

# Gut microbial ecosystems: from bench to bedside, and beyond

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Researcher Live Event

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#GutMicrobiomeLIVE

# Disclosure

- I am co-founder and CSO of NuBiyota LLC, a company that aims to create 'microbial ecosystem therapeutics' to treat a range of indications with gut microbial imbalance as a root cause.
- I will mention the work of this company (at a high level) in my talk today





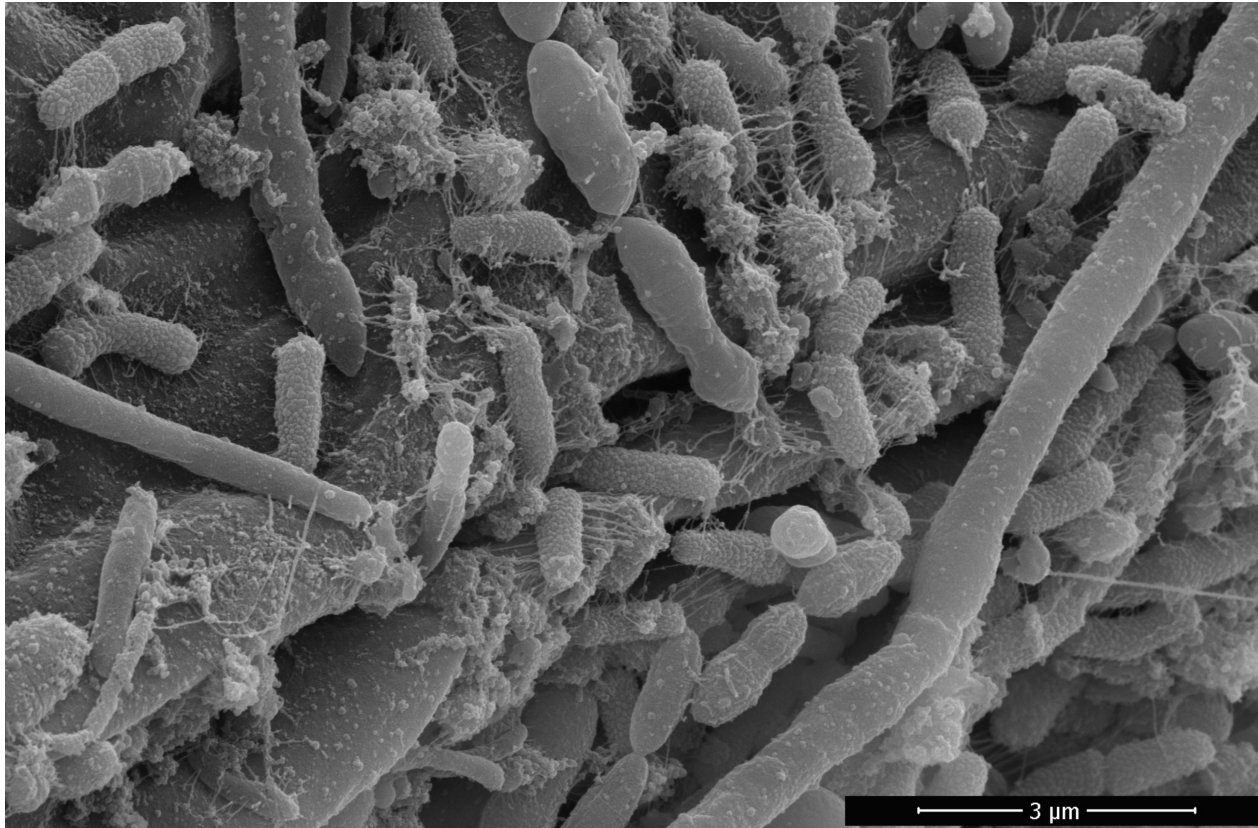
We are not (just) human!

We are complex *super-organisms* of human and microbial cells  
We exist in a delicate host : microbe equilibrium

Most of our microbes reside in our gut

Each gram of feces contains ~  $10^{11}$  bacterial cells  
of ~200 species

# How can we study something as complex as the gut microbiota?



Gut microbes digesting a kernel of corn. SEM credit: Dr. Amber Park, U of G

As a *complex* microbial ecosystem, its function and behaviour is *best studied as a whole*



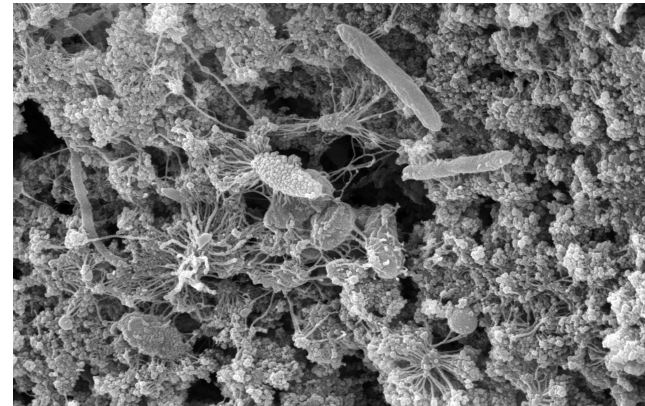
### Microbes in a microbiology lab...

- Almost always exist on their own as part of a pure culture
- Usually have to adapt to survive this way
- Are often grown logarithmically
- Are usually given access to rich nutrient sources



### Microbes in nature...

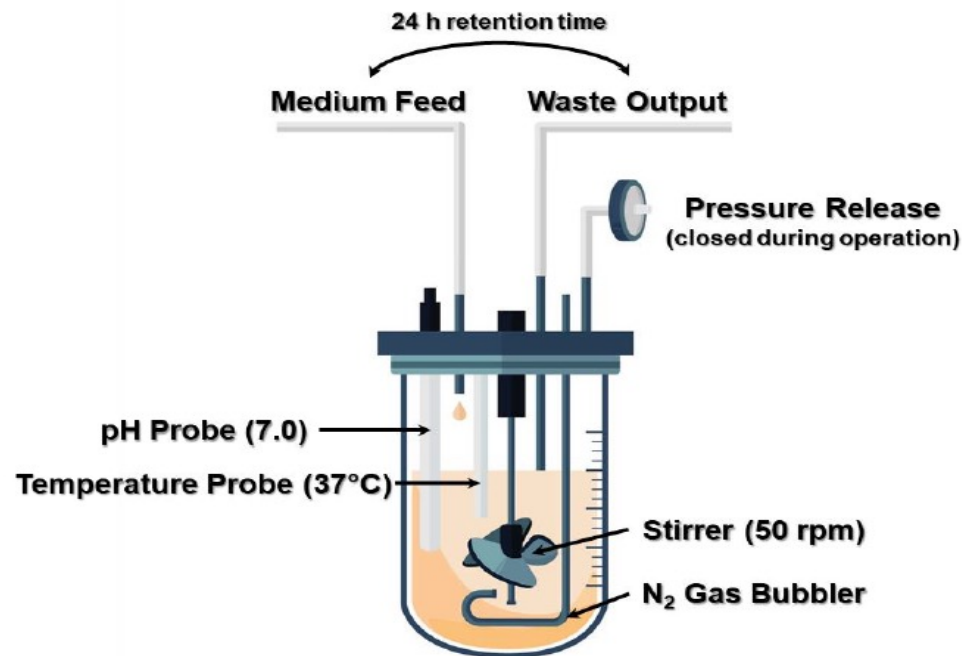
- Almost always exist as part of microbial communities
- Benefit from their microbial friends (& host)
- Rarely grow logarithmically
- Rarely have access to rich nutrient sources



So, why not just emulate nature to culture microbes?



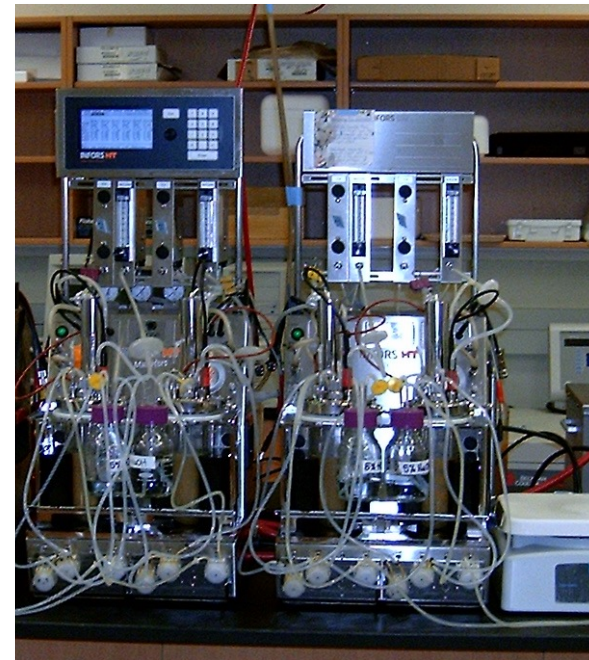
The human colon is a sophisticated bioreactor...



...thus,  
chemostat  
bioreactors can  
be used to  
approximate the  
human colonic  
environment

“But most gut microbial species are unculturable,  
aren’t they?”

- Seeded with fresh feces, this system supports broad ecosystem growth for several weeks
  - Bacteria, fungi, archaea, viruses
- Host-free system
  - But can add host components
- Can test effects of stressors on the ecosystem
  - No one gets hurt!
- Can easily measure metabolic output

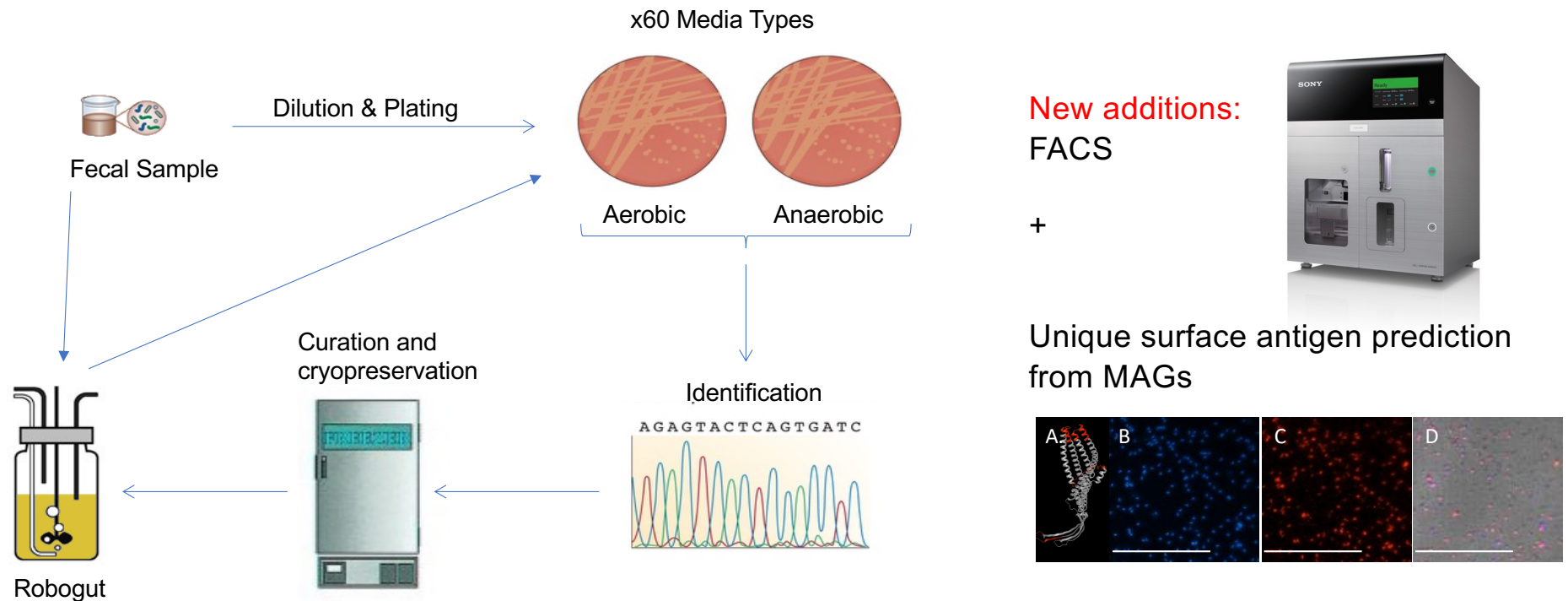


“Roboguts”

J. McDonald et al., 2013, 2015  
Brown and Allen-Vercoe, SURG 2011

# Creating model ecosystems

- It's not always easy to get fresh poop for experiments!
- It can be more reproducible to do experiments with defined ecosystems



Model ecosystems aren't necessarily simple - some have >100 strains



# Working with microbial ecosystems

Some vignettes from my lab



**BENCH:** microbes and ecosystems, and their responses to human milk oligosaccharides



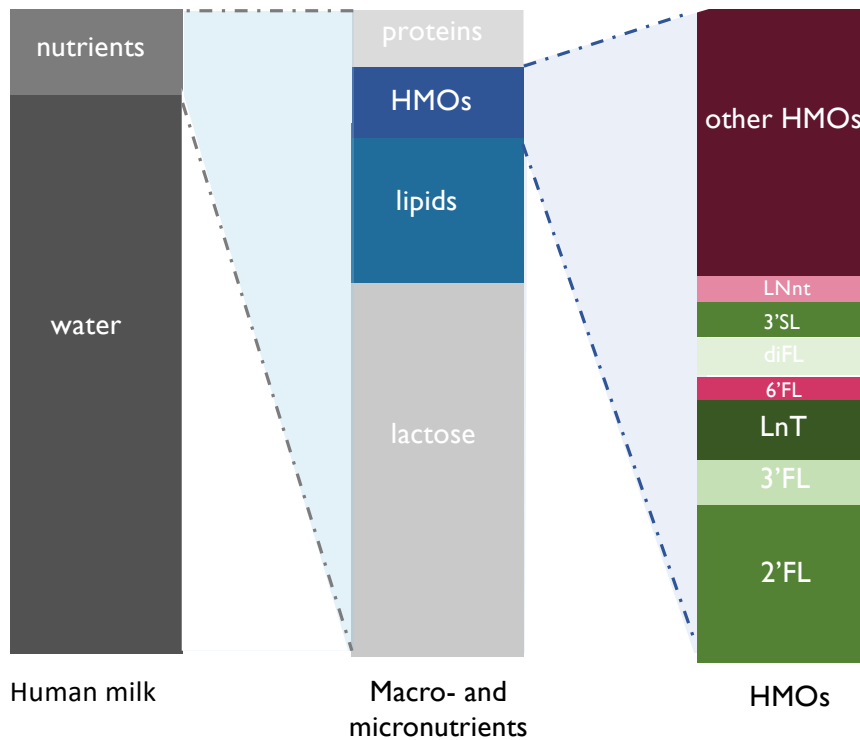
**BEDSIDE:** Moving defined microbial ecosystems to the clinic



**BEYOND:** Hunting for 'missing microbes' in the Amazon Jungle

# How do human milk oligosaccharides modify the microbiome?

## Human milk oligosaccharides (HMOs)



2'-fucosyllactose (2'FL)



Dr. Simone Renwick

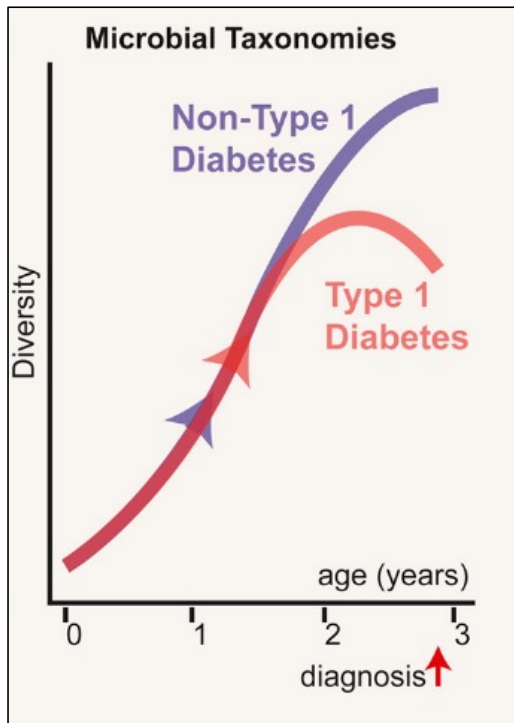


Bode 2015. Early Human Dev.  
Berger et al. 2020 mBio.  
Goehring et al. 2016 J. Nutr.

# Diabimmune Microbiome project



Dr. Jayne Danska,  
Sick Kids



Kostic et al. 2015

Seroconversion: production of autoantibodies

- 4 controls (NS) and 3 cases (S)
- Age-, sex-, and HLA-haplotype matched

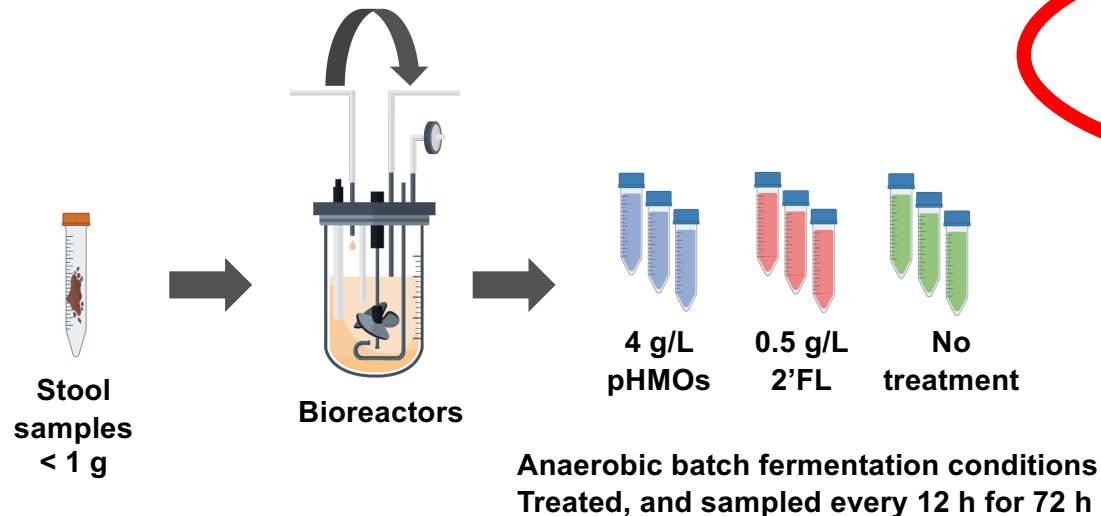
## Donor characteristics

Community ID	Case/Control	Pair	Age at Collection (months)	Sex	Serum Autoantibodies	Duration of Breastfeeding (months)
NS_0	Control	Pilot	18	M	Negative	9
NS_1	Control	1	24	F	Negative	9
S_2	Case	1	24	F	Negative	16
S_3	Case	2	18	M	Negative	7
NS_4	Control	2	24	M	Negative	10
S_5	Case	3	23	M	IAA and ICA	12
NS_6	Control	3	24	M	Negative	2

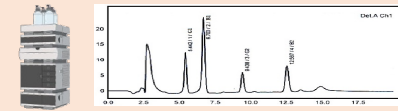
**AIM: Investigate impact of HMOs on T1D-gut derived microbiota**

# How do infant gut microbial ecosystems respond to HMOs?

For 7 infant donors  
(4 controls & 3 cases):



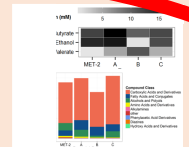
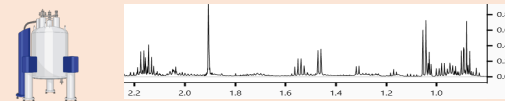
## HMO glycoprofiling (Bode lab, UCSD)



Dr. Lars Bode

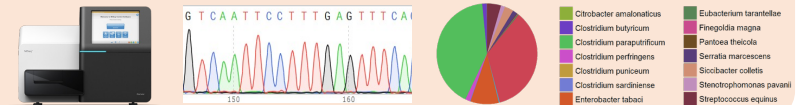
## Study HMO structure preferences

## Metabonomics using 1D <sup>1</sup>H NMR



## Study the metabolites produced by the communities

## Metataxonomics using 16S rRNA sequencing (V4 region)



## Study changes in taxonomic composition

## Metaproteomic analysis of secretome using LC-MS/MS

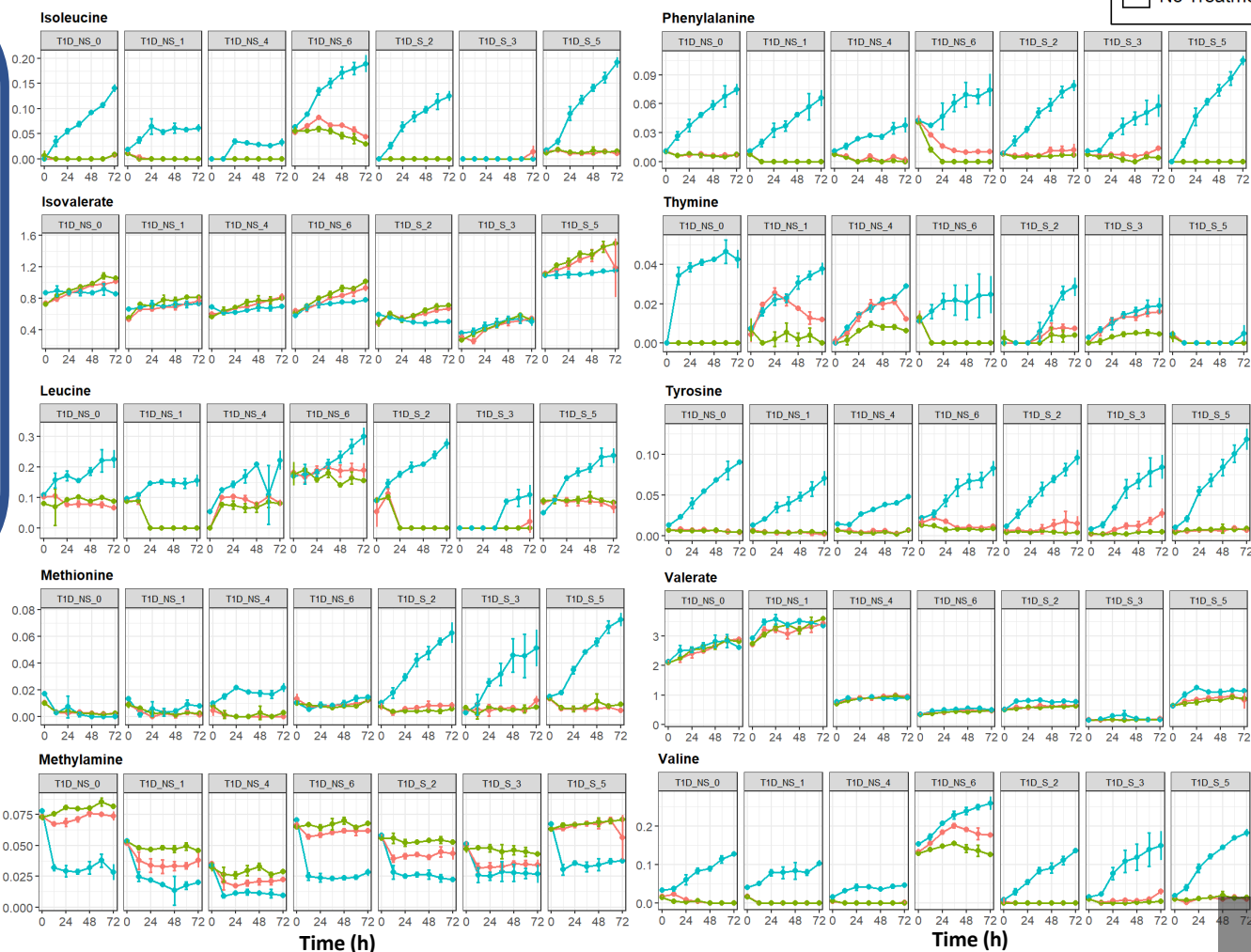
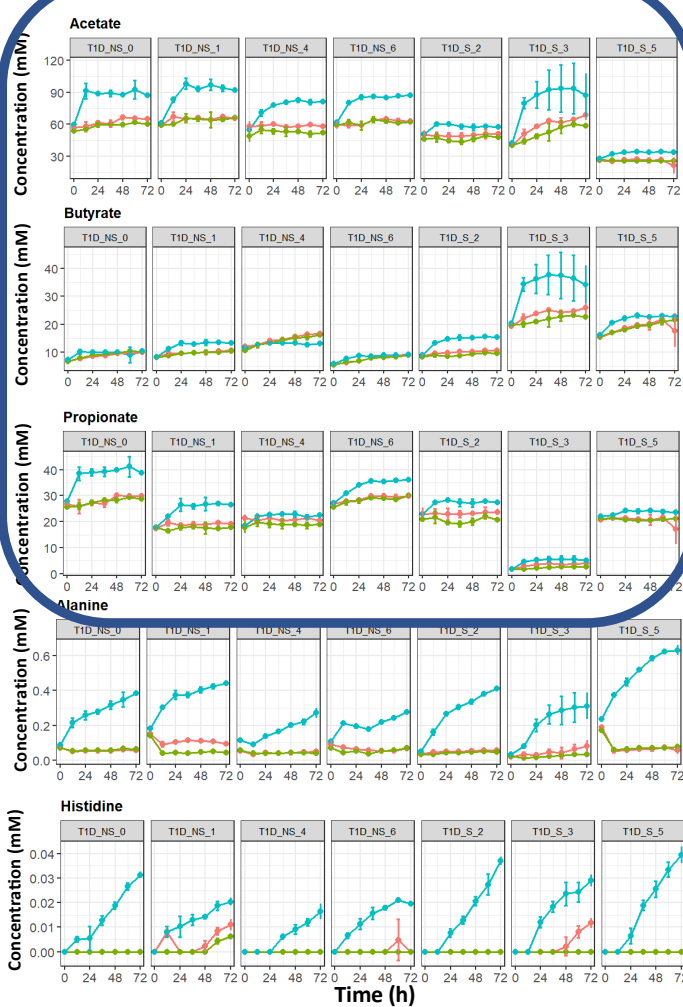
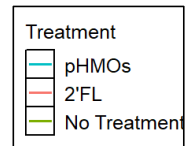


## Study changes in protein expression



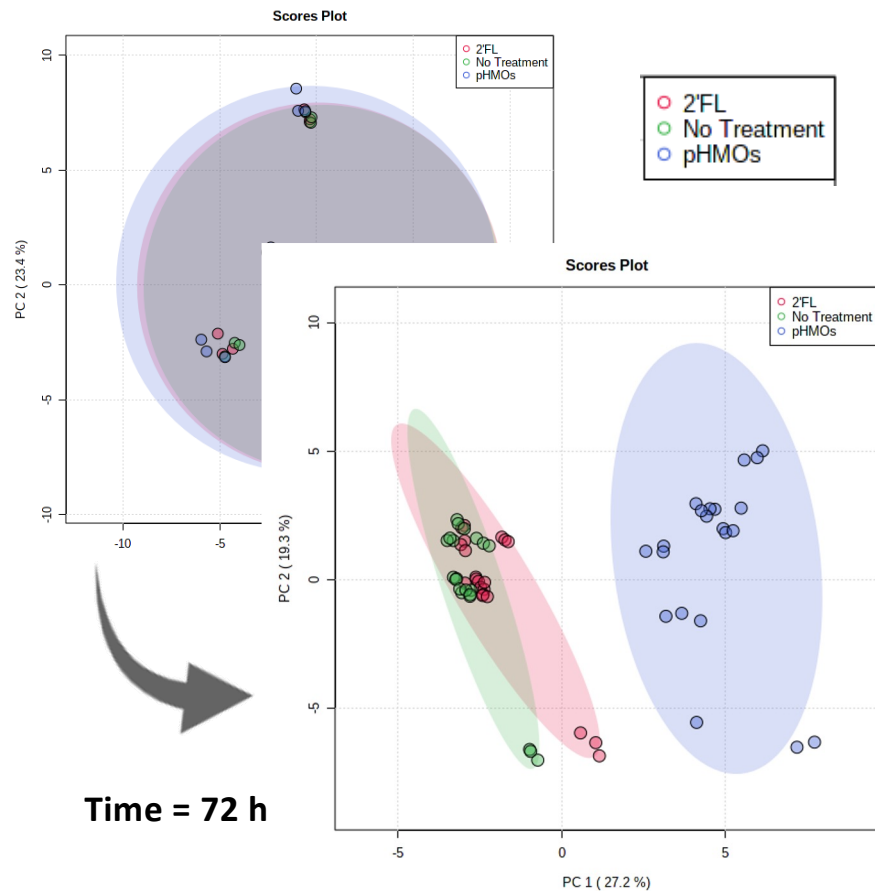
# SCFA

## Metabolic output of 7 different infant fecal ecosystems

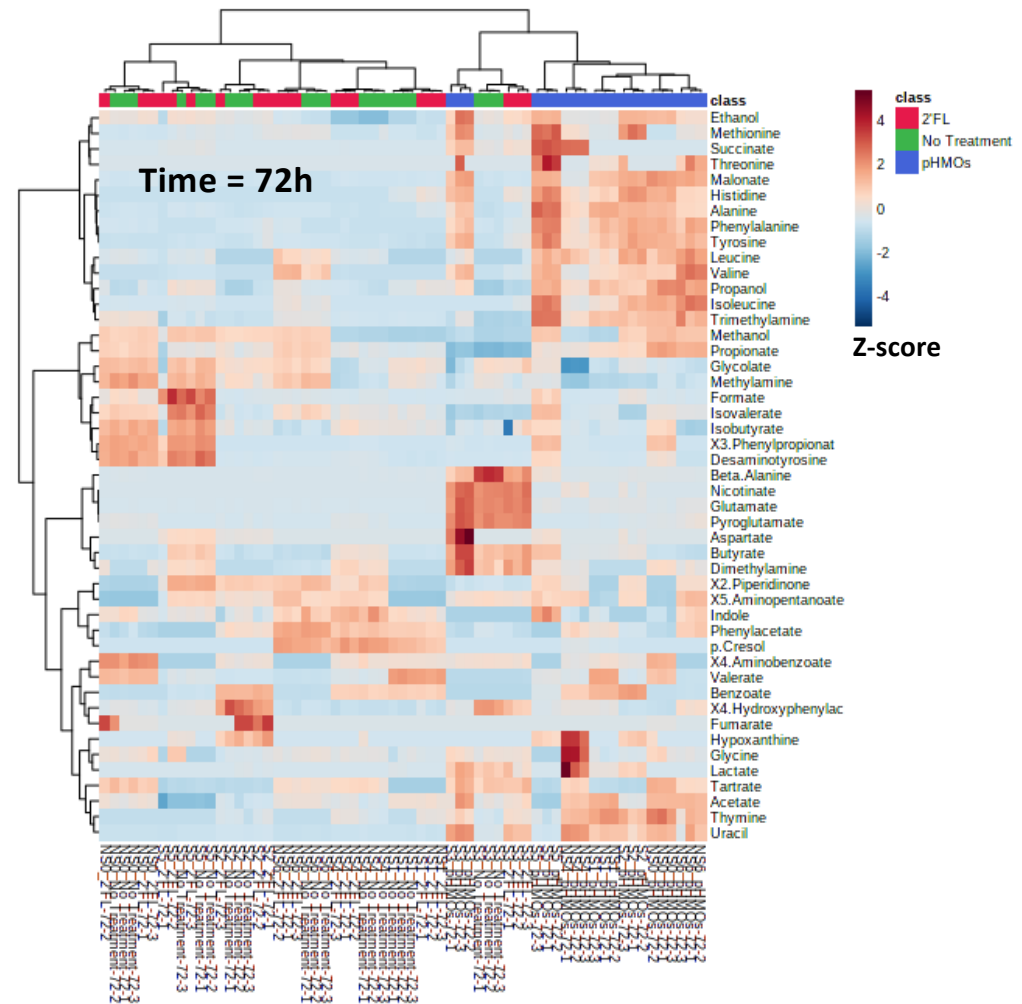


# Treatment with pHMOs results in metabolically distinct patterns

Time = 0 h

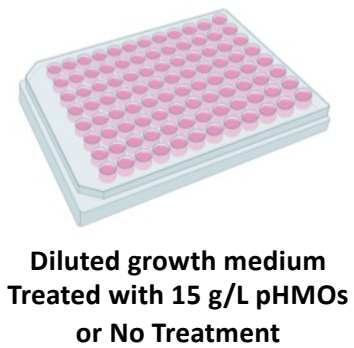


Time = 72 h

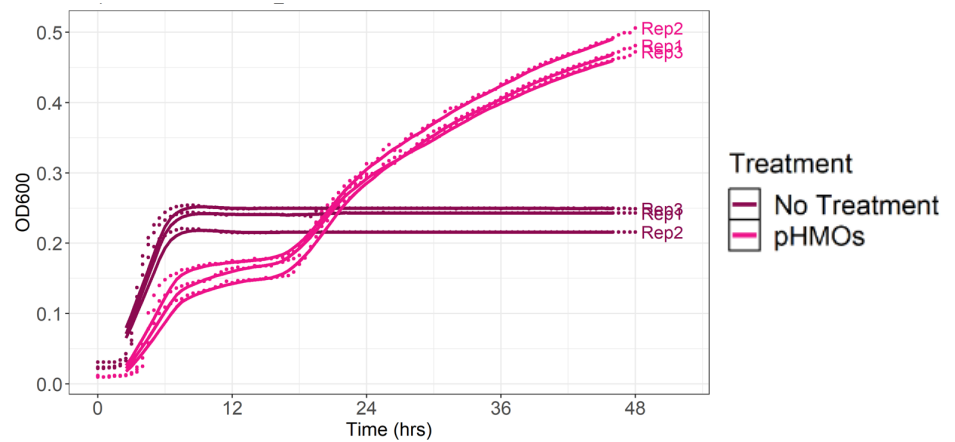


# Effect of pHMOs on *individual* bacterial isolates

330 isolates (spanning 7 donors):



Anaerobic gas  
48 h, 37°C  
OD<sub>600</sub> every 30 min

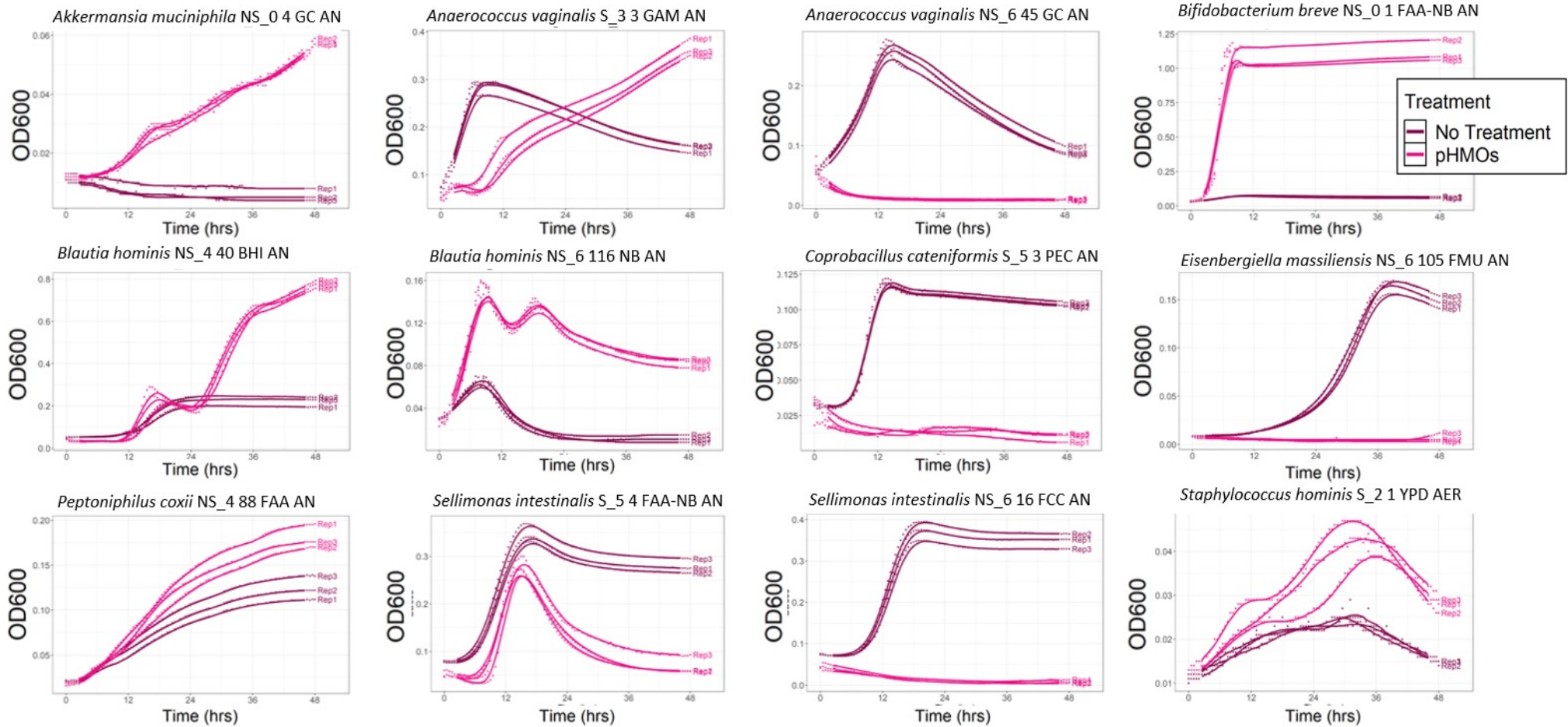


T=0h and T=48h  
Glycoprofiling by:



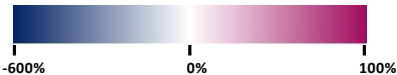
Dr. Lars Bode, UCSD

# A variety of growth curve patterns observed...



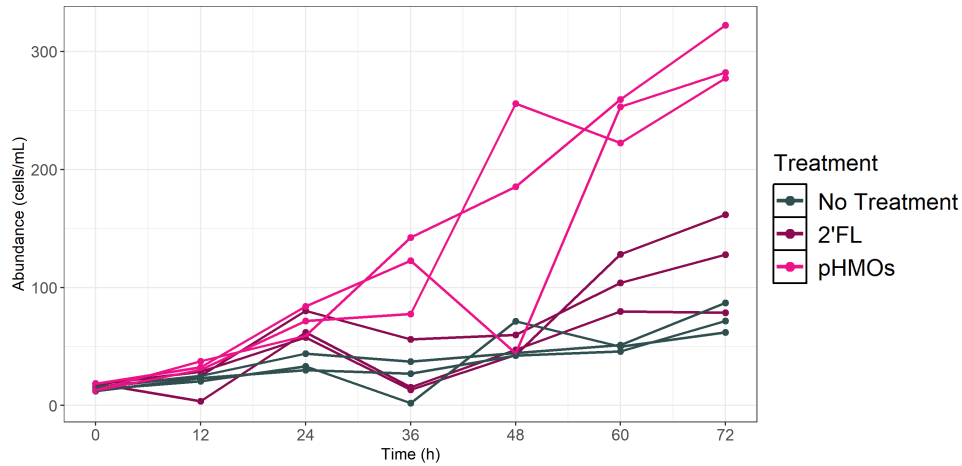


# Glycoprofiling



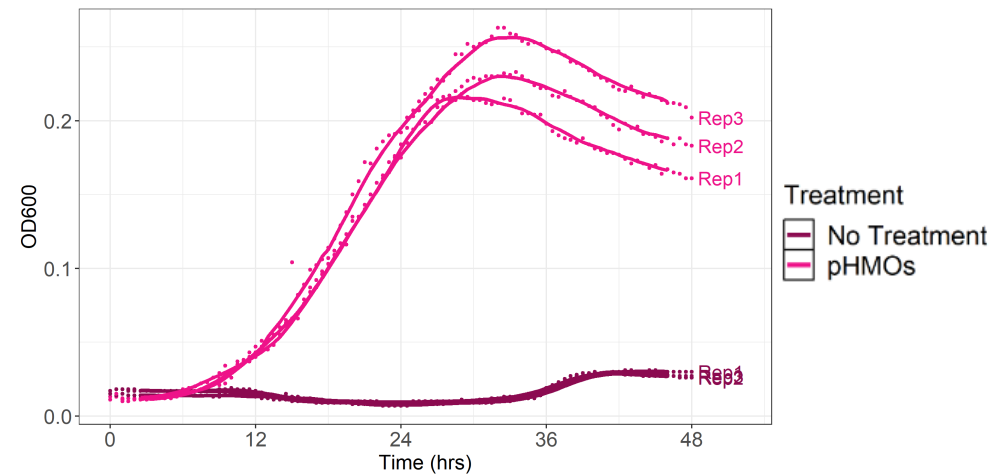
# Strain behaviour in communities vs. monocultures

*Akkermansia muciniphila* S\_3 community



**Microbial Community**  
(HMO community experiments –  
16S rRNA sequencing)

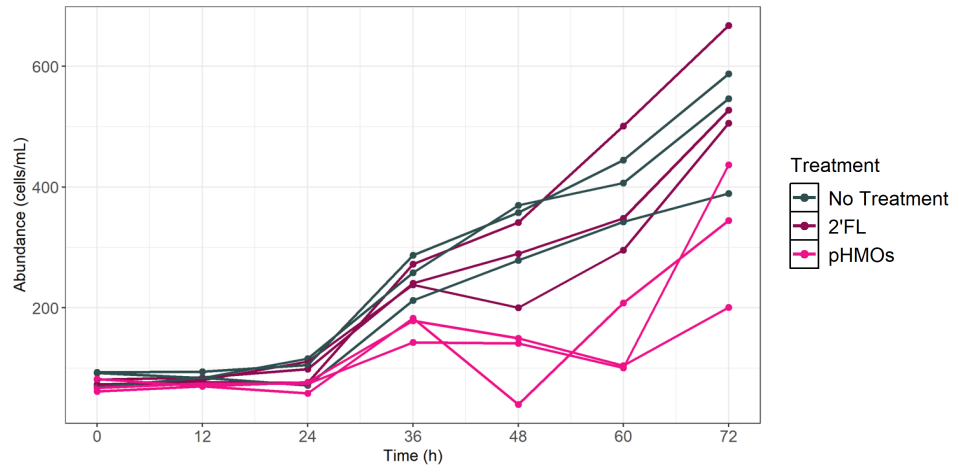
*Akkermansia muciniphila* S\_3 3 FMU AN



**Monoculture**  
(HMO assay growth curve)

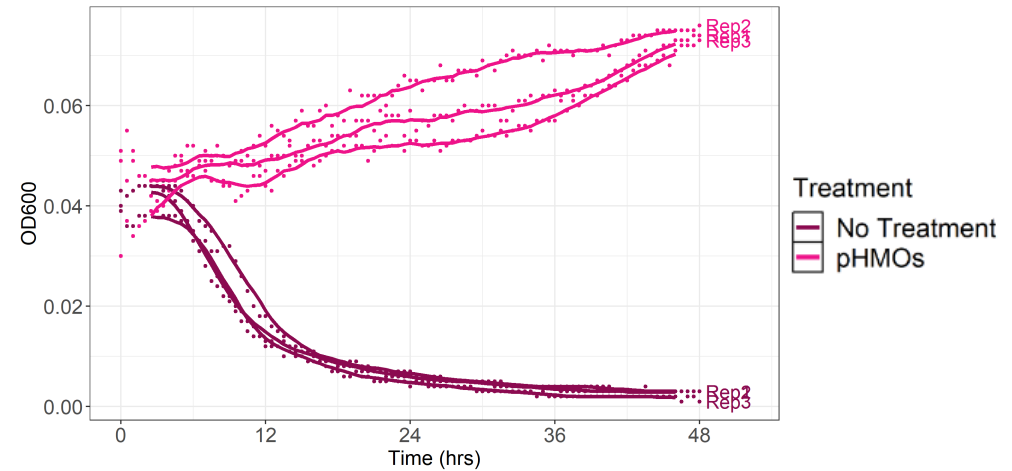
# Strain behaviour in communities vs. monocultures

*Alistipes fingoldii* NS\_4 community



**Microbial Community**  
(HMO community experiments –  
16S rRNA sequencing)

*Alistipes fingoldii* NS\_4 36 FMU AN



**Monoculture**  
(HMO assay growth curve)

## SUMMARY\*



pHMOs significantly alter the metabolic profile of microbial communities

Communities treated with 2'FL did not significantly differ from control

A wider variety of bacterial strains are able to degrade HMOs than was previously known

Some strains demonstrate differing growth properties when treated with HMOs in monocultures compared to microbial communities

\*Based on preliminary findings



## Bedside: moving defined microbial ecosystems to the clinic

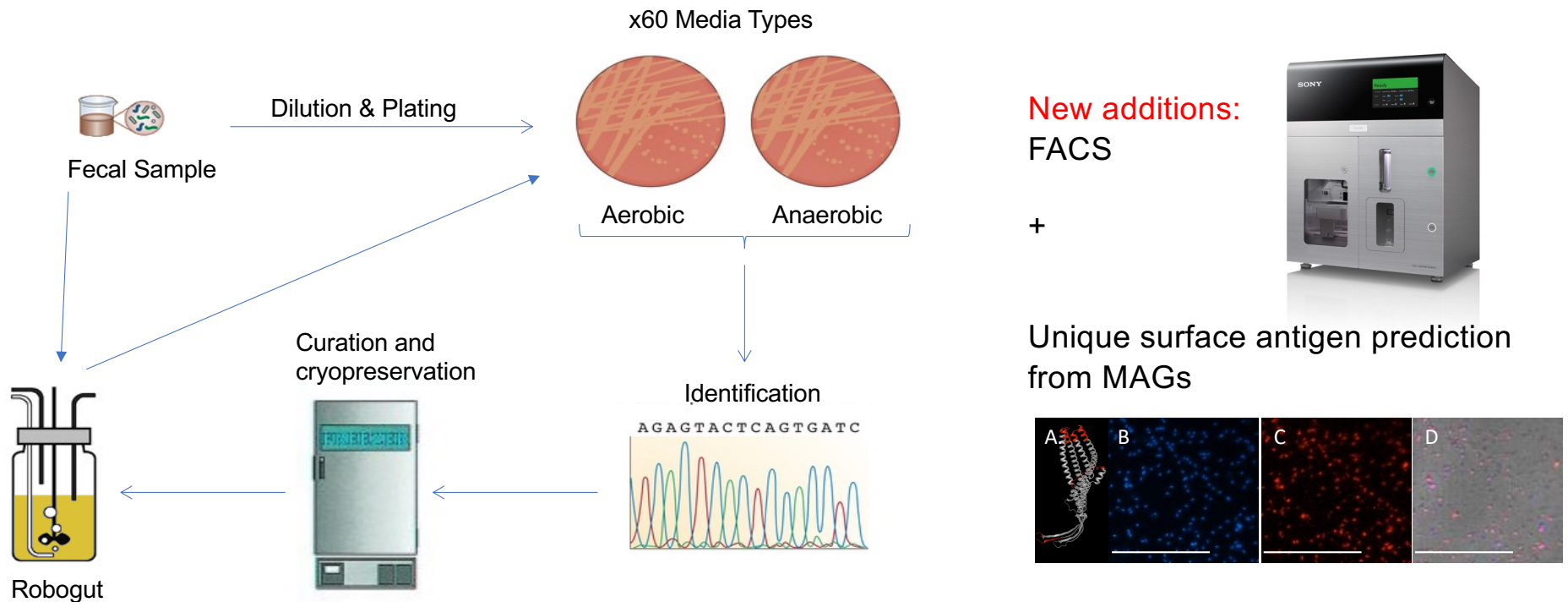


Can we develop 'microbial ecosystem therapeutics' to enhance health?

**NUBIYOTA**  
BETTER ECOSYSTEM. BETTER HEALTH

# Creating model ecosystems

- It's not always easy to get fresh poop for experiments!
- It can be more reproducible to do experiments with defined ecosystems



Some defined ecosystems may be therapeutically useful

# The journey to therapeutically useful ecosystems



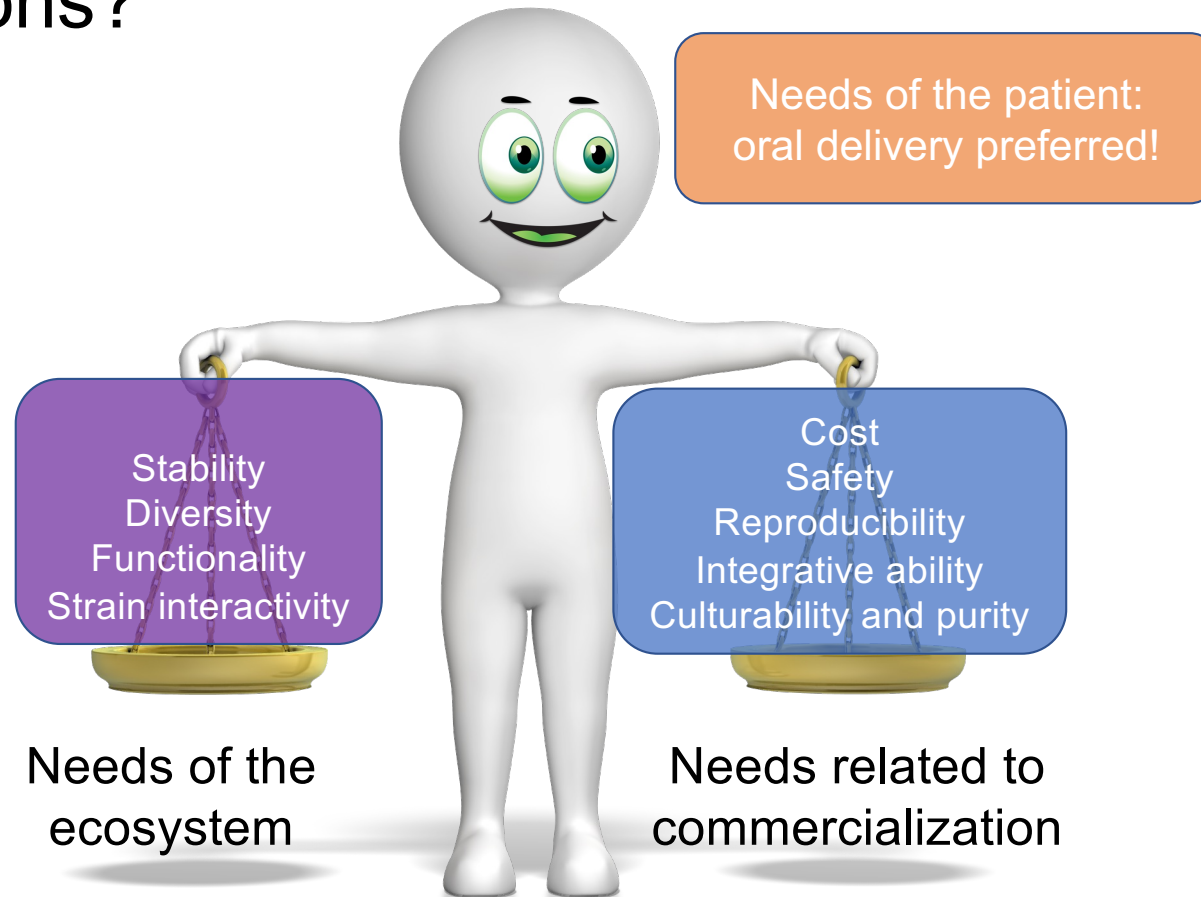
**How can we translate derived defined ecosystems to the clinic?**

Start with the Robogut to build ecosystems and test their dynamics under perturbational stress

**“RePOOPulate”  
prototype 33 strain ecosystem**

*Petrof et al. Microbiome 2013*

# What are the commercial and patient considerations?



Sweet spot: 30-40 species, as long as phylogenetic diversity is upheld

# How do we select which microbial species to include?

Does the selected ecosystem show stability in the Robogut?  
(suggests metabolic diversity)

Is it free from known virulence genes?  
(incl. antibiotic resistance genes of concern)

What does the literature tell us about the gut microbiome for a given disease indication  
(is something missing? Over-abundant?)

How does the predicted function/metabolic network look? How does the actual metabolic output compare?

What can we glean from actual patient data from our trials? (Sorry, can't discuss!)

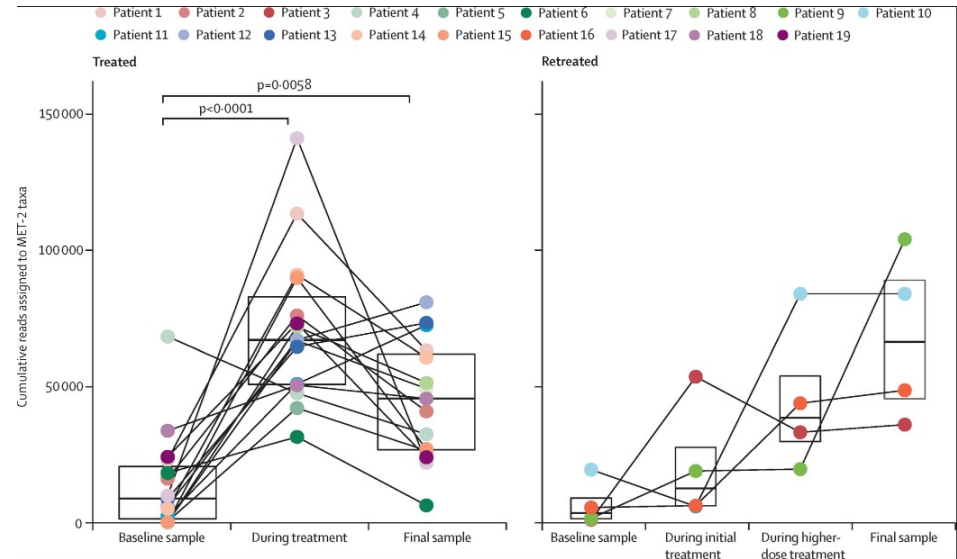
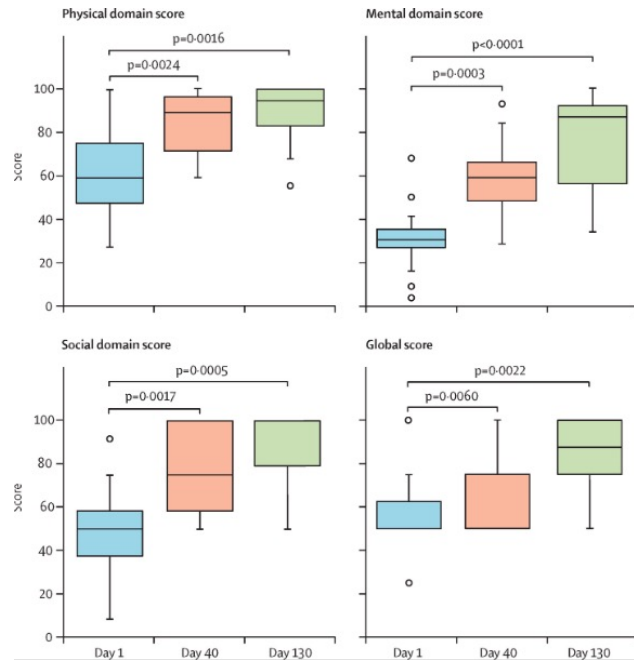




Articles

# The effect of a microbial ecosystem therapeutic (MET-2) on recurrent *Clostridioides difficile* infection: a phase 1, open-label, single-group trial

Dina Kao MD <sup>a, \*</sup>, Karen Wong MD <sup>a</sup>, Rose Franz RN <sup>a</sup>, Kyla Cochrane PhD <sup>c</sup>, Keith Sherriff MSc <sup>c</sup>, Prof Linda Chui PhD <sup>b</sup>, Colin Lloyd BSc <sup>b</sup>, Brandi Roach RN <sup>a</sup>, Anthony D Bai MD <sup>c</sup>, Elaine O Petrof MD <sup>f</sup>, Prof Emma Allen-Vernoe PhD <sup>c, d</sup>




- MET-2 – improvement on RePOOPulate
  - 40 strains, 40 species
- Signatures associated with MET-2 microbes appeared to increase with treatment and persist after treatment
- Patient QoL scores consistently improved
- Data is helping us to define keystone members of MET communities

# NuBiyota's current drug portfolio

Drug formulation	Indication (s)	Trial phase	Clinical Trial Numbers
MET-1	<i>C. difficile</i> infection	1 (pilot)	NCT01372943
MET-2	<i>C. difficile</i> infection; ulcerative colitis; Depression & anxiety	1, 2	NCT04052451 NCT04602715 NCT03832400 NCT02865616
MET-3	Metabolic syndrome, obesity	1, 2 (3 starting in US)	NCT04507971 NCT03660748
MET-4	Checkpoint inhibitor potency booster in cancer chemotherapy	1, 2	NCT03838601 NCT03686202
MET-5	Metabolic syndrome	1	NCT04507971
MET-6	Under development	-	-

## WHERE WE ARE



These are biologic drug products, **not** probiotics  
Much higher barrier to approval

Each individual isolated component is considered a drug product  
So, we do QC on 40 different drugs to make our end-product

Growth rates for unpredictable microbes need to be predicted  
Lots of work on understanding microbial physiology!

Currently trying to implement novel molecular approaches to QC

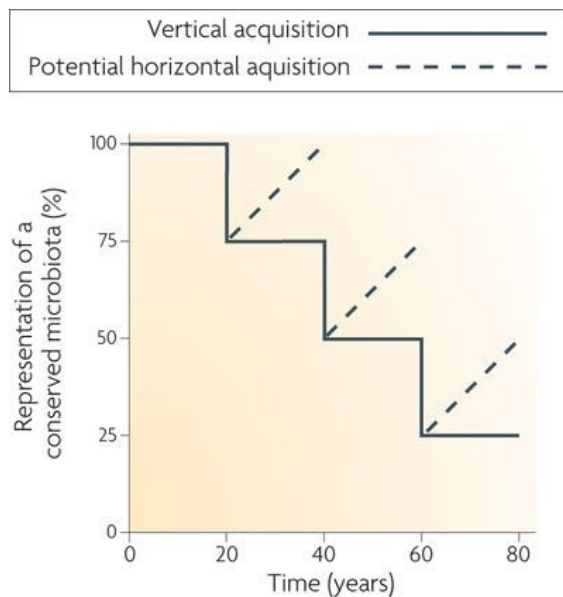
- Difficult, because Health Canada and FDA have set protocols
- Our products do not 'fit the box'
- Lots and LOTS of reporting and evidence presented to review panels!

# Beyond: hunting for missing microbes in the Amazon jungle



# Has 'industrialized' microbiome diversity been eroded?

- Missing microbiota hypothesis
- (Blaser & Falkow, Nature Rev Microbiol 2009)
  - Loss of microbiota generally compounds over generations, and *recent changes in lifestyle* have greatly exacerbated this loss



Nature Reviews | Microbiology





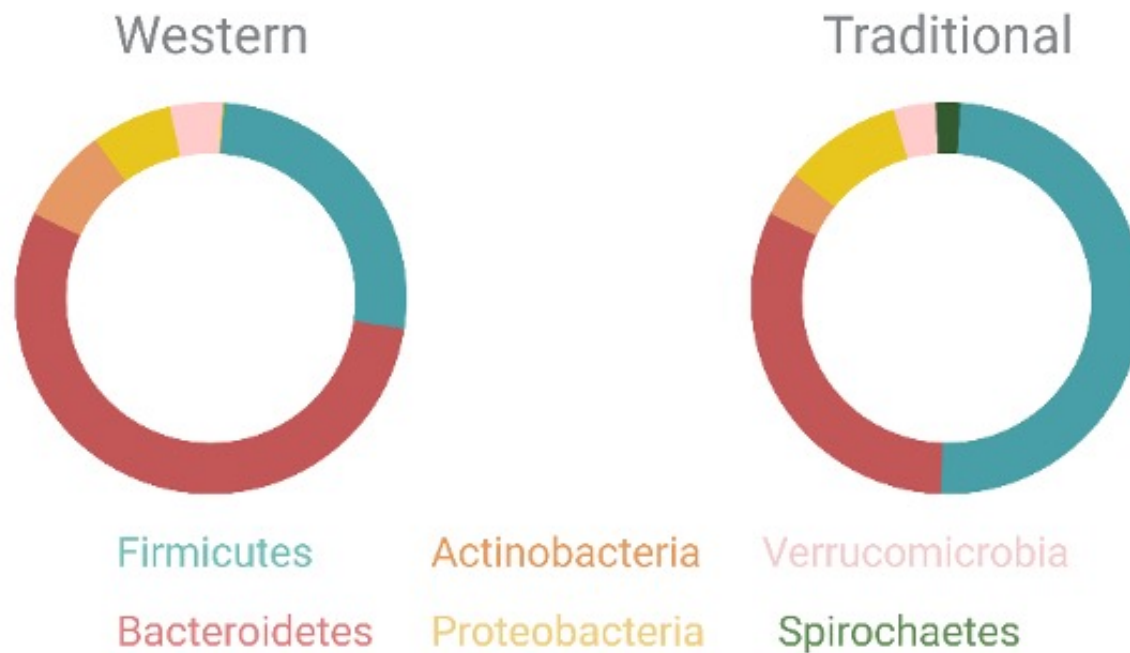
# How do we *know* that industrialized people have low gut microbial diversity?

- We **can't** go back in time to look at microbiomes pre-antibiotics/refined foods
- We **can** look at indigenous peoples who have not had exposure to these things



Their gut microbiomes are much more diverse than ours!

# Typical findings

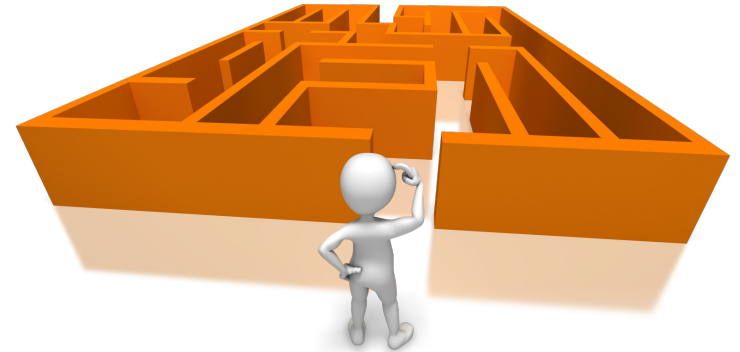


Similar across traditional populations in Africa and S. America

– suggests the Western microbiome overall lost species rather than the traditional microbiome overall gained them

# So, why not just culture microbes from these indigenous peoples?

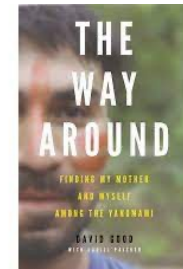
- That way, we can better understand what we are missing
- Unfortunately, that's actually very difficult to do!
  - Remoteness and difficult access
  - Dangerous terrain/endemic disease
  - Culture and language barriers
  - Political turmoil
  - Ethical challenges
  - Preservation of samples
    - Need to keep gut microbial samples cool and free of oxygen



# The Good Project



Photo with permission from David Good



David Good – a biologist with a unique family heritage

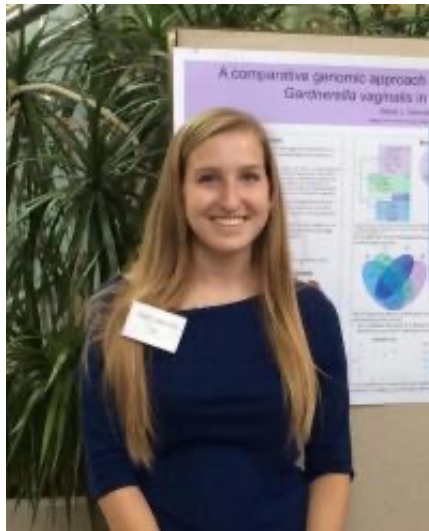
Yanomami – a group of indigenous South Americans, many of whom still live as nomadic hunter-gatherers

The Good Project – non-profit organization founded by David and dedicated to help support the future of the Yanomami people





# Isolations performed using a *lot* of specialist media (and many 'tricks')!



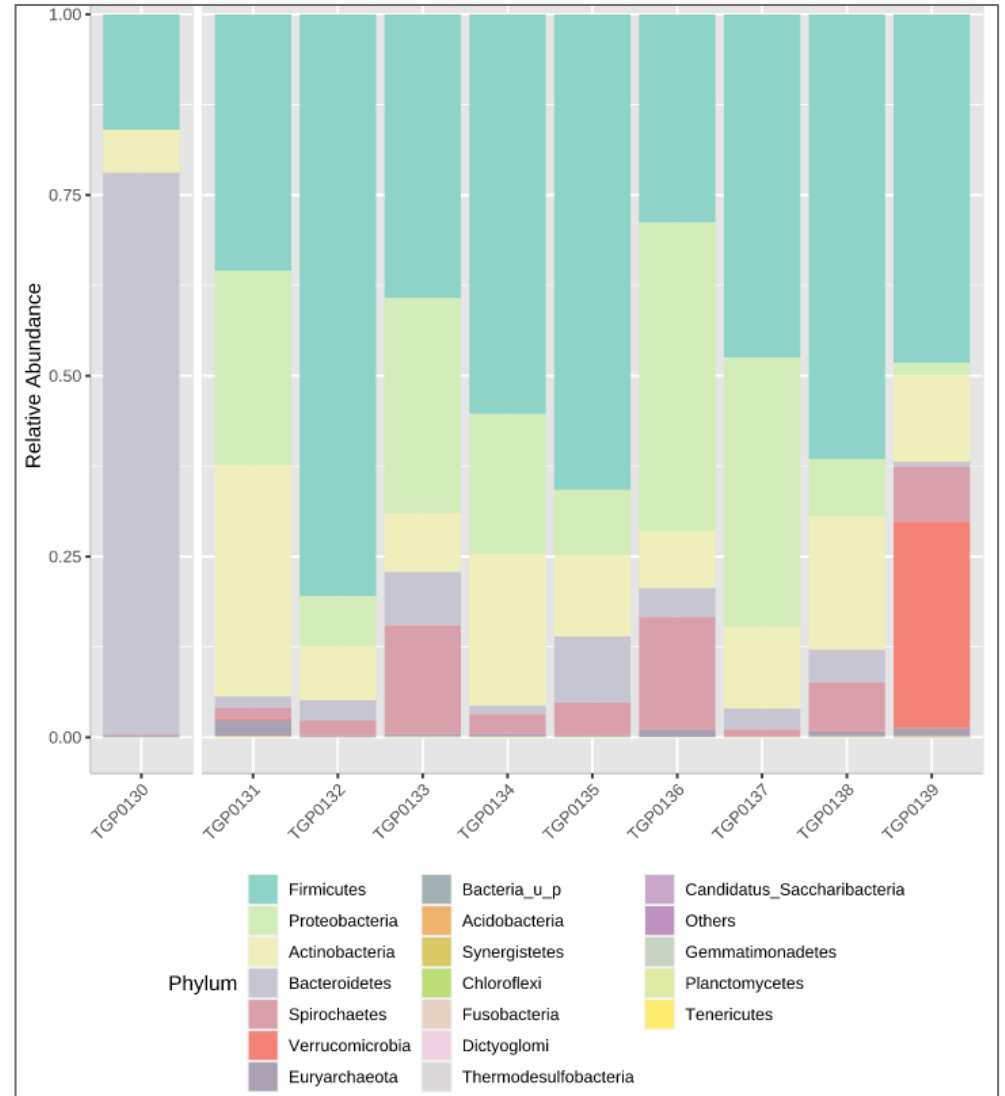
Sarah Vancuren





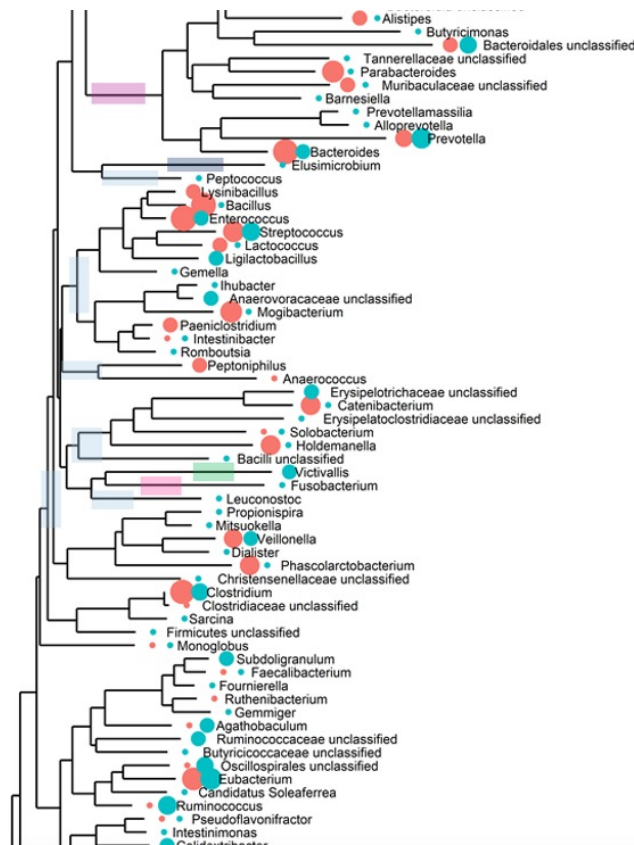
# What *should* we find?

- Shotgun metagenomics
  - (just bacteria shown)
- Western microbiome is different from the Yanomami microbiome
  - The Yanomami gut microbiome is far more diverse, as expected
- DNA profiling gives us a snapshot of species present that we can target
  - But does not distinguish dead microbes from live ones



# So far, what have we been able to grow?

- 5 samples so far: yield of **>1000 unique strains**, **>200 unique species**.



Phyla cultivated:

Firmicutes

Lentisphaera

Proteobacteria

Spirochaetes

Actinobacteria

Bacteroidetes

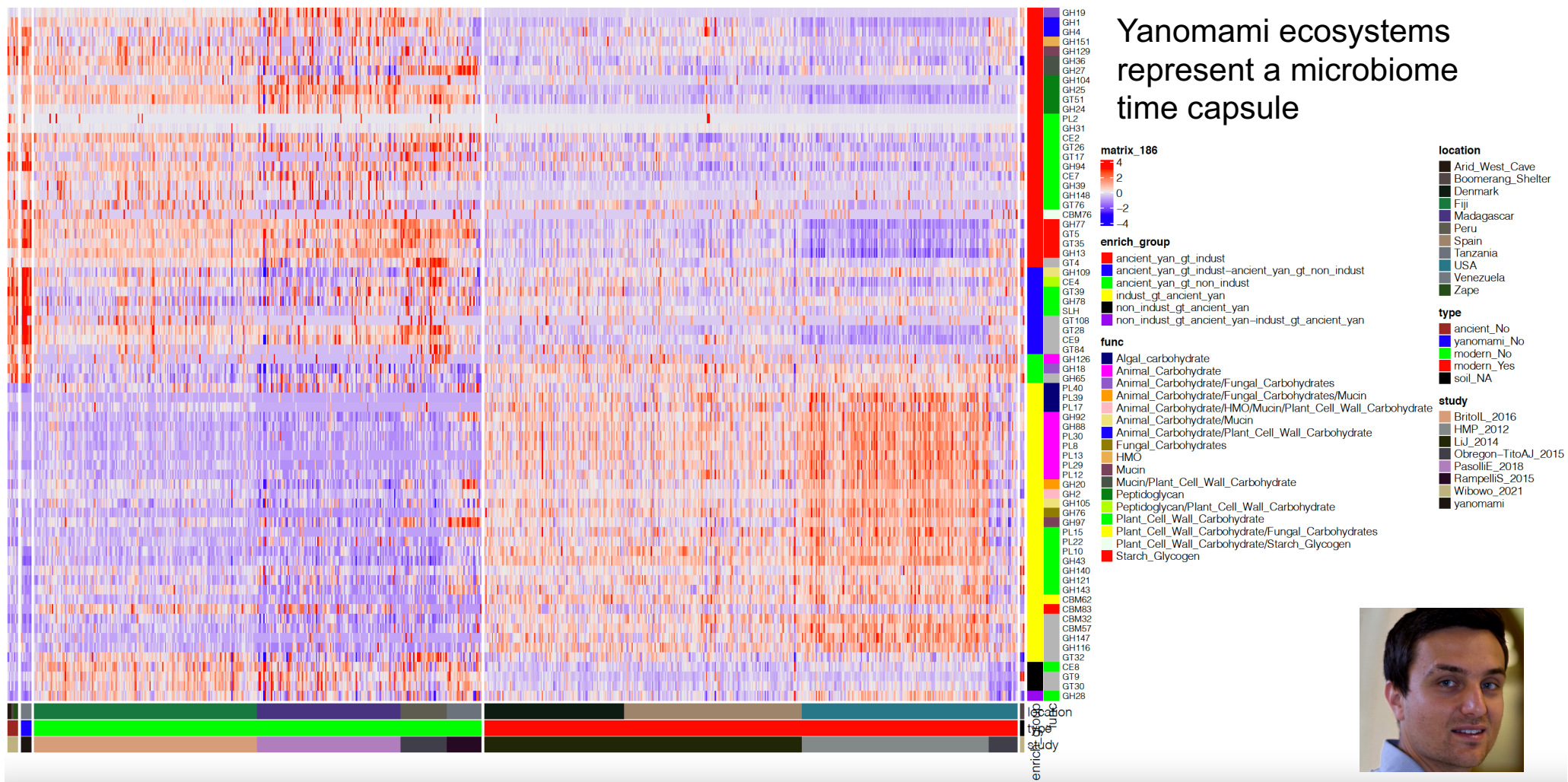
Verrucomicrobia

For comparison: Clemente *et al.*, 2015:  
**27 unique species** from 12 fecal  
samples using 7 media types.

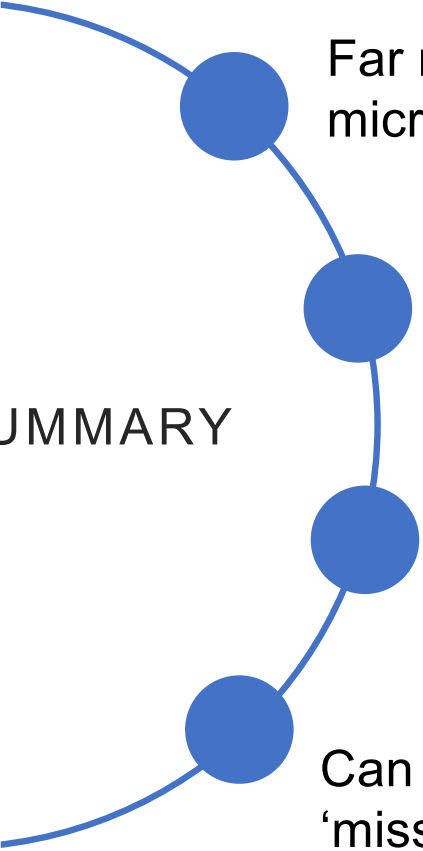
# From just 5 samples, >60 suspected novel bacterial species, and several novel genera

- Including several novel species of *Treponema* from the human gut
  - So far, *Treponema* spp. have only ever been seen in hunter-gatherer people from around the globe, and only by looking at DNA samples
  - Never seen in Westerners
  - Representative of a 'missing microbe'?





## SUMMARY



Far more diversity of bacterial species in Yanomami gut microbiomes than those of typical healthy Westerners

Many novel species cultivated (in the process of characterization)

Yanomami ecosystems may represent a time capsule, a window to the ancient human microbiome

Can we use these ecosystems to better understand the roles of 'missing microbes'?

# Acknowledgements



## EA-V lab members

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Liz Mallory  
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Avery Robinson  
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Heather Dang  
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Craig Moore  
Dr. Kathleen Schroeter  
Emerald Sheridan  
Keith Sherriff  
Co-founders  
Shawn Langer  
Nissim Mashiach  
Elaine Petrof

## Collaborators

### UCSD

Dr. Lars Bode  
Annalee Loeffler

### Sick Kids

Dr. Jayne Danska  
Dr. Alessandra  
Granato

### Joslin Diabetes Center

Dr. Alex Kostic  
Sam Zimmerman



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