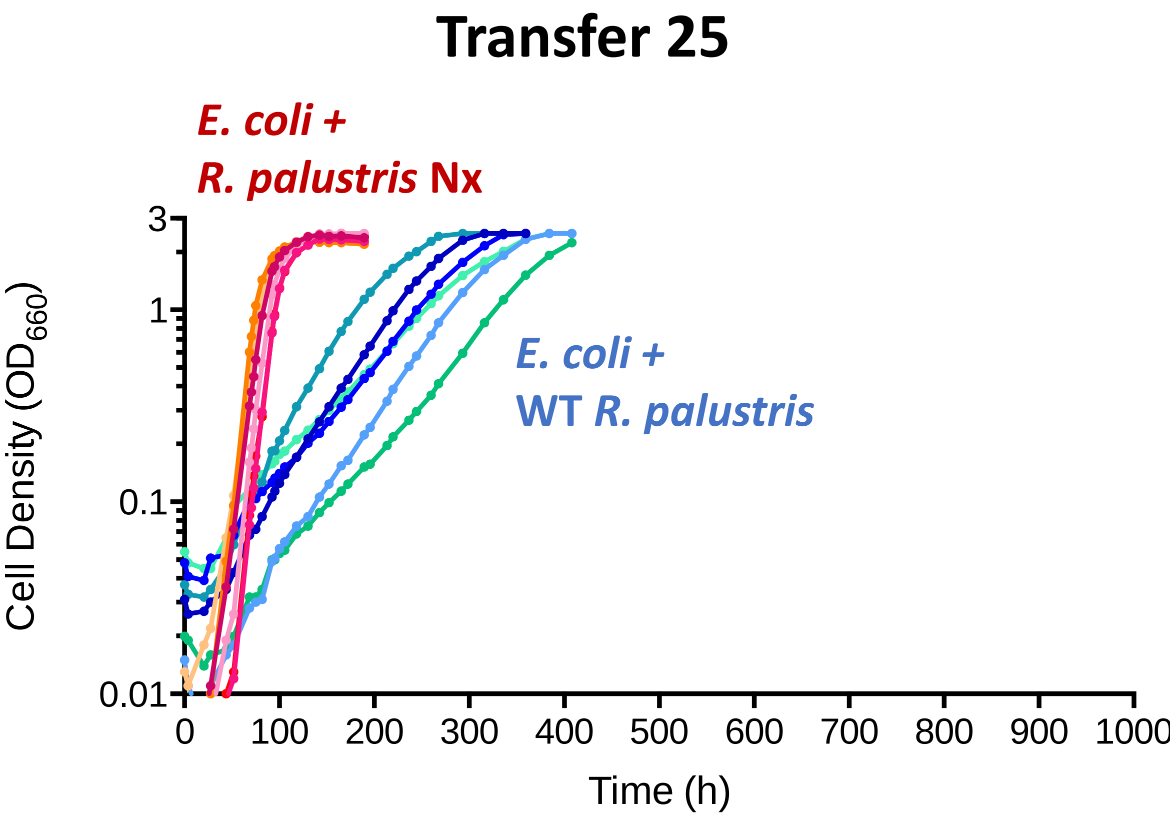
Cocultures with wildtype *R. palustris* eventually grow



- Nascent mutualistic cross-feeding can emerge relatively quickly

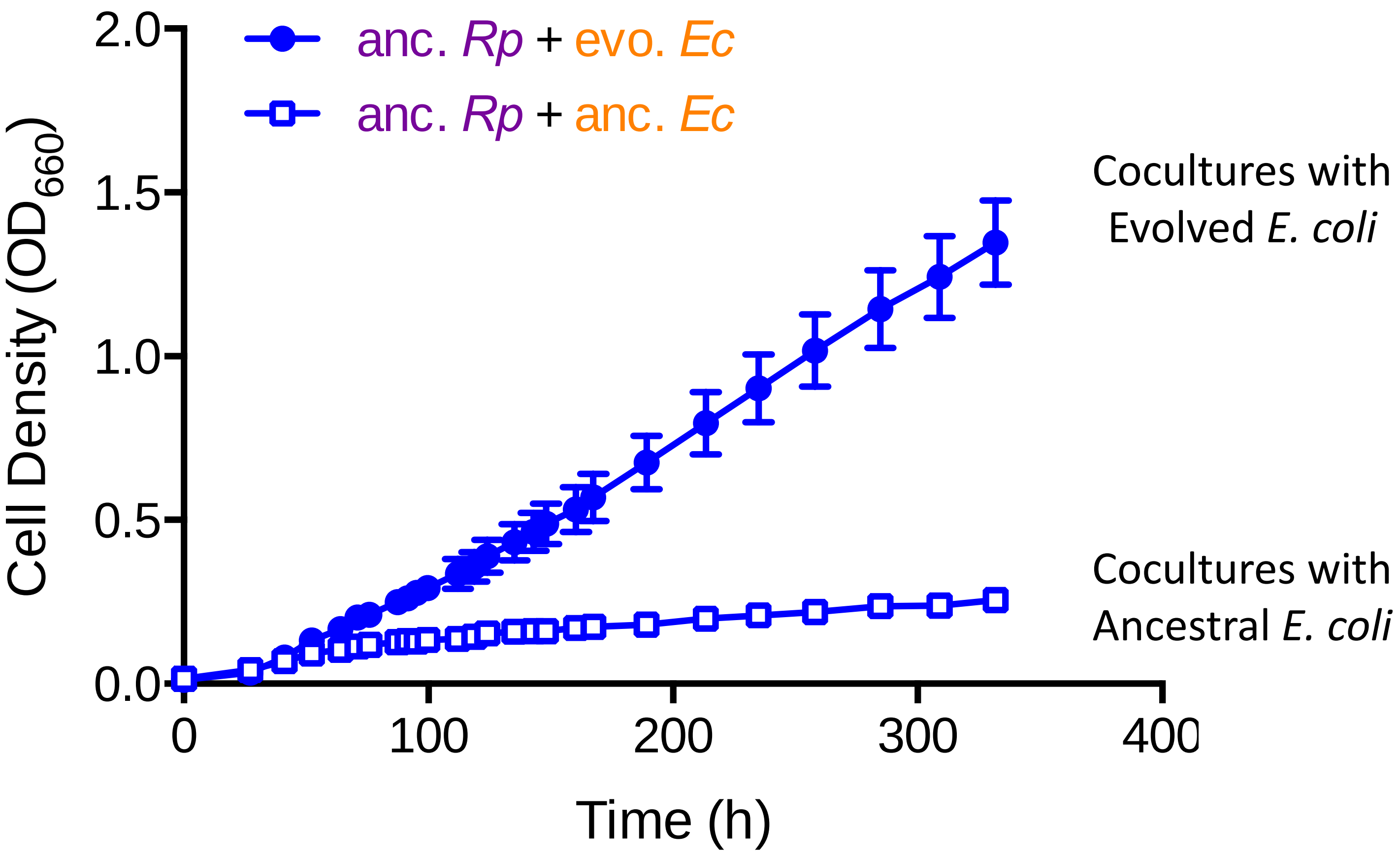
Coculture growth improves through serial transfers





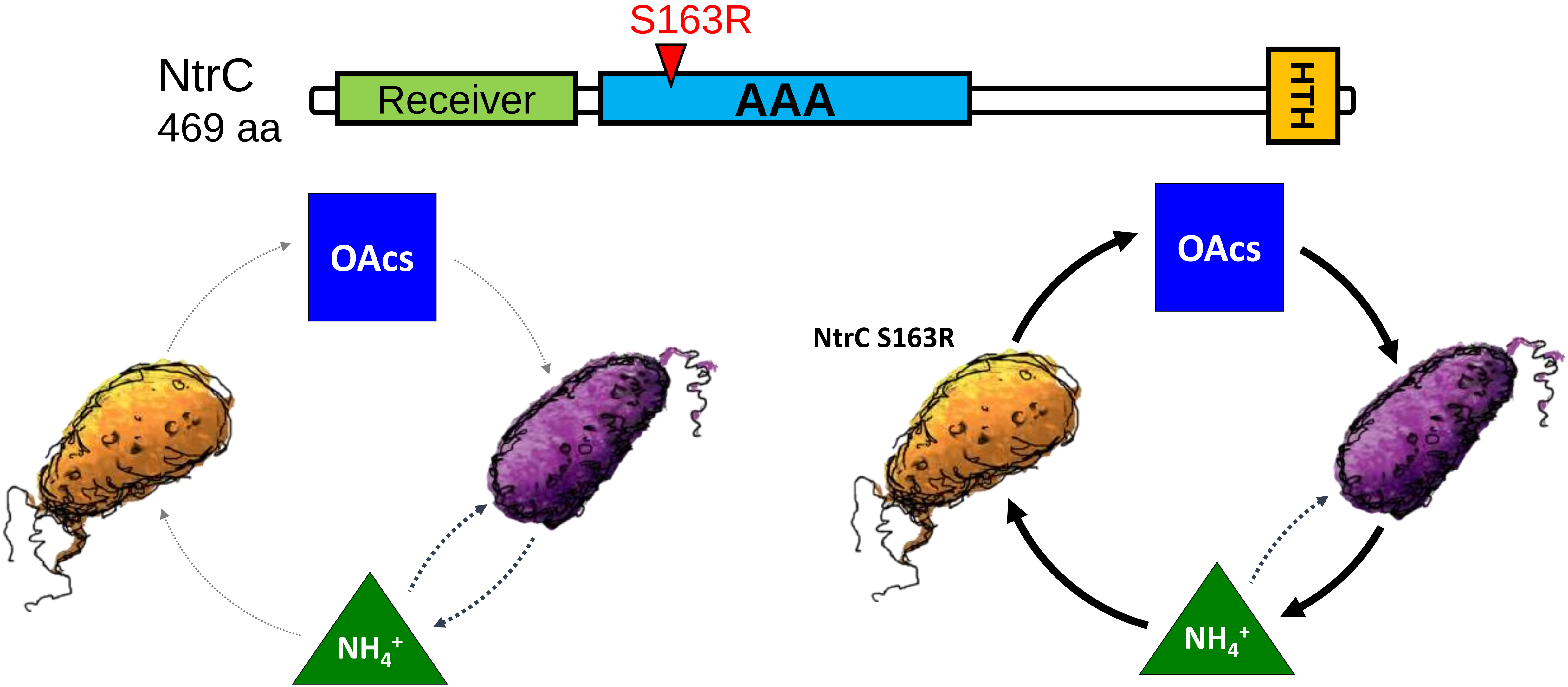
- **What mutations are responsible for establishing nascent cross-feeding?**

Evolved *E. coli* alone is sufficient to support cross-feeding!



- Genetically-enhanced *R. palustris* ammonium excretion is not required

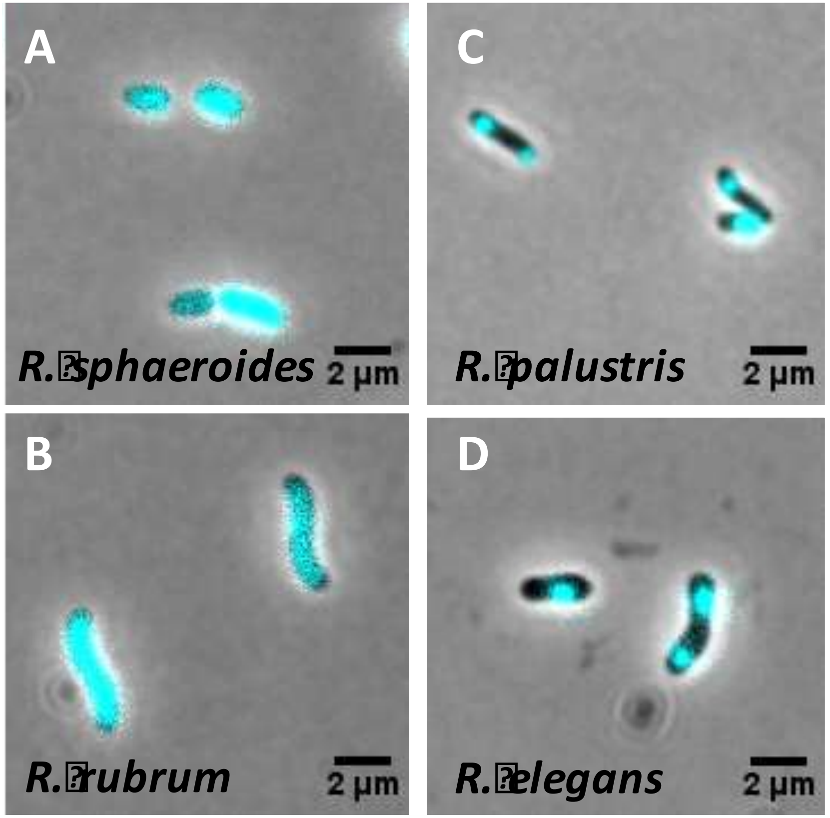
Evolved *E. coli* lines have a common mutation in NtrC



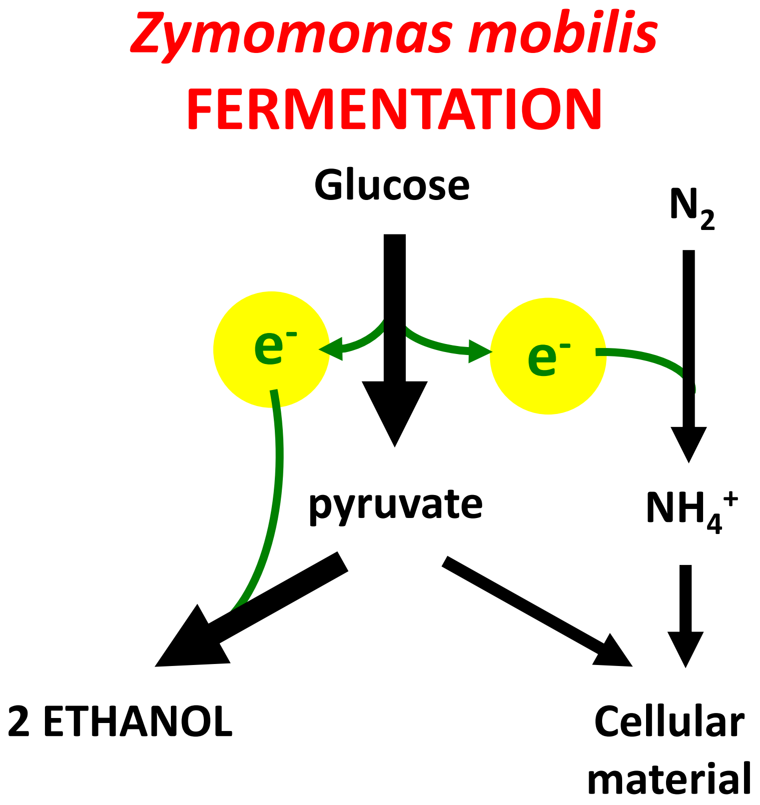
Unanticipated projects stemming from the coculture system

1. Polar localization of *R. palustris* photosystems

2. Using N_2 as an inexpensive nitrogen source for ethanol production

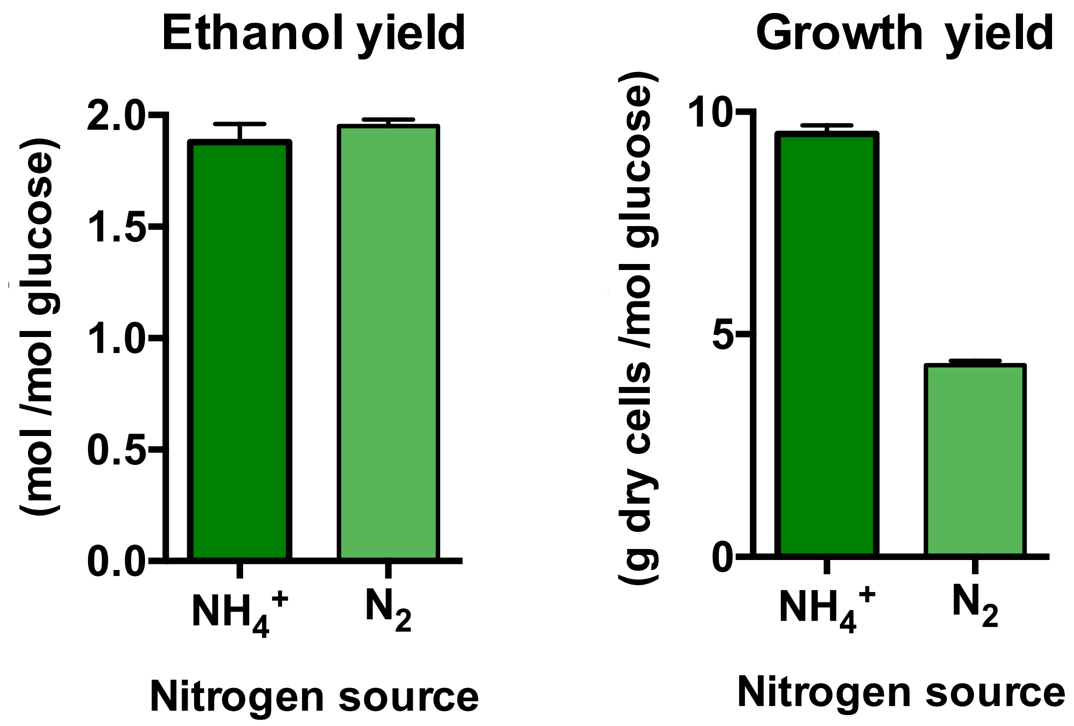


N_2 fixation in ethanol-producing *Z. mobilis*



- **How does *Z. mobilis* partition electrons between ethanol production and N_2 fixation/growth?**

N_2 as a fertilizer for ethanol production



- **Using N_2-fixing *Z. mobilis* could save an ethanol facility >$1 million per year**

*Kremer, *LaSarre, Posto, and McKinlay. 2015. PNAS *equal contribution

Also supported by an Oak Ridge Associated Universities Ralph E. Powe Junior Faculty Enhancement Award

Impact of Early Career award on my research program

Grants

- JGI Community Science Program (PI)
  - Sequencing of naturally-evolved cross-feeding relationships
- DoD Multidisciplinary University Research Initiative (co-PI)
  - Evolution of cooperative microbial communities
- Defense University Research Instrumentation Program (PI)
  - High-throughput capabilities in anaerobic microbiology
- NSF CAREER (PI)
  - Impact of bacterial motility and adhesion on cross-feeding interactions

Impact of Early Career award on my research program

People

- Dr. Breah LaSarre
  - Joined lab as a postdoc in 2013
  - Applied to lab after reading DOE award press release
  - NIH NRSA fellowship from 2014-2017
- Dr. Alexandra ‘Ali’ McCully
  - Joined lab as a graduate student in 2014
  - Graduated in 2018 with numerous awards
  - Now a postdoc with Prof. Alfred Spormann at Stanford
  - Recently received a Simon’s Foundation Postdoctoral Fellowship
- Ryan Fritts

The McKinlay Lab

Coculture collaborators:

Lennon Lab – Jay Lennon
Lynch Lab – Mike Lynch, Megan Behringer
Drummond Lab – Allan Drummond, Evgeny Pilipenko

Coculture funding:

US Department of Energy
Army Research Office
National Science Foundation
Joint Genome Institute
IU College of Arts and Sciences

@mckinlab
@JakeMcKinlay

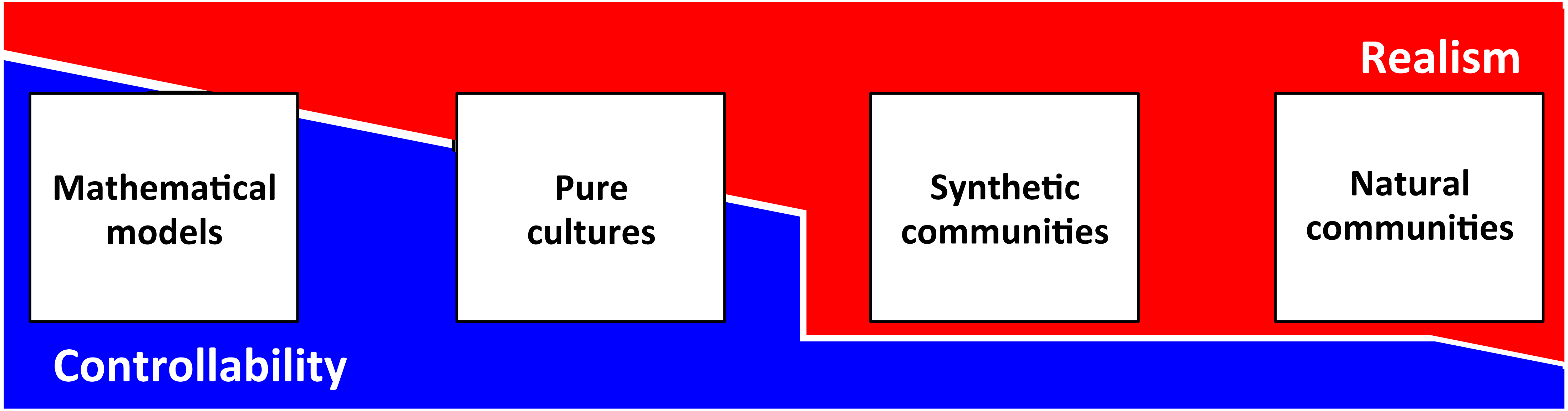
Impact of Early Career award on my research program

Publications and Patents

1. **McKinlay**, Oda, Rühl, Posto, Sauer, Harwood. 2014. Non-growing *Rhodopseudomonas palustris* increases the hydrogen gas yield from acetate by shifting from the glyoxylate shunt to the tricarboxylic acid cycle. J Biol Chem. 289: 1960-1970.
2. Gordon and **McKinlay**. 2014. Calvin cycle mutants of photoheterotrophic purple non-sulfur bacteria fail to grow due to an electron imbalance rather than toxic metabolite accumulation. J Bacteriol. 196: 1231-1237.
3. Kremer, LaSarre, Posto, **McKinlay**. 2015. N_2 gas is an effective fertilizer for bioethanol production by *Z. mobilis*. PNAS. 112: 2222-2226.
4. McCully and **McKinlay**. 2016. Disrupting Calvin cycle phosphoribulokinase activity results in proportional increases to both H_2 yield and specific H_2 production rate. Int J H2 Energy. 41: 4143-4149.
5. LaSarre, McCully, Lennon, **McKinlay**. 2017. Microbial mutualism dynamics governed by dose-dependent toxicity of cross-fed nutrients. ISME J. 11:337–348.
6. Fritts, LaSarre, Stoner, Posto, **McKinlay**. 2017. A *Rhizobiales*-specific unipolar polysaccharide adhesin contributes to *Rhodopseudomonas palustris* biofilm formation across diverse photoheterotrophic conditions. Appl Environ Microbiol. 83: doi:10.1128/AEM.03035-16
7. McCully, LaSarre, **McKinlay**. 2017. Growth-independent cross-feeding modifies boundaries for coexistence in a bacterial mutualism. Environ Microbiol. 19: 3538-3550.
8. McCully, LaSarre **McKinlay**. 2017. Recipient-biased competition for an intracellulary generated cross-fed resources is required for coexistence in a bacterial mutualism. mBio. 8: e01620-17
9. McCully, Behringer, Gliessman, Pilipenko, Mazny, Lynch, Drummond, **McKinlay**. 2018. A nitrogen starvation response is important for *E. coli* to coexist in a mutualistic cross-feeding relationship with *Rhodopseudomonas palustris*. Appl Environ Microbiol. 84:e00404-18

2015. McKinlay, JB, TA Kremer, B LaSarre, AL Posto. Culture conditions that allow *Zymomonas mobilis* to assimilate N_2 gas as a nitrogen source during bio-ethanol production. (submitted). https://patents.google.com/patent/WO2016109286A1/en

Synthetic microbial communities are useful experimental systems…



… provided that a reasonable level of control can be achieved

Adapted from Momeni et al. 2011. Cell Mol Life Sci