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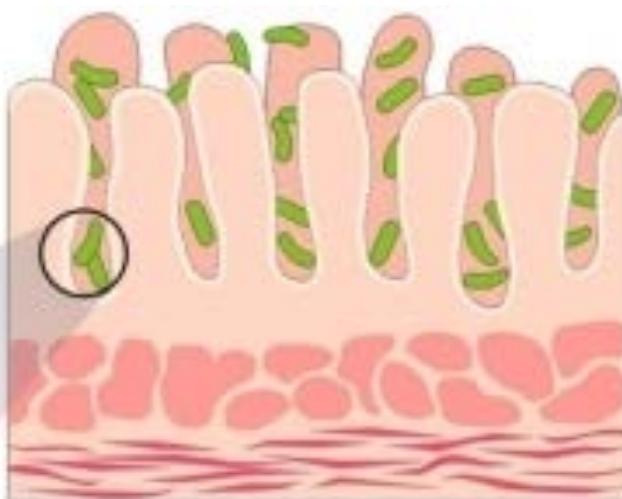


boakley@westernu.edu

Modern food safety: Managing the microbiome of food animals for performance and food safety goals

Importance of microbiota

Protective Functions
Pathogen displacement
Nutrient competition
Receptor competition
Production of anti-microbial factors

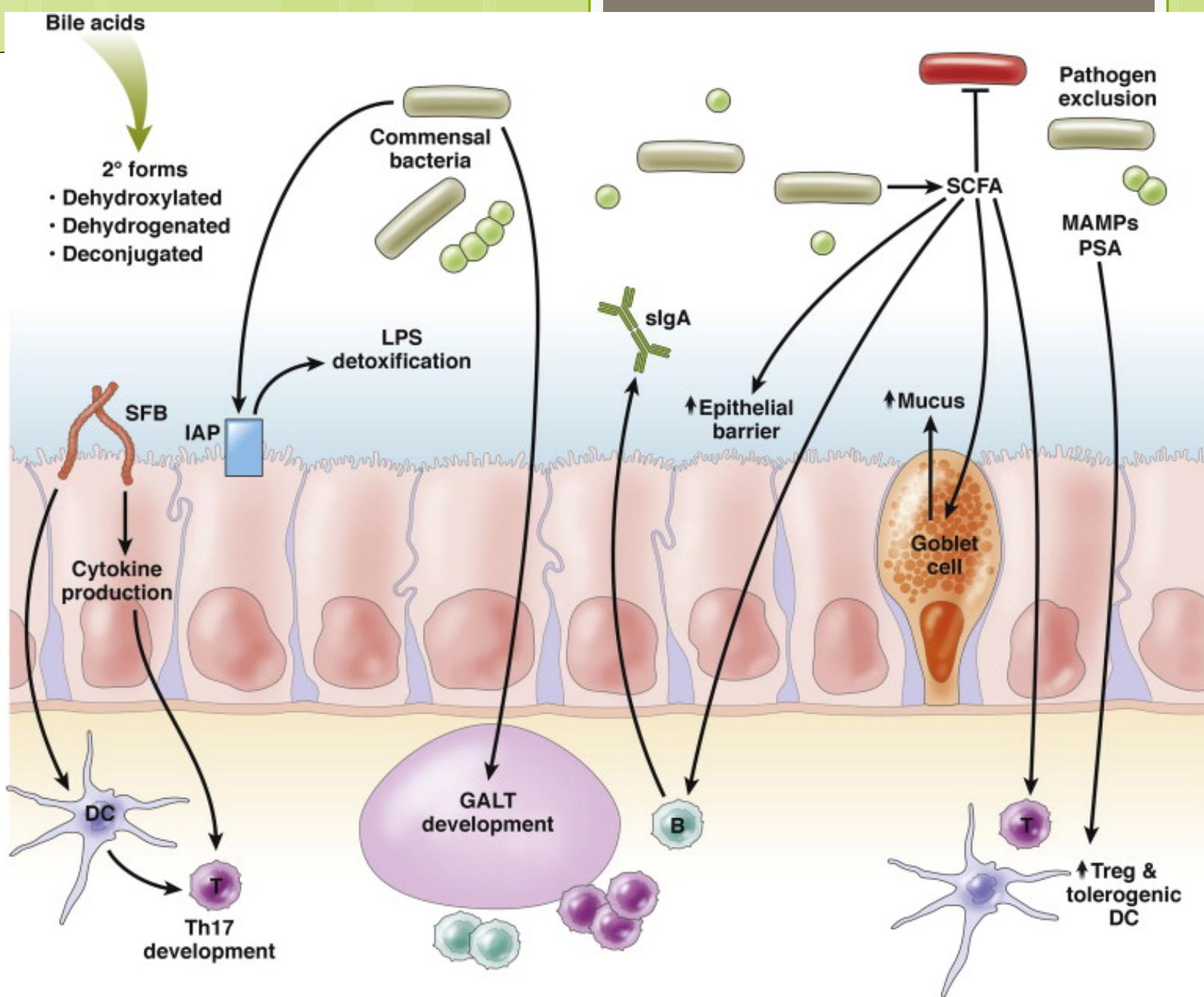


Structural Functions

Barrier fortification
Induction of IgA
Apical tightening of tight junctions
Immune system development

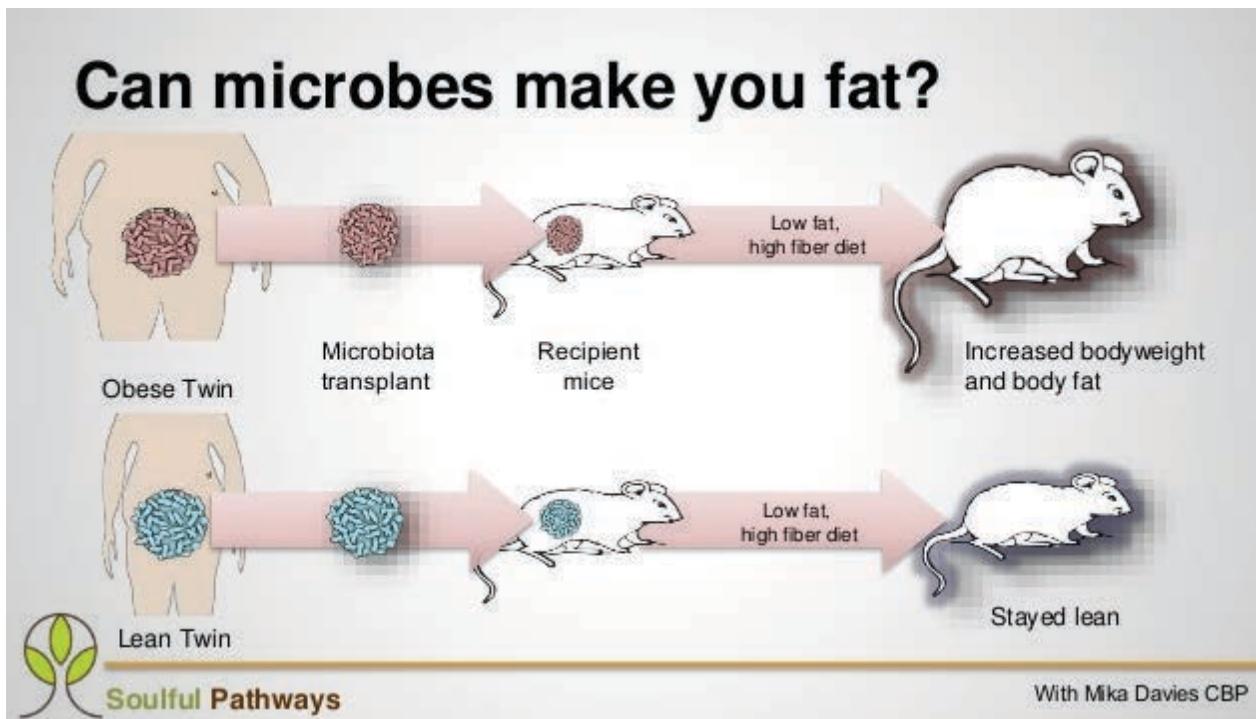
Metabolic Functions

Control of epithelial cell differentiation and proliferation
Metabolism of dietary carcinogens
Synthesis of vitamins
Fermentation of non-digestible dietary residue and epithelial-derived mucus
Ion absorption
Salvage of energy



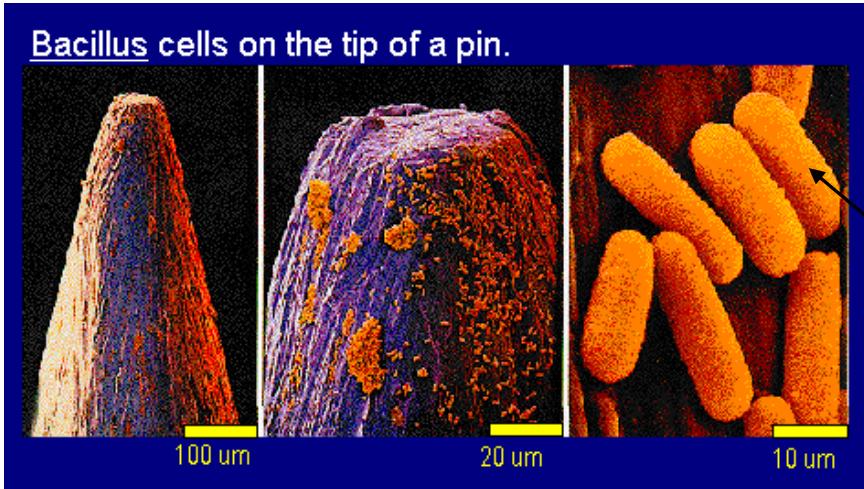
Importance of microbiota

GI microbiome helps control energy balance



Turnbaugh et al. 2006 Nature (444)

Bacteria truly inhabit a “Micro” Biome



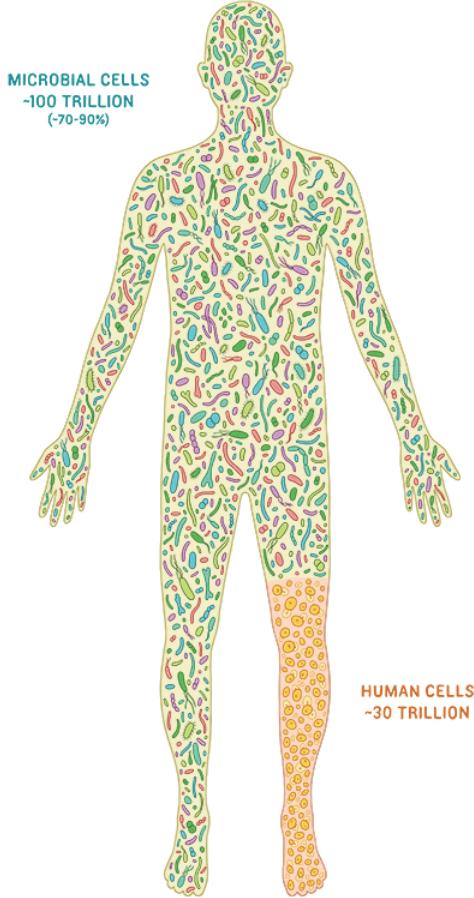
	Zebra	Bacterium
Scale of individual	2 m	2 μM
Scale of biome	25,000 km^2	40 m^2
Ratio biome:individual	1.3E+07	2.0E+07



Surface Areas:

Mara-Serengeti = 25,000 km^2
Human GI tract = 40 m^2

All animals are mostly microbial



Animals are:

- **50-90% bacterial by cell count**
- **90-99% bacterial by gene count**



Veterinary & Food Animal Medicine MUST consider what comprises the majority of the organism

by Gaby D'Allesandro / © AMNH

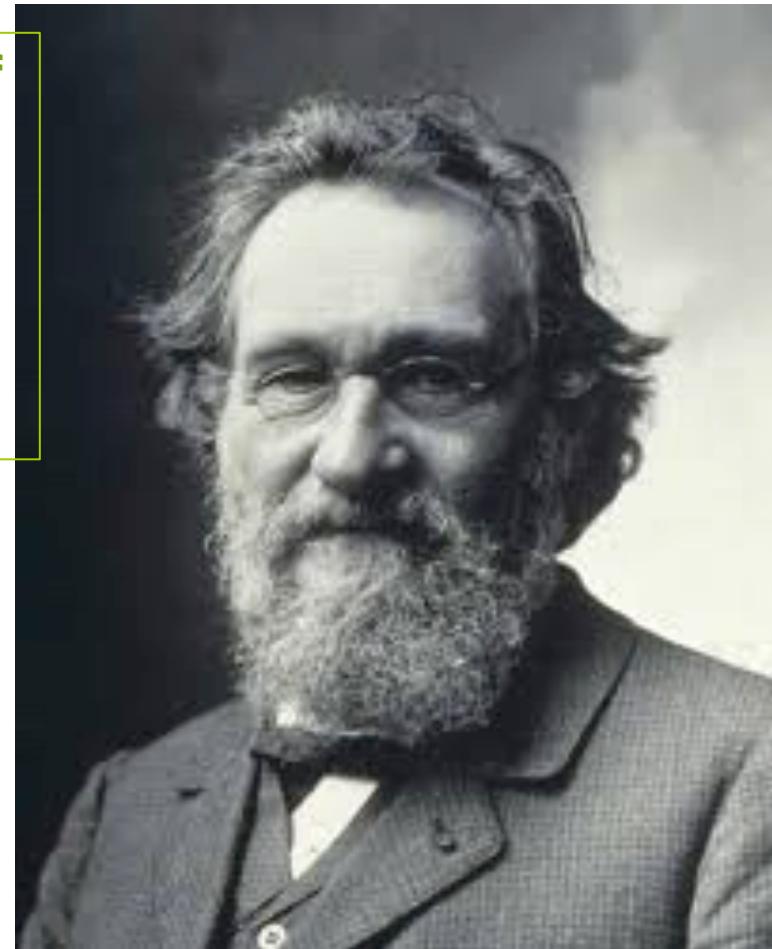
Can modern food safety go back to the future?

Q: Can we move from a mentality of 'killing bad bugs' to Ecosystem Management?

Current state of the art is development of "Microbial Agriculture"

"The dependence of the intestinal microbes on our food intake makes it possible to adapt measures to modify the flora in our bodies and to replace harmful microbes by useful microbes"

E. Metchnikoff
Optimistic Studies, NY:
Putnam's Sons, 1908.

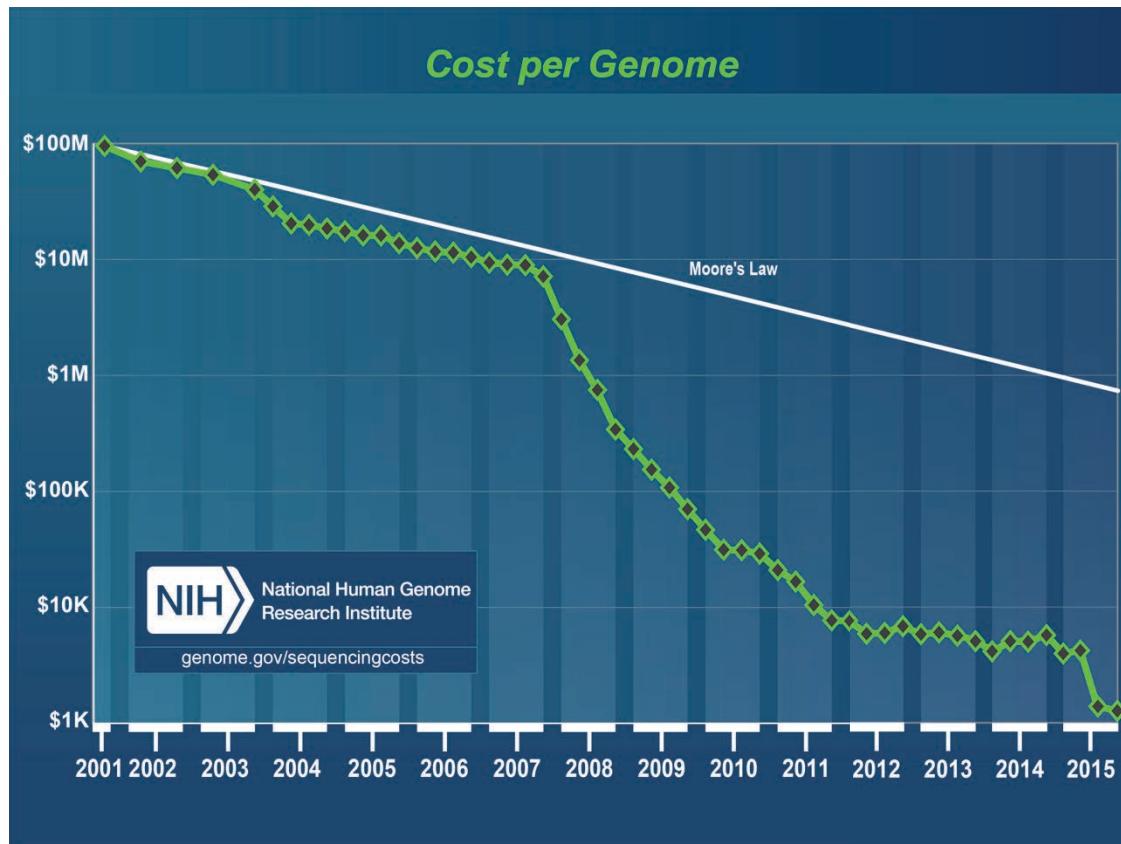


Microbial Ecology – Nascent field

- ~~Who is there?~~
- ~~How many of each?~~
- ~~What are they doing?~~

Renaissance of Microbial Ecology

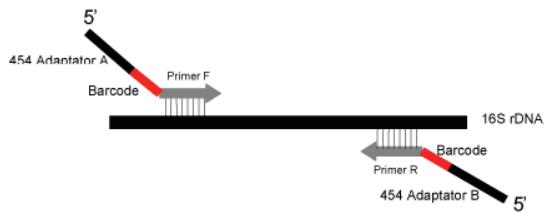
- DNA sequencing costs are decreasing faster than Moore's law:



Toolkit of molecular microbial ecology

Revolutionized by high-throughput sequencing

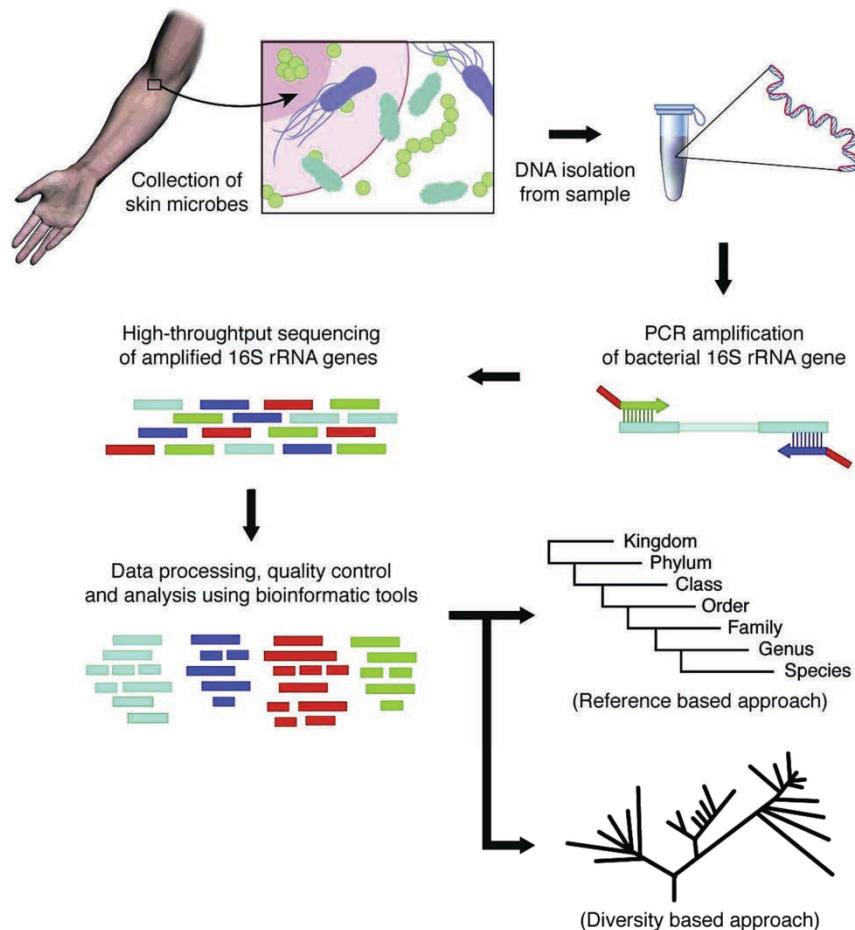
- Compositional census
 - 16S rRNA
 - Taxonomic ID



- Functional census
 - Metagenomics
 - Metabolic potential
- Metatranscriptomics
- Metabolic activity

Toolkit of molecular microbial ecology

- Compositional census
 - 16S rRNA
 - Taxonomic ID



Toolkit of molecular microbial ecology

- Functional census
- Metagenomics

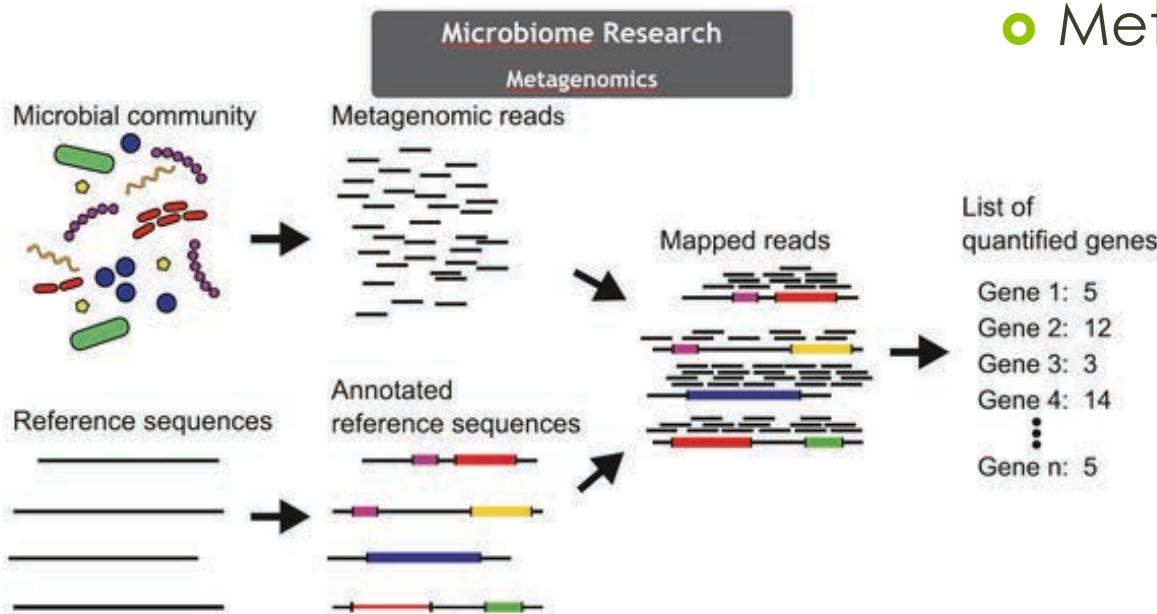


Figure 1: Overview of gene quantification in metagenomics. DNA is extracted and randomly sequenced from a microbial community. The resulting reads are then mapped to reference sequences that have been annotated according to their gene content. Each read that matches a gene is counted as an occurrence of that gene. The end result is a list of counts for each sample providing the relative abundance of each gene.

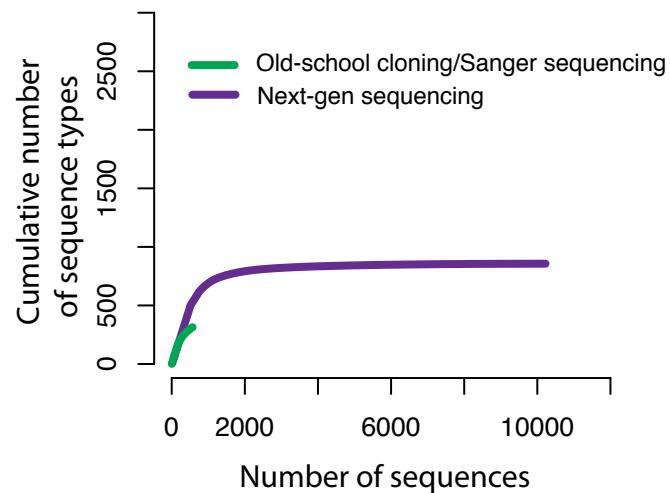
Modified from Jonsson, Viktor. Statistical analysis and modelling of gene count data in metagenomics. Diss. Göteborgs universitet, 2017.
https://gupea.ub.gu.se/bitstream/2077/48788/1/gupea_2077_48788_1.pdf

Renaissance of Microbial Ecology

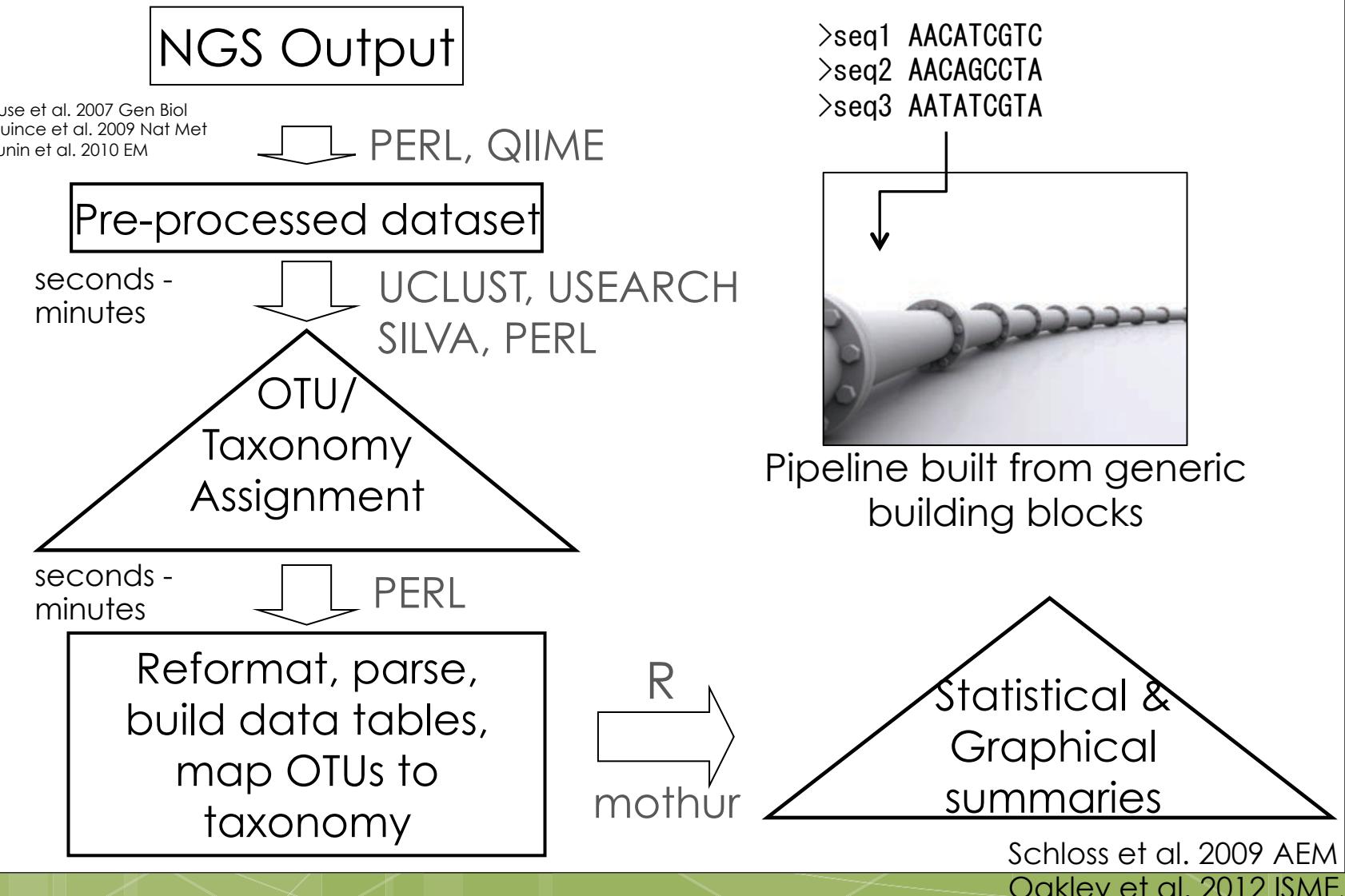
High-throughput sequencing

- Great plate count anomaly - Has largely removed previous limitations
 - Only 0.1 – 1% of taxa cultivable
 - 'uncultured majority' – Only 26/52 phyla in cultivation
- Comprehensive census once impossible, now routine
- Bioinformatic and Computational Advances

Staley and Konopka 1985. Ann Rev Micro
Rappe and Giovannoni 2003. Ann Rev Micro



Bioinformatics gives the keys to the kingdom

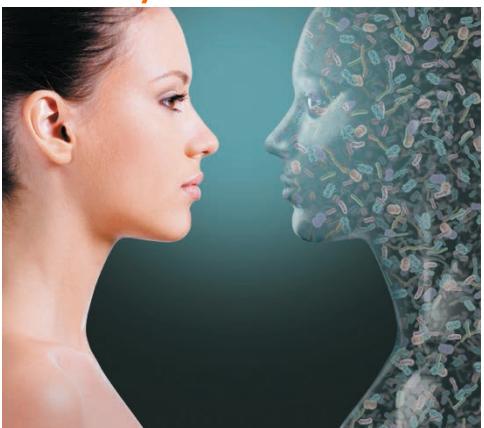


Importance of Microbial Ecology

Can now answer:

- **Community composition – who's there**
- **Community structure – how many of each**
- **Community function – what are they doing**

HMP – Humans as microbial ecosystems



- Humans as 'super-organisms'
 - New tools applied to microbial ecosystems in and on our bodies
 - "Second genome"
 - ~10x more microbial than human cells
 - ~100x more genes
 - **Critical roles in nutrition, health, disease**

Renaissance of Microbial Ecology

Poultry microbiology and its importance for food safety

- ~ 9,000,000,000 broilers processed per yr in U.S.
- ~ 40,000,000 tons of feed per yr
- Carbon footprint
~450,000,000,000 car miles yr⁻¹
- Major source of protein & foodborne illness
- Microbiome of birds **and** production system as 'super-organism'



Broiler Production 101

Hatchery



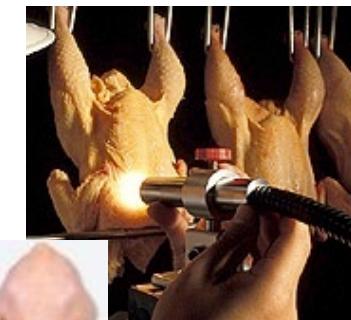
Grow-out



Transport



Processing



Consumer

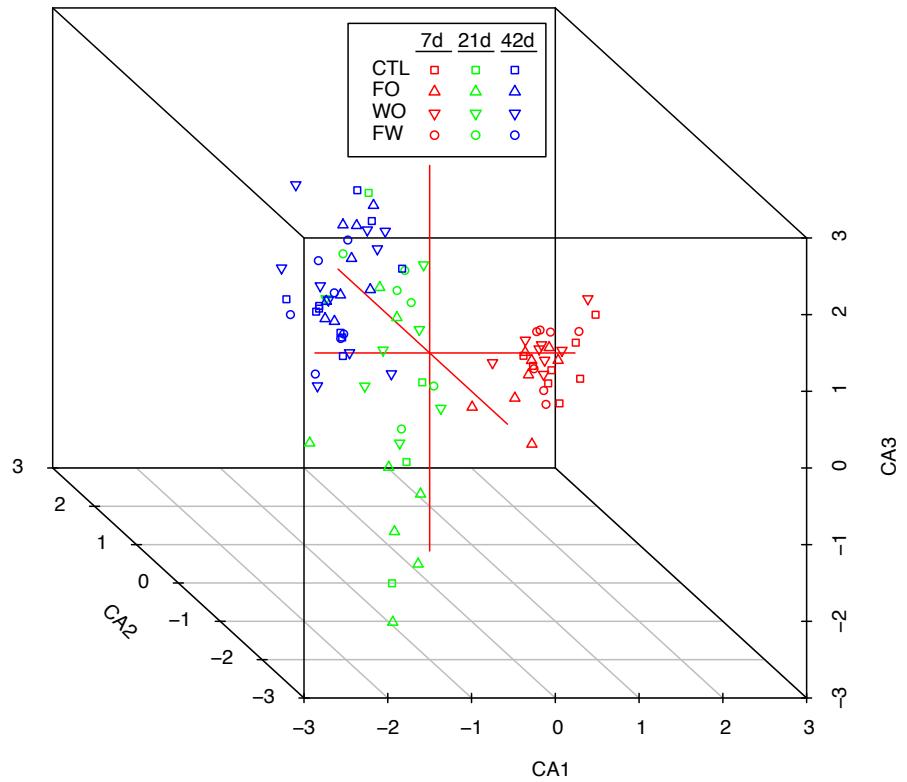


Can we manage the micro biota?

- What do natural communities look like?
- New tools & approaches needed
- Alternative antimicrobials
- Can we put it all together? “Seed, Feed, & Weed”

What do natural communities look like?

Community changes significantly as bird grows

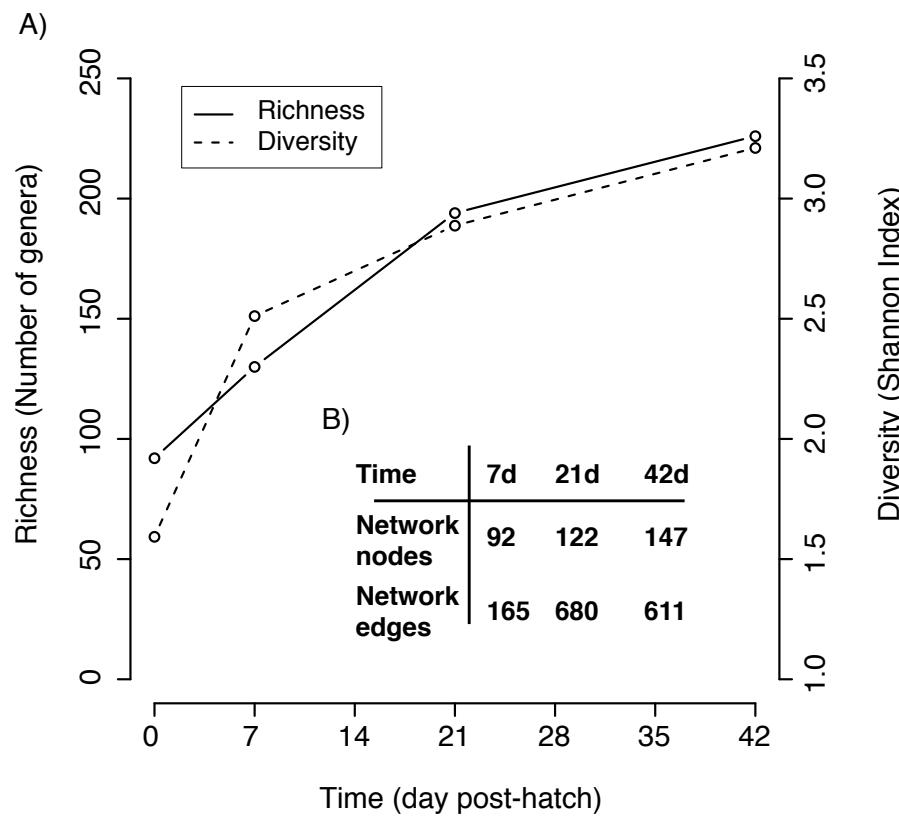


Temporal differences > feed additives

	Degrees of Freedom	Sum of Squares	Mean Squares	F.Model	R ²	Pr(>F)
Time	1	3.457	3.457	8.986	0.0912	0.0001
Treatment	3	1.137	0.379	0.985	0.0300	0.4935
Time:Treatment	3	1.019	0.340	0.883	0.0269	0.8455
Residuals	84	32.310	0.385		0.8520	
Total	91	37.922			1.0000	

What do natural communities look like?

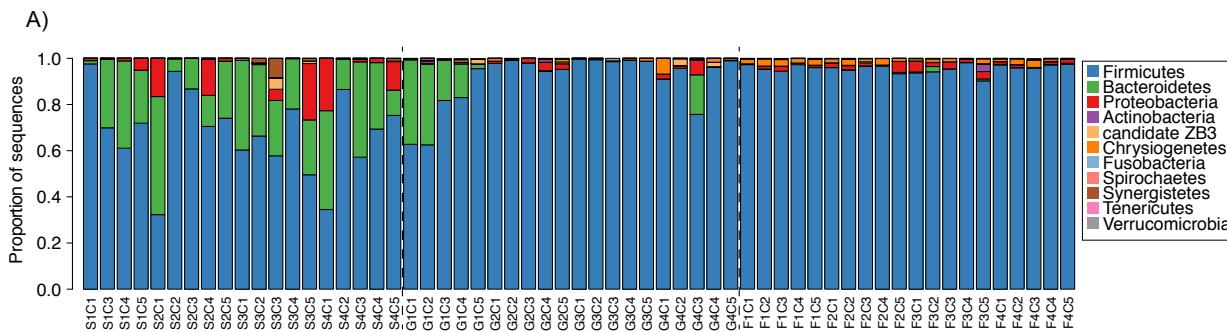
Community changes significantly as bird grows



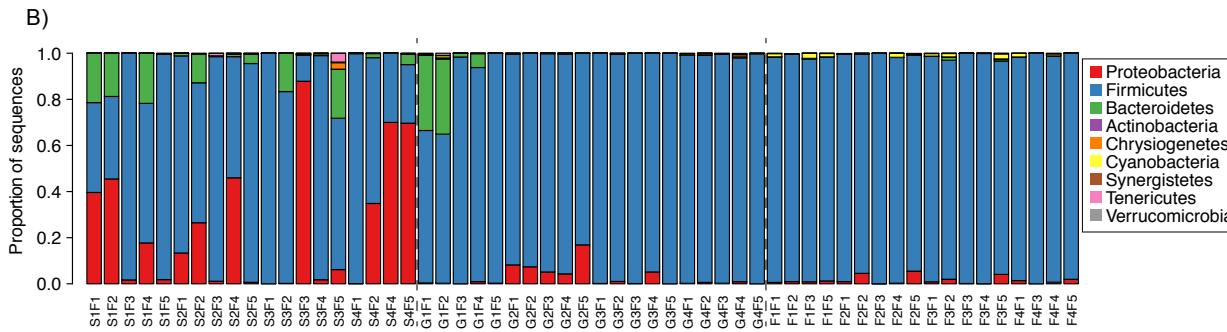
What do natural communities look like?

Community changes significantly through time
...and space

Cecal



Fecal



Oakley et al. 2016

What do natural communities look like?

Knowledge of community composition can guide management strategies

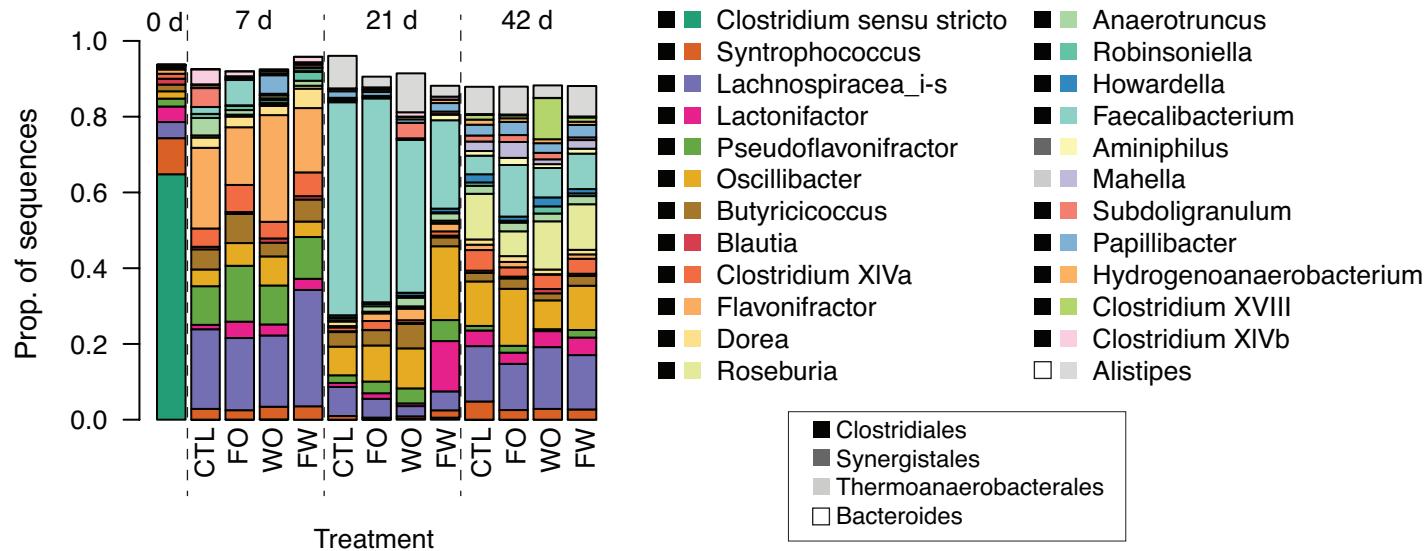


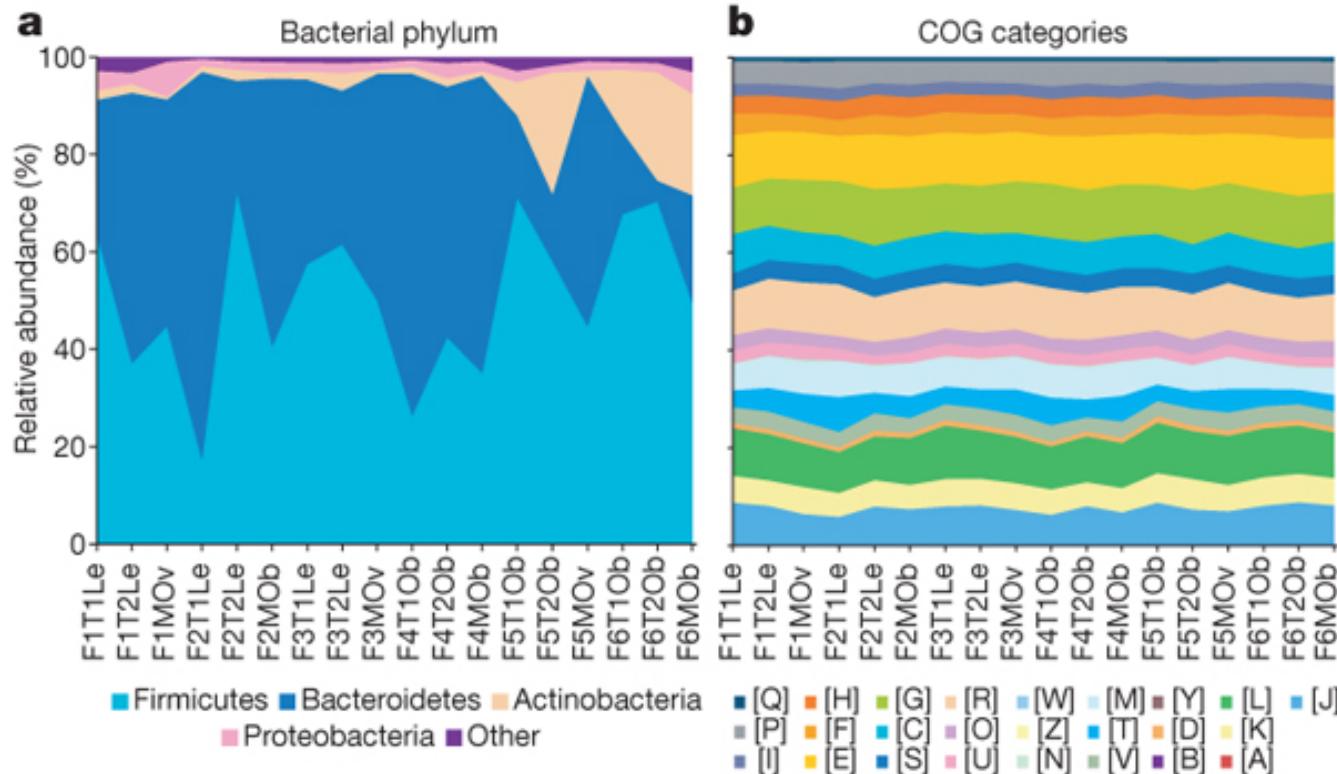
Figure 2.

Birds exposed to wide range of environmental bacteria, but reproducibly colonized by limited subset.....

Oakley et al. 2015 BMC Vet Res.

What do natural communities look like?

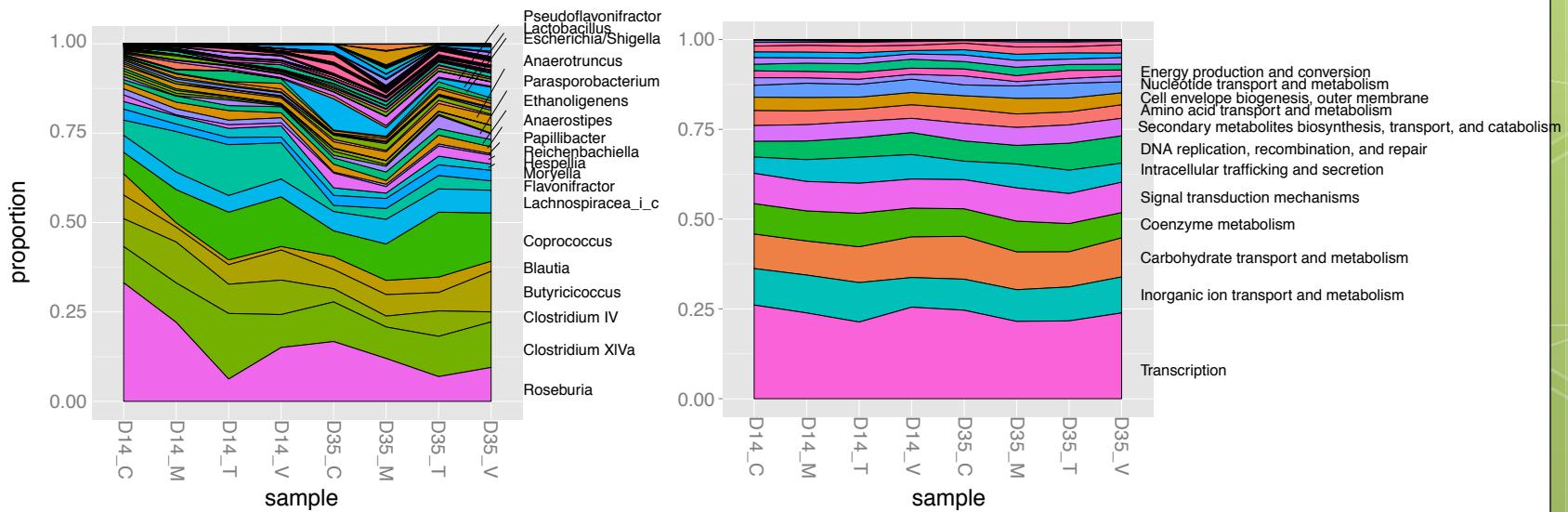
Humans: Taxonomic variability but functional conservation



What do natural communities look like?

Poultry: Taxonomic variability but functional conservation

- Why is this important?
 - Functional redundancy
 - Possible to construct minimal community?



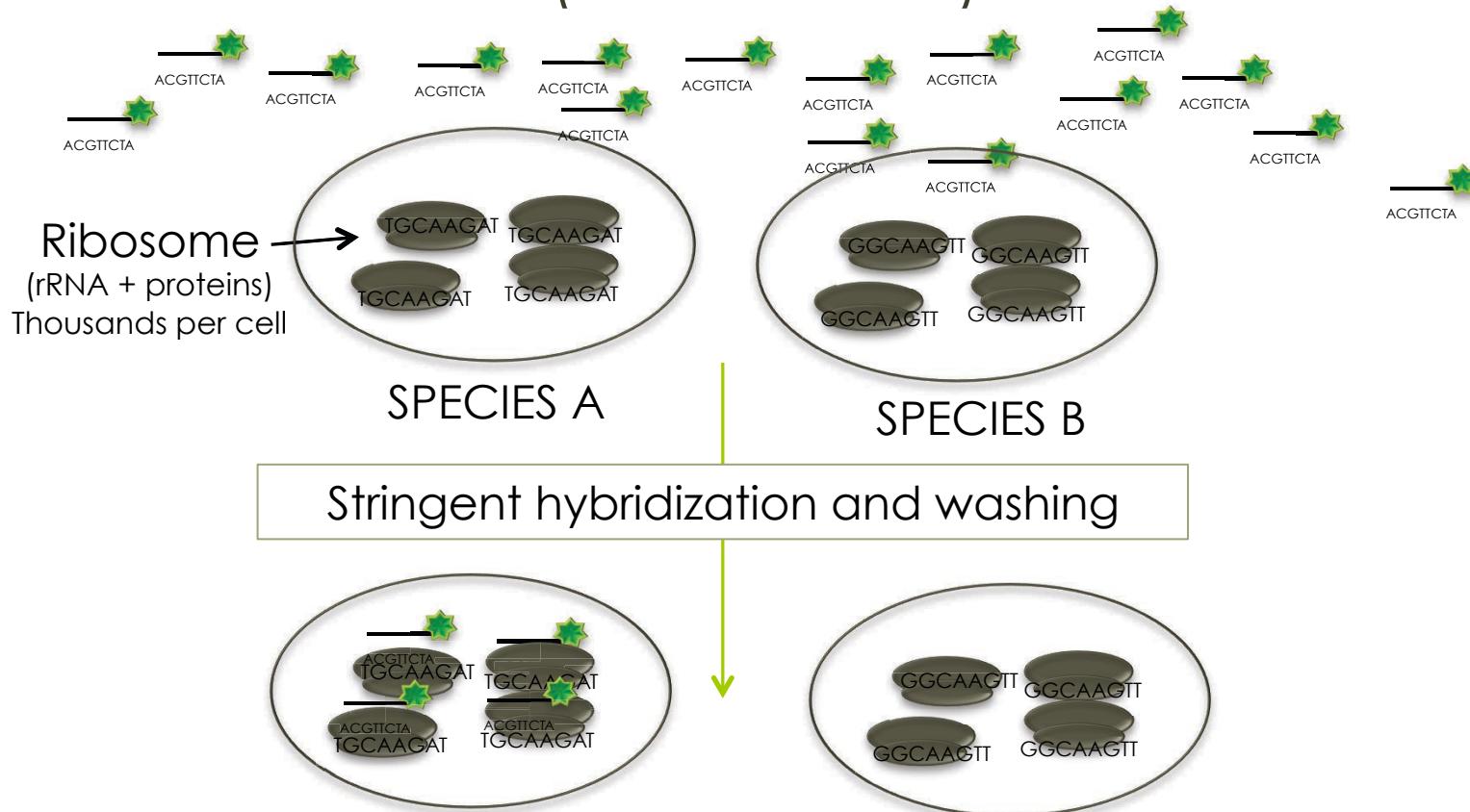
Danzeisen et al. PLoS ONE 2011
Oakley et al. FEMS Micro Let 2014

Can we manage the microbiota?

- What do natural communities look like?
- New tools & approaches needed
- Alternative antimicrobials
- Can we put it all together? “Seed, Feed, & Weed”

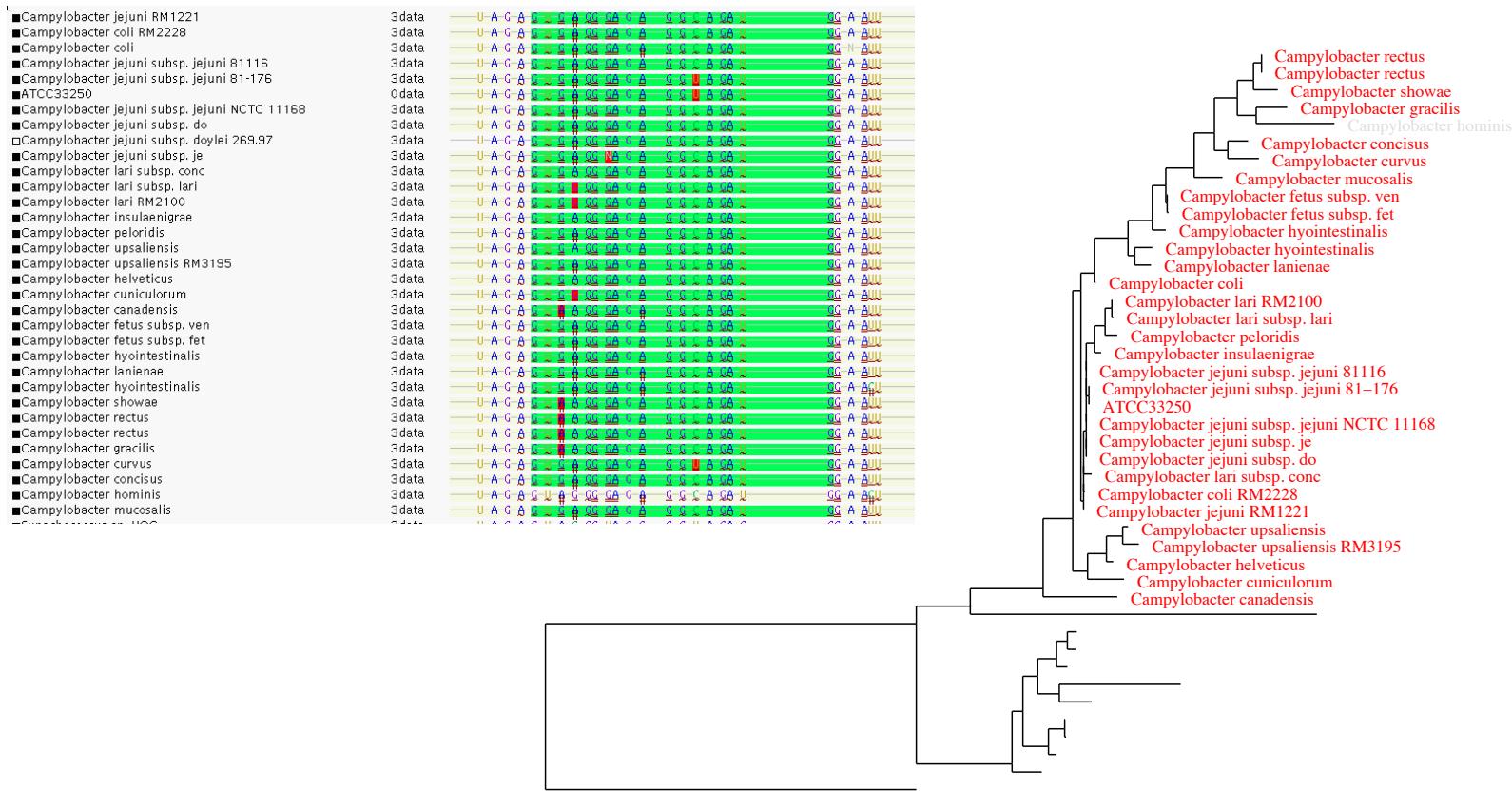
Fluorescence in-situ hybridization (FISH)

- Sequence-based discrimination of bacterial cells (and colonies)

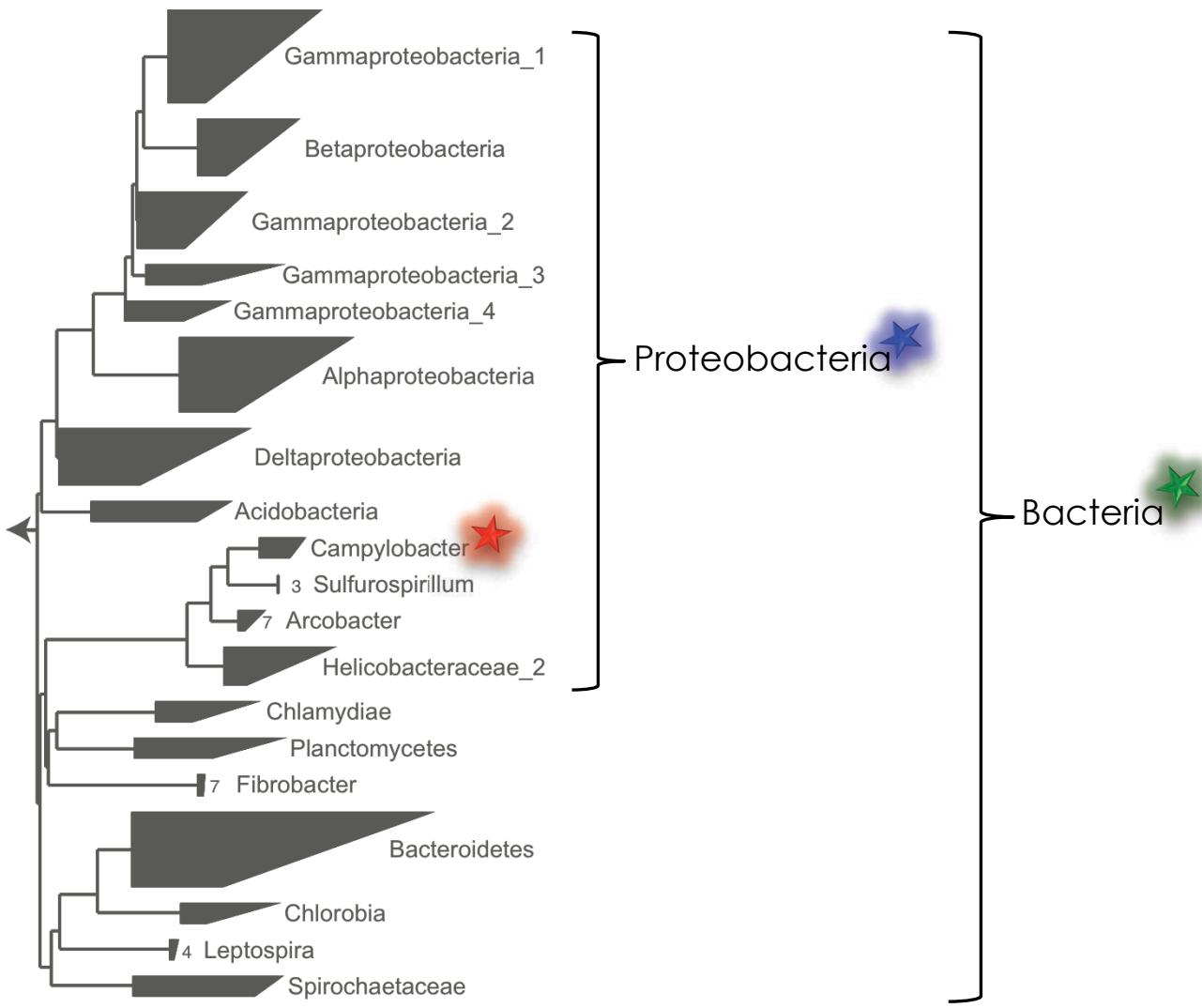


Fluorescence in-situ hybridization (FISH)

- Can use massive rRNA sequence repositories to design specific and sensitive probes:

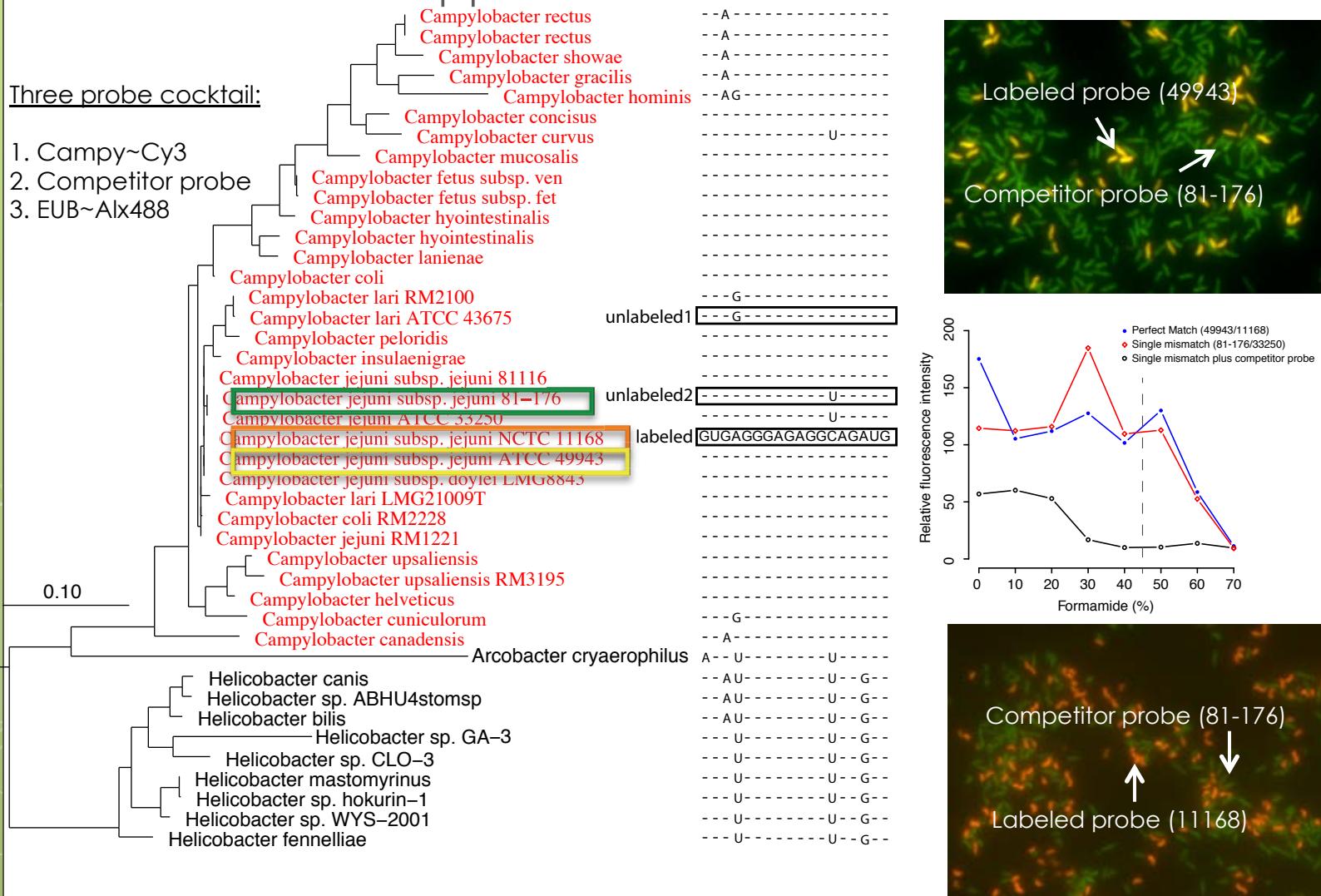


Hierarchical probe concept



Can we manage the microbiota?

- New tools & approaches

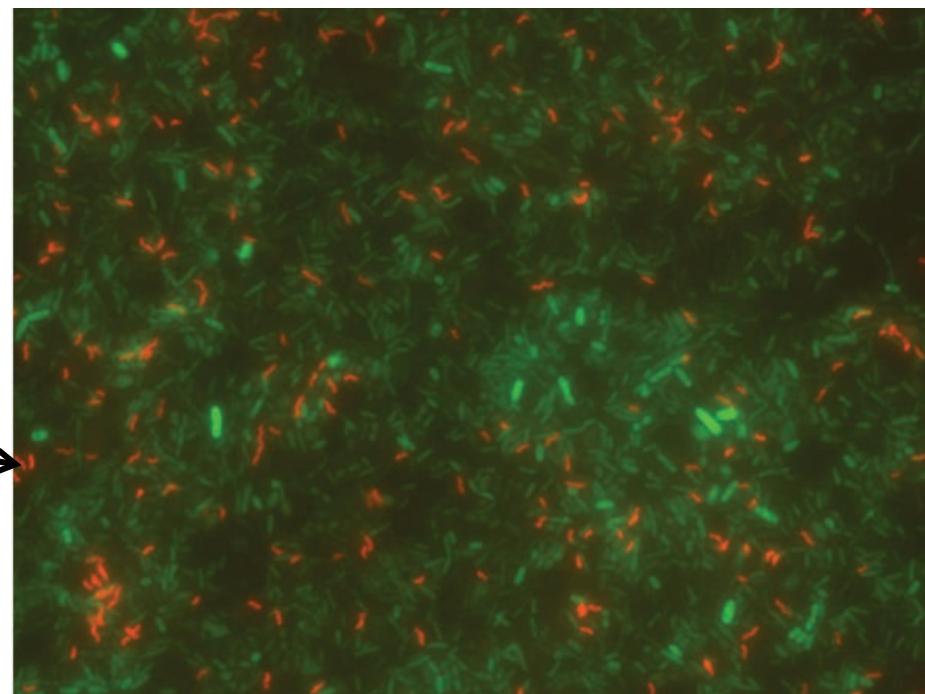
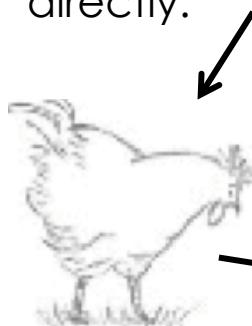


Can we manage the microbiota?

- New tools & approaches

- Now have direct window to follow individual strains in G-I tract
- Developed high-throughput image analysis tools

C. jejuni 11168 gavage,
image cecal sample
directly:

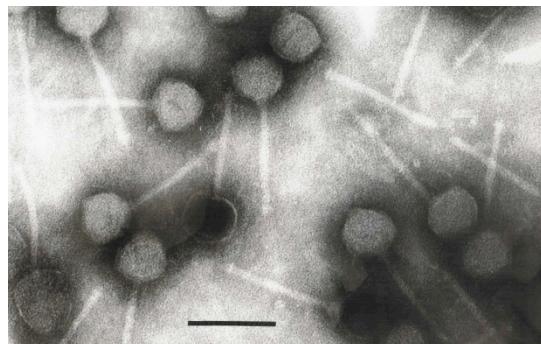
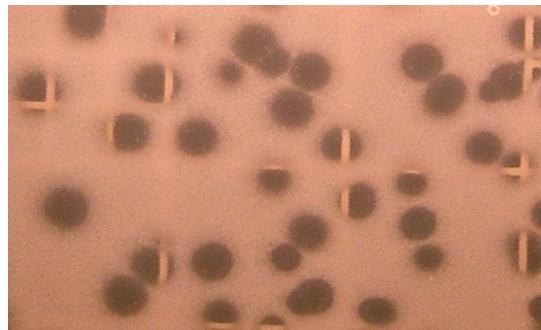
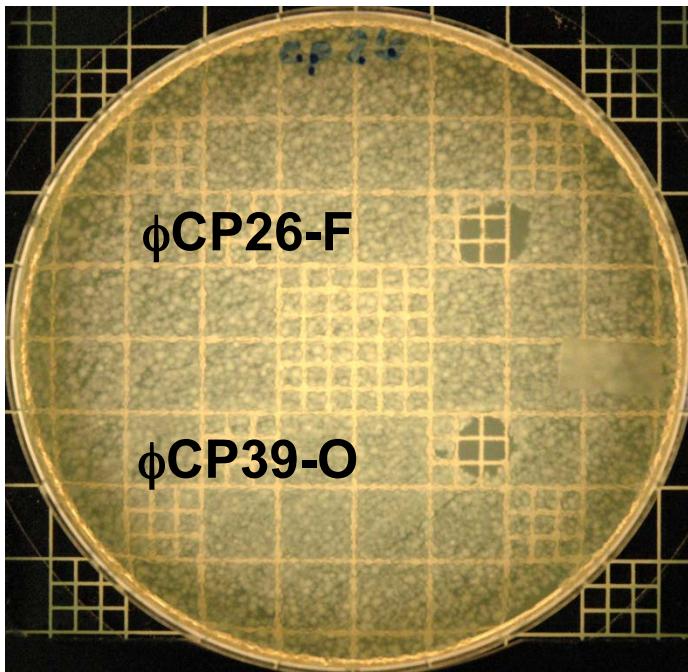


Can we manage the microbiota?

- What do natural communities look like?
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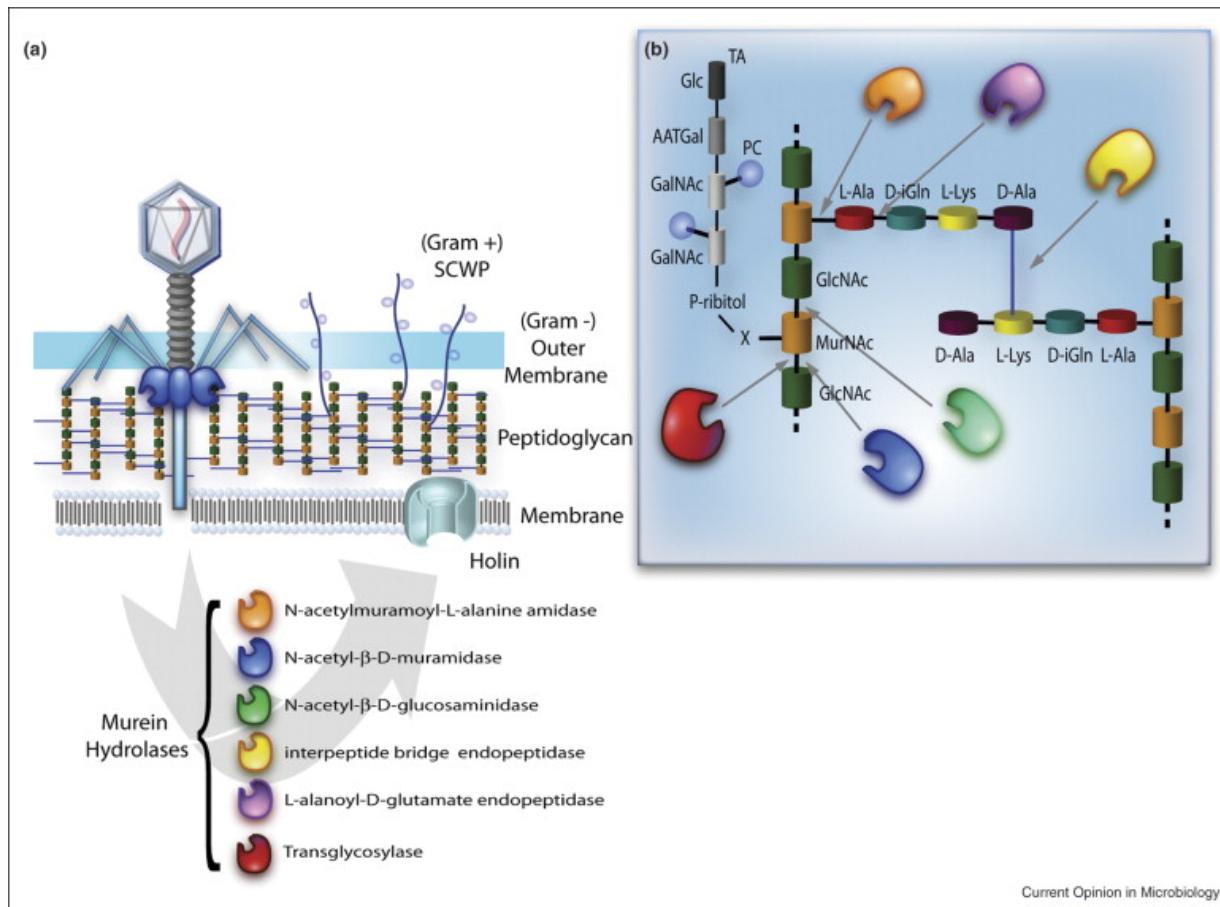
Can we manage the microbiota?

- Alternative antimicrobials



Can we manage the microbiota?

■ Alternative antimicrobials

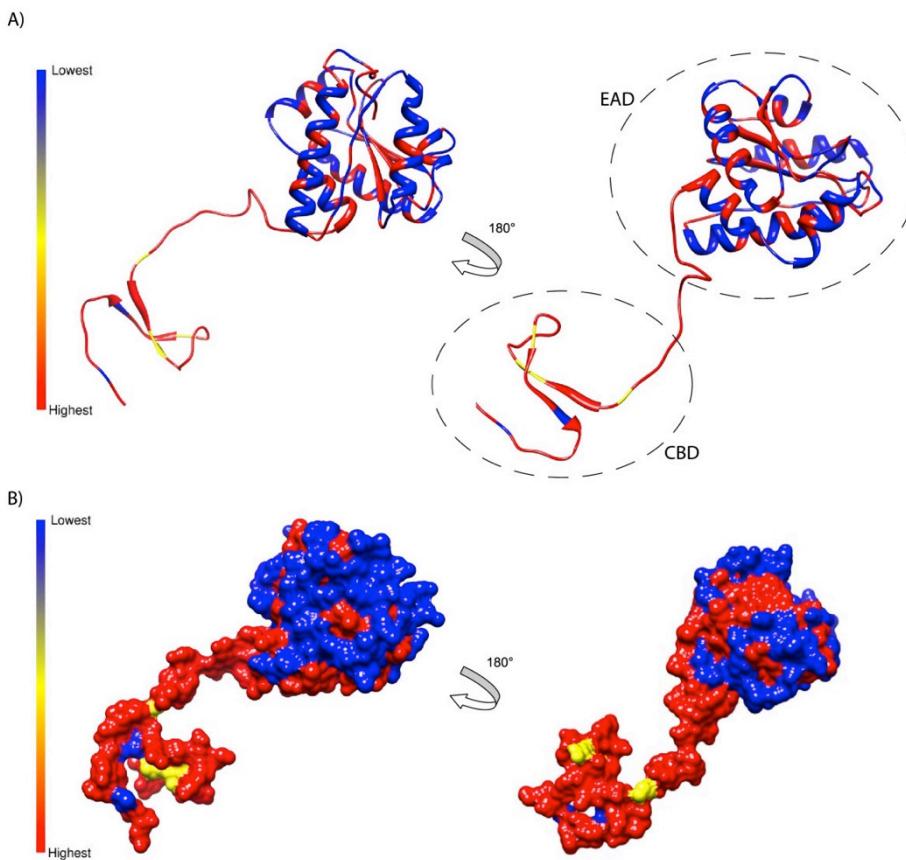


- Amidases
- Muramidases (Lysozyme)
- Glucosaminidases
- Endopeptidases
- Holins form lesions in bacterial membranes
- Autolysins and prophage enzymes present in bacterial genomes

Can we manage the microbiota?

■ Alternative antimicrobials

Structural modeling suggest potential for ‘mix-and-match’ use of phage lytic enzyme domains to tailor specificity as alternative antimicrobial agents:

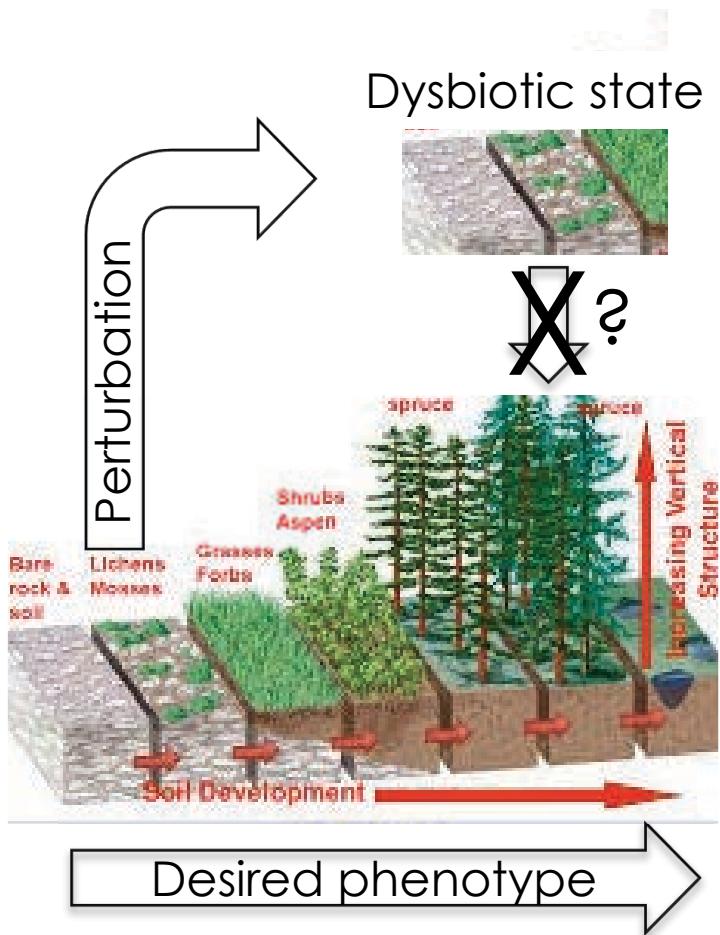


- N-acetylmuramoyl-L-alanine amidase sequence conservation varies by domain
- Percent protein sequence conservation is shown from highest 100% (red) to lowest 60% (blue).

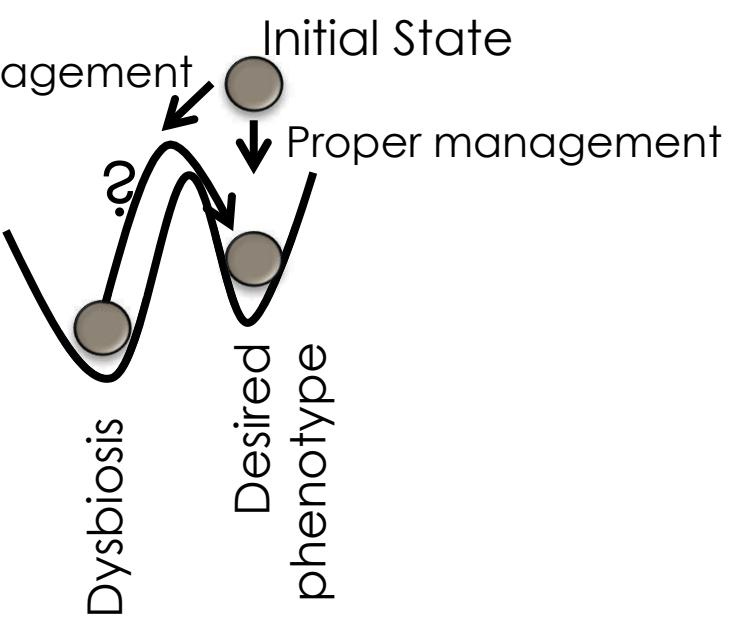
Can we manage the microbiota?

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Importance of early development



May not be possible to return to desired condition from dysbiotic state



Managing the micro biota...

Knowledge of community composition can guide management strategies

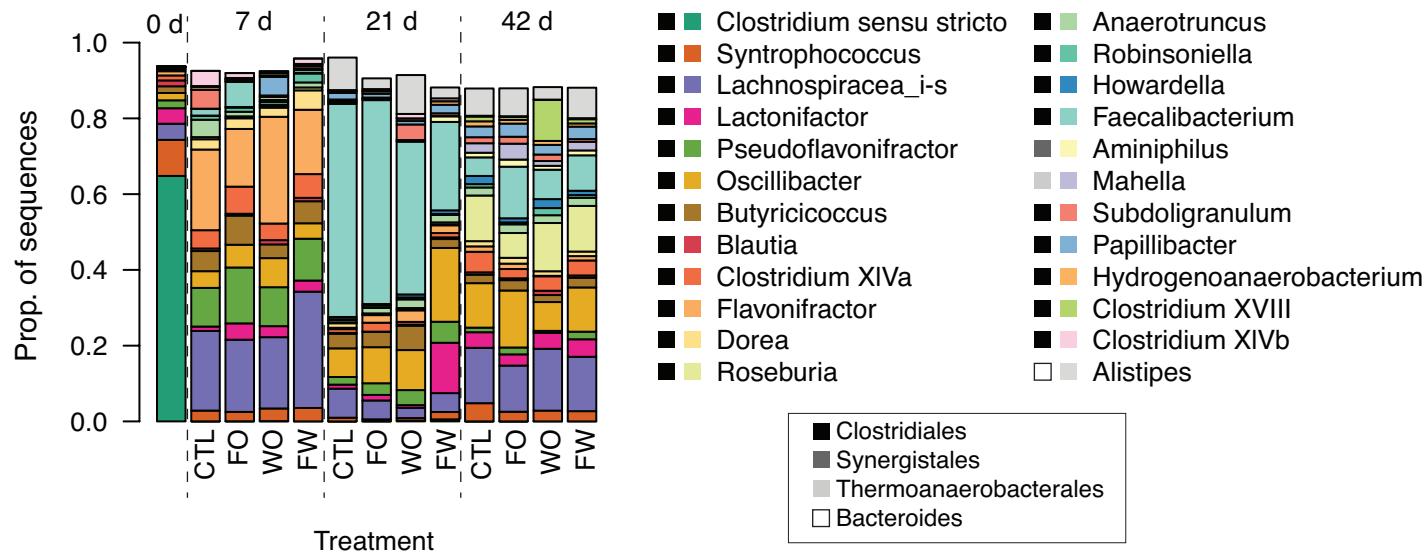


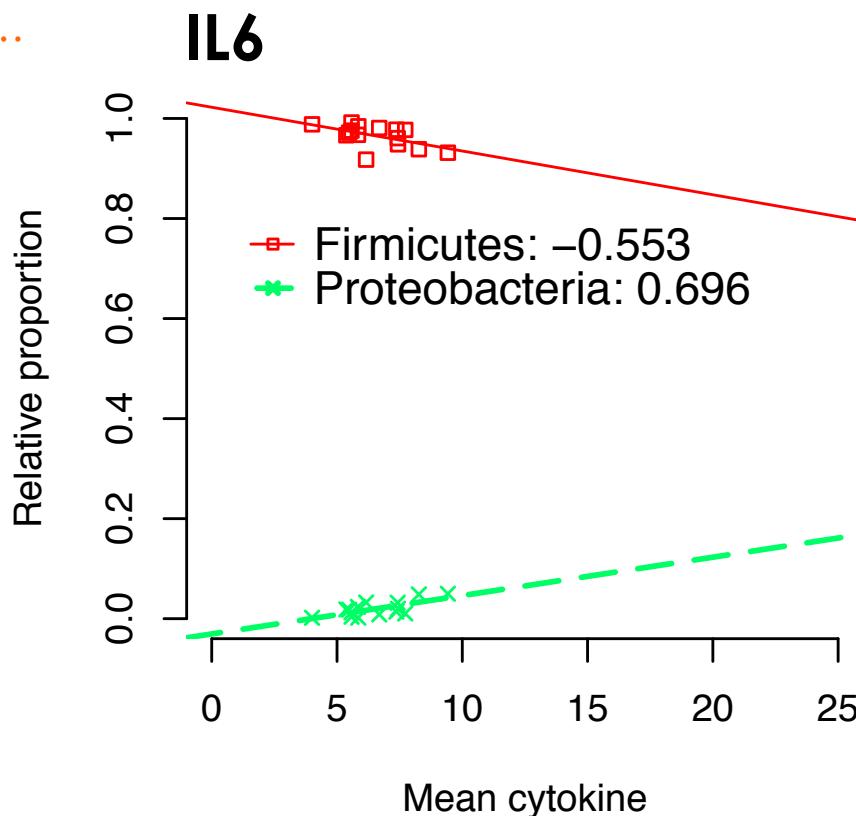
Figure 2.

Managing the micro biota...

Microbiome datasets are amenable to high-throughput data mining to identify potentially desirable & undesirable taxa.

Statistical associations of particular taxa with pro- and anti-inflammatory cytokines...

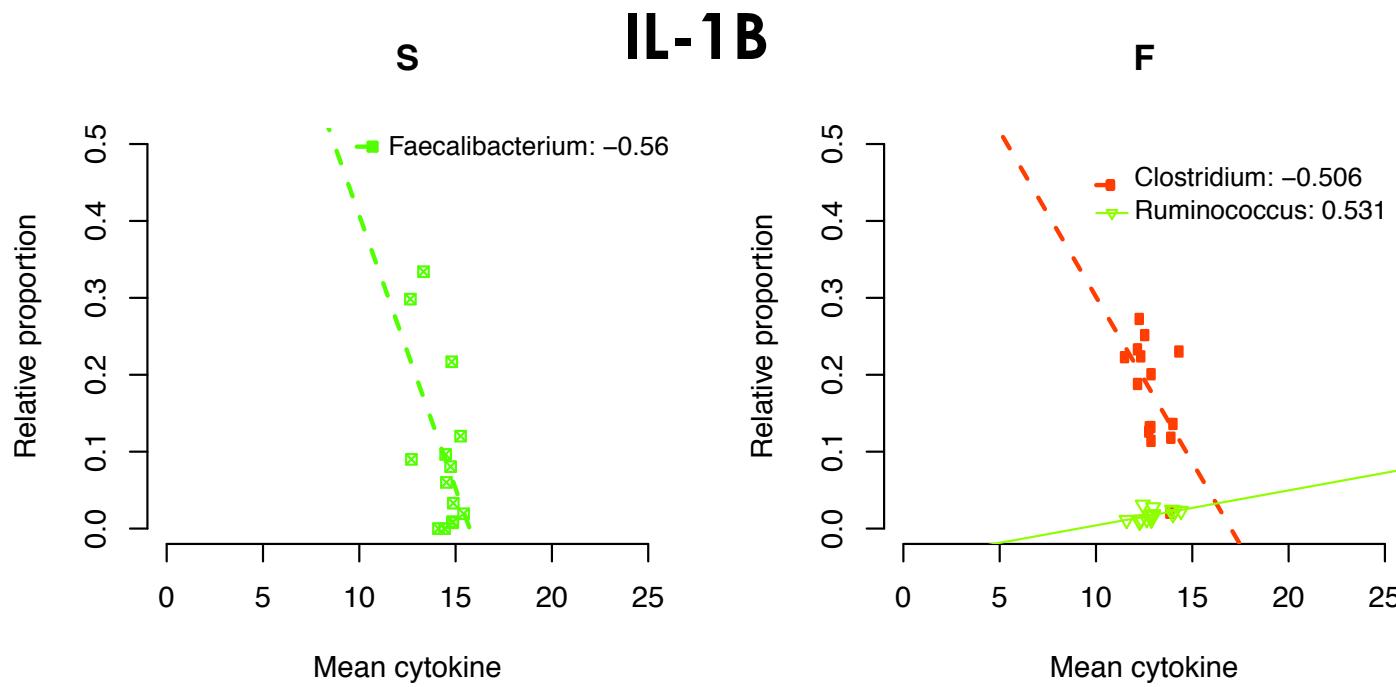
Ultimate objective is to manipulate taxonomic composition to achieve desired immune responses...



Managing the micro biota...

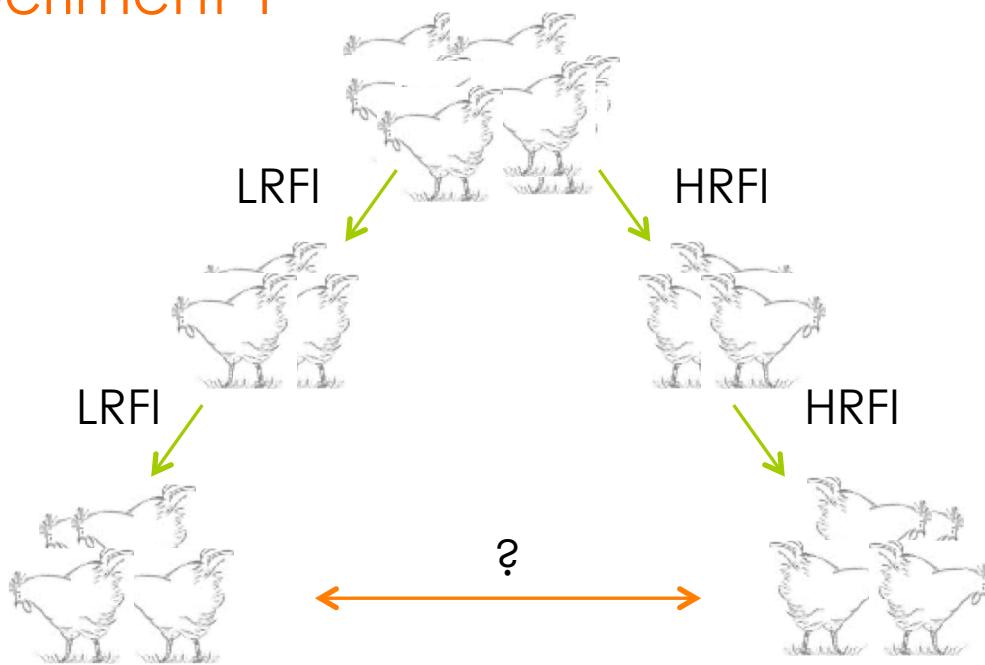
Microbiome datasets can reveal associations...

Answer may be different for different age birds:

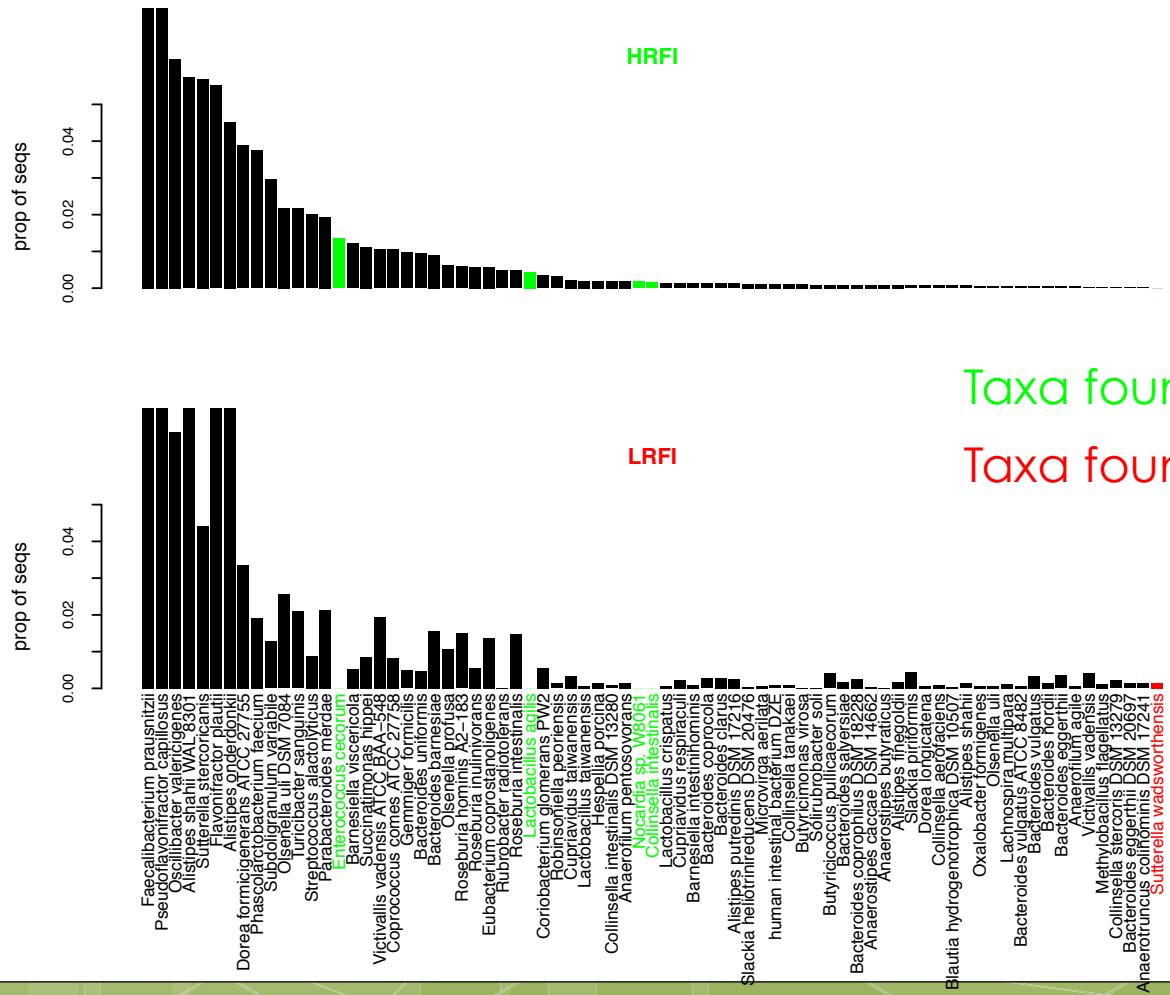


Can we identify a microbial contribution to FCR?

Experiment 1



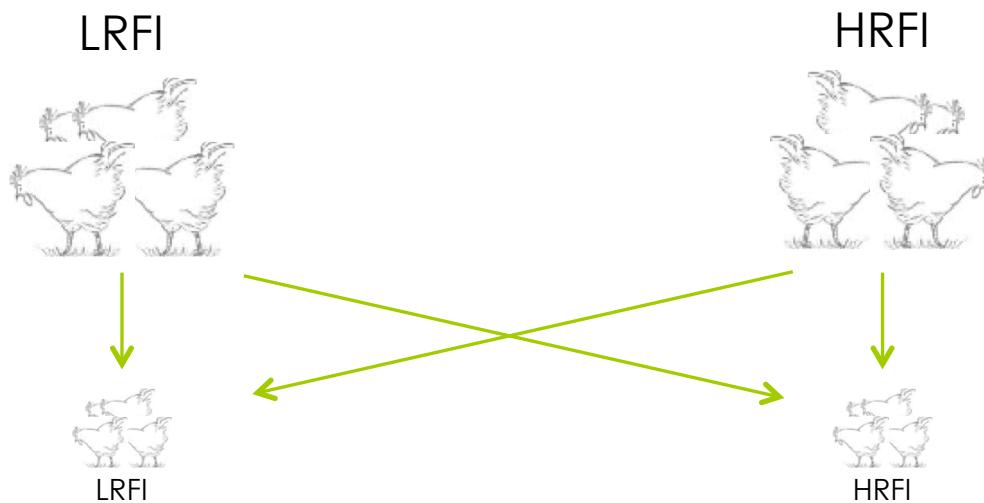
Can we identify a microbial contribution to FCR?



Can we identify a microbial contribution to FCR?

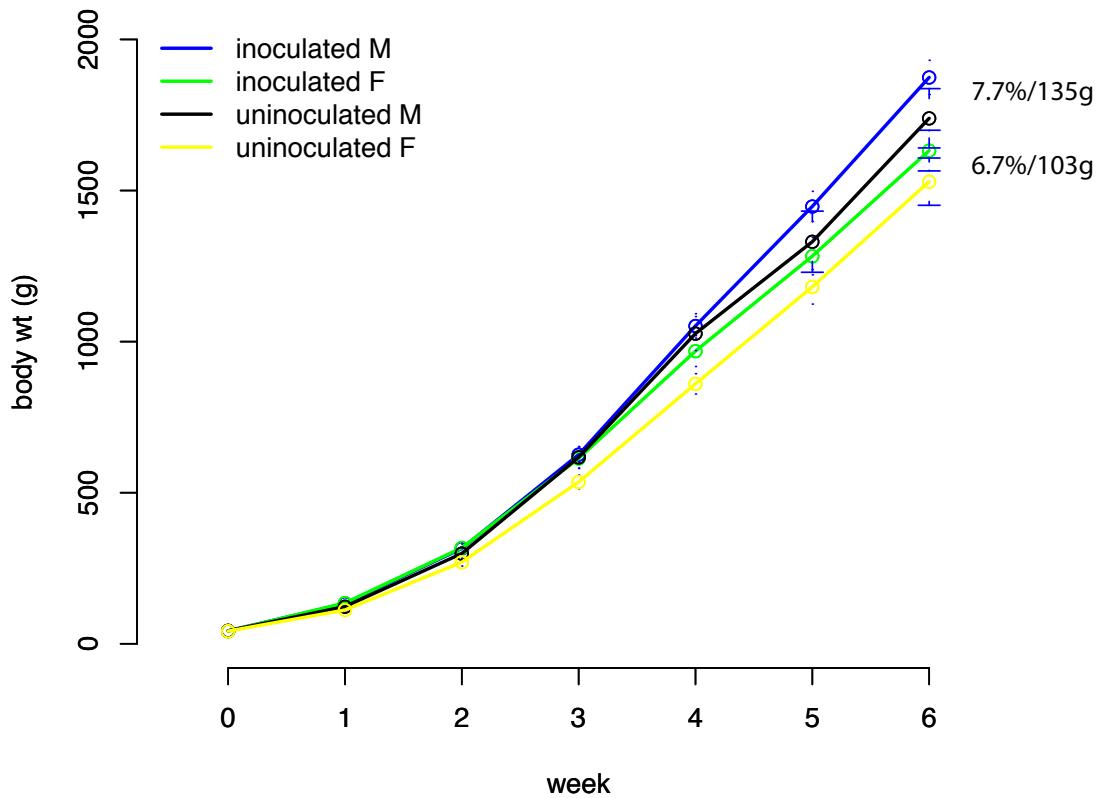
Experiment 2: Genetics vs. microflora

Reciprocal transplant of fecal material from adults to chicks:

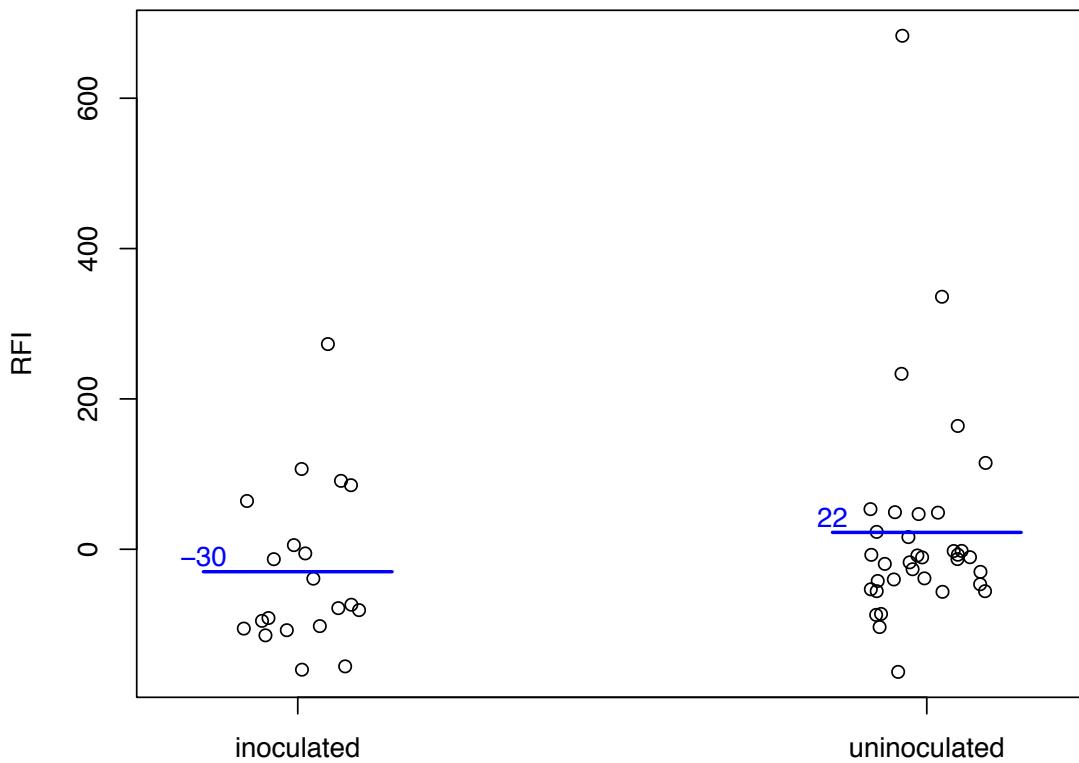


Plus uninoculated controls...

Inoculum improves weight gain

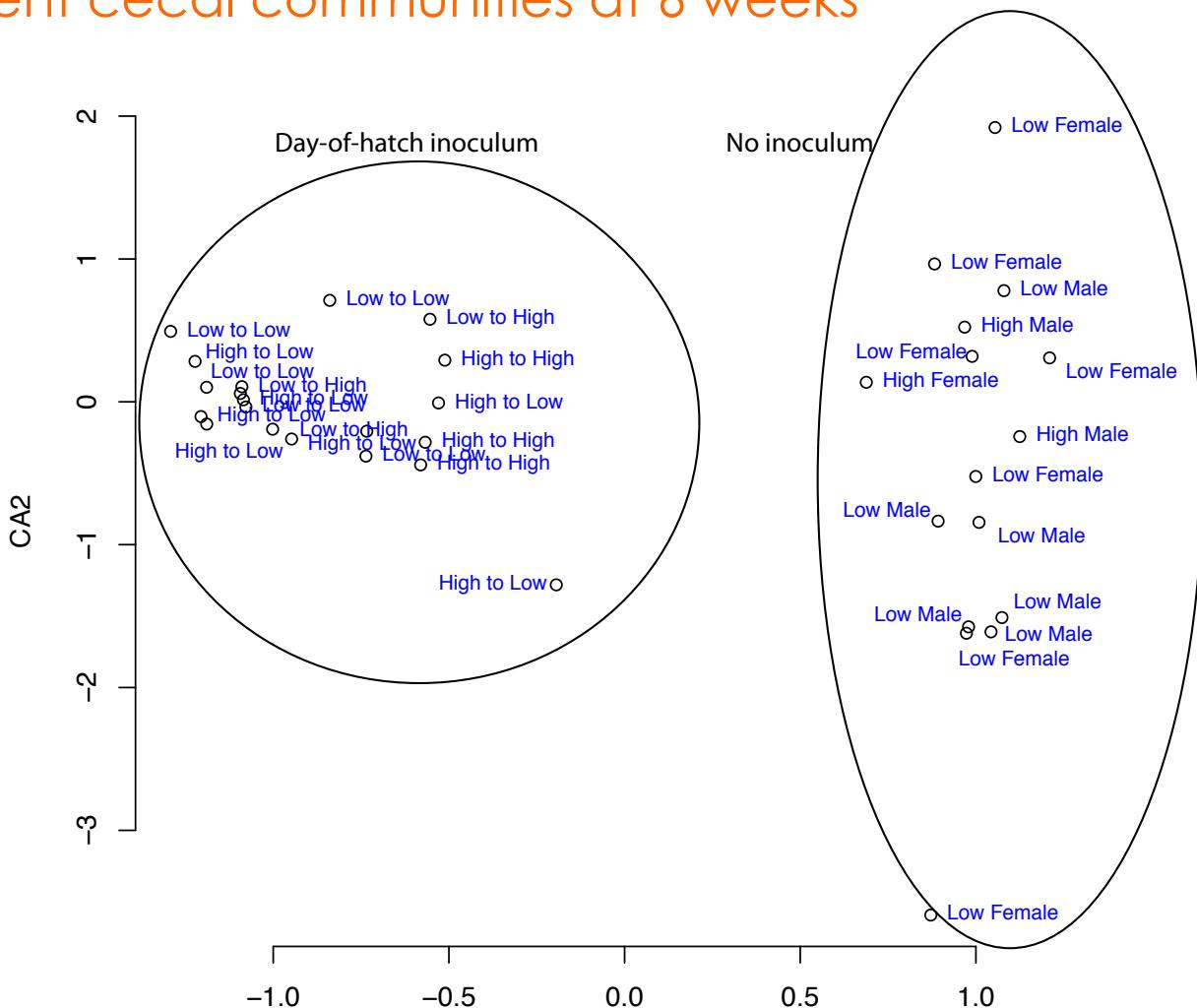


Inoculum improves FCR



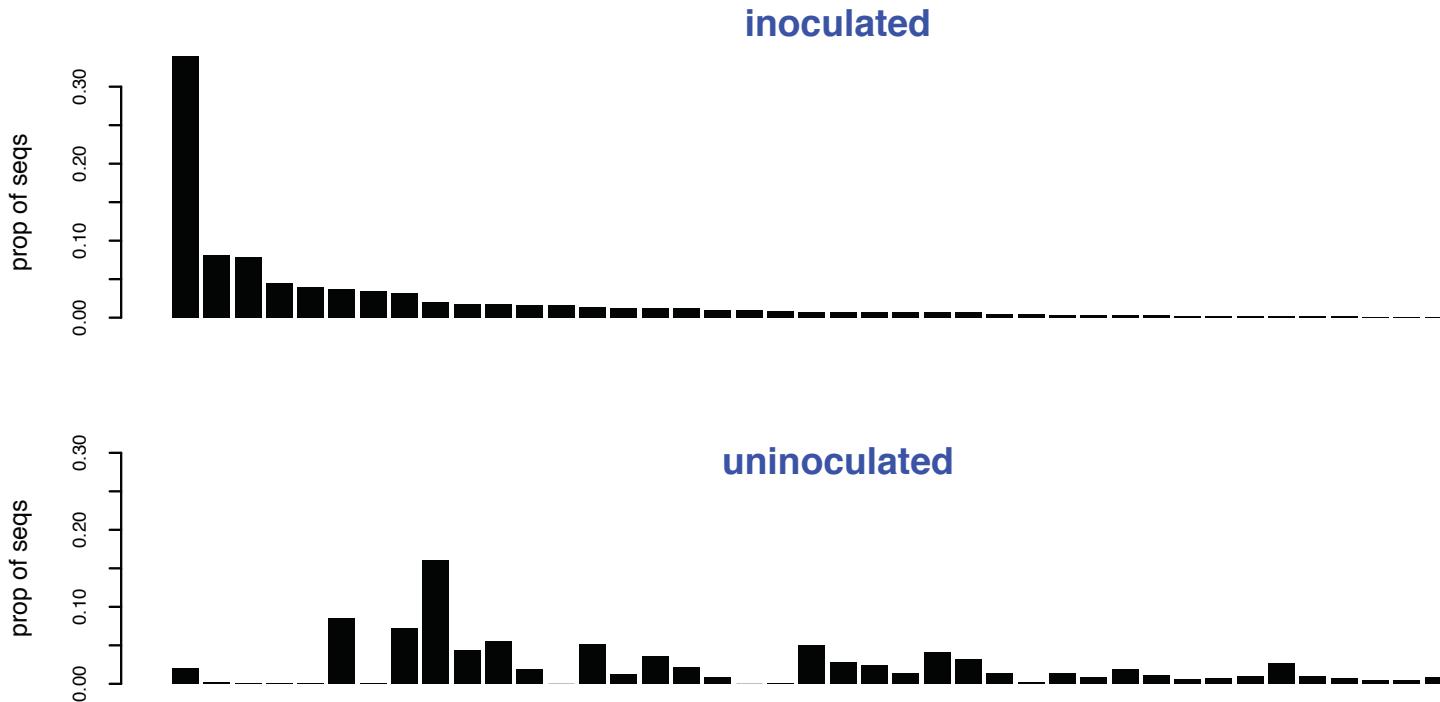
Can we manage the microbiota?

- Different cecal communities at 6 weeks

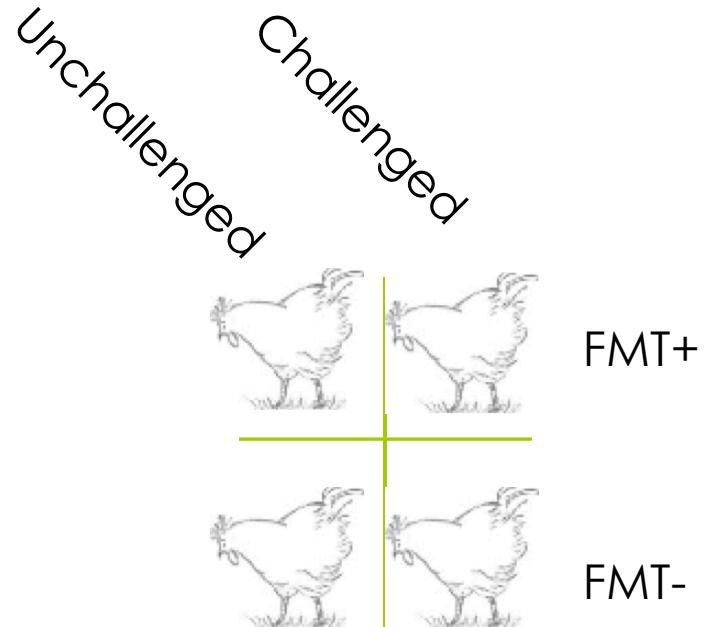
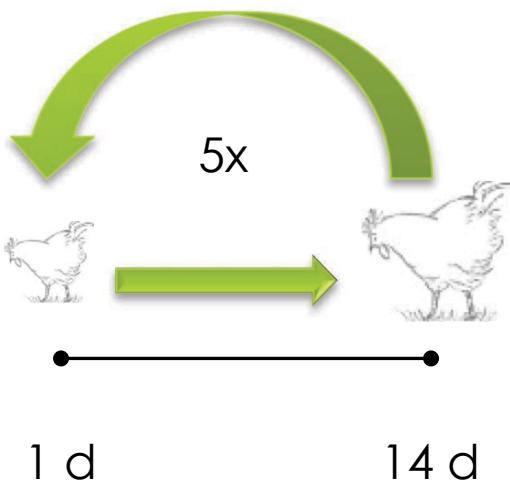


Strain Identification

- Different cecal communities at 6 weeks

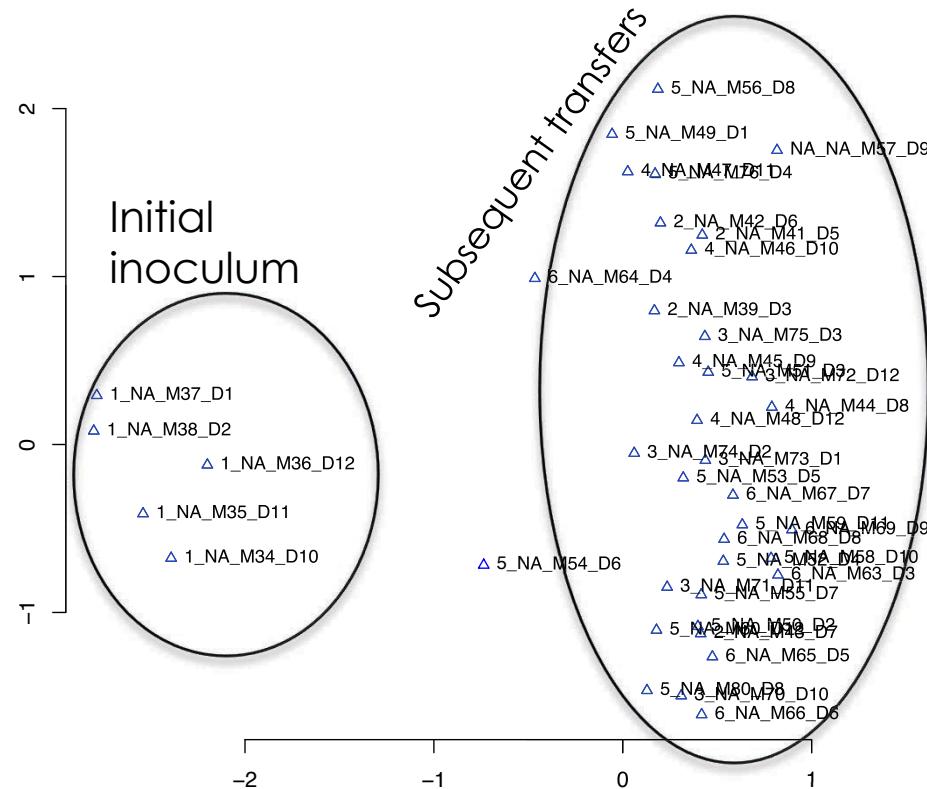
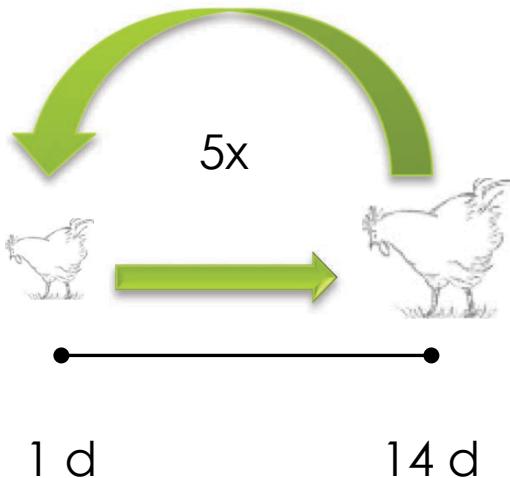


Can FMT 'lay the golden egg'?

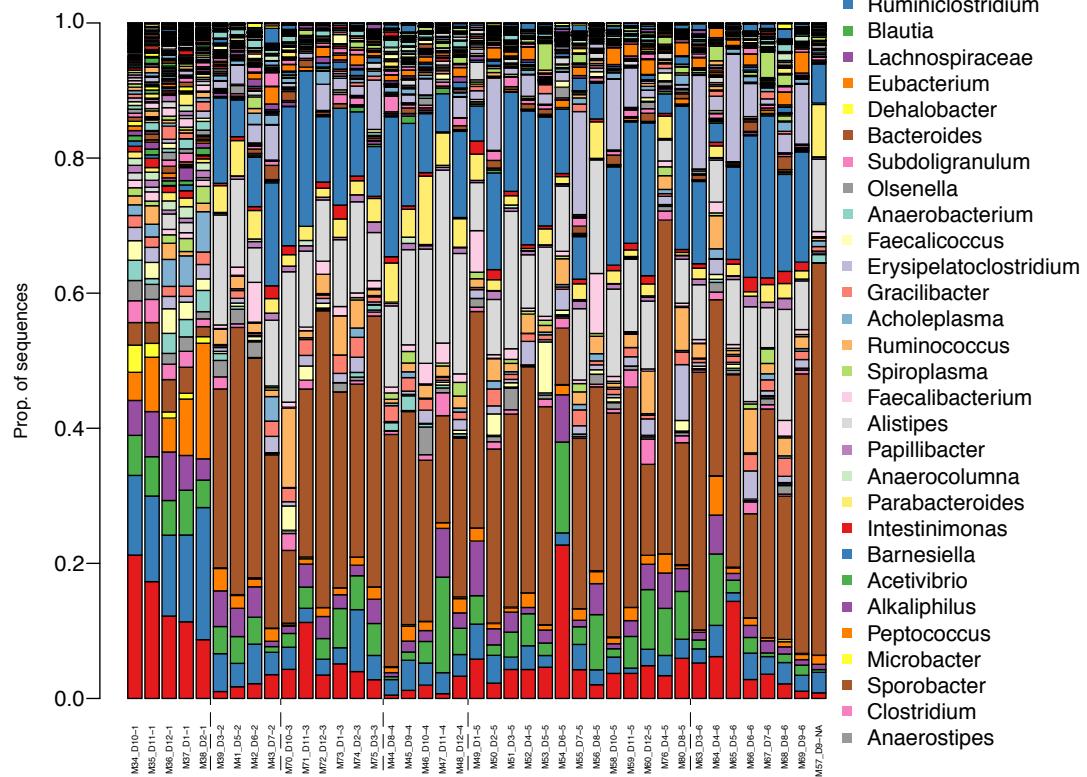
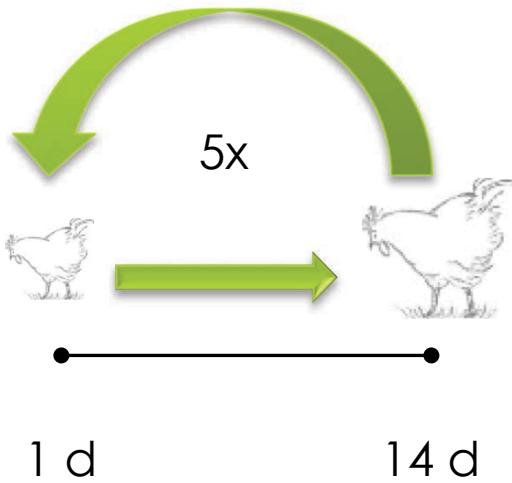


- Community composition
- Metagenome
- Host Transcriptome
- Immune response (M1 vs. M2)
- AME
- Pathogen load
- Growth

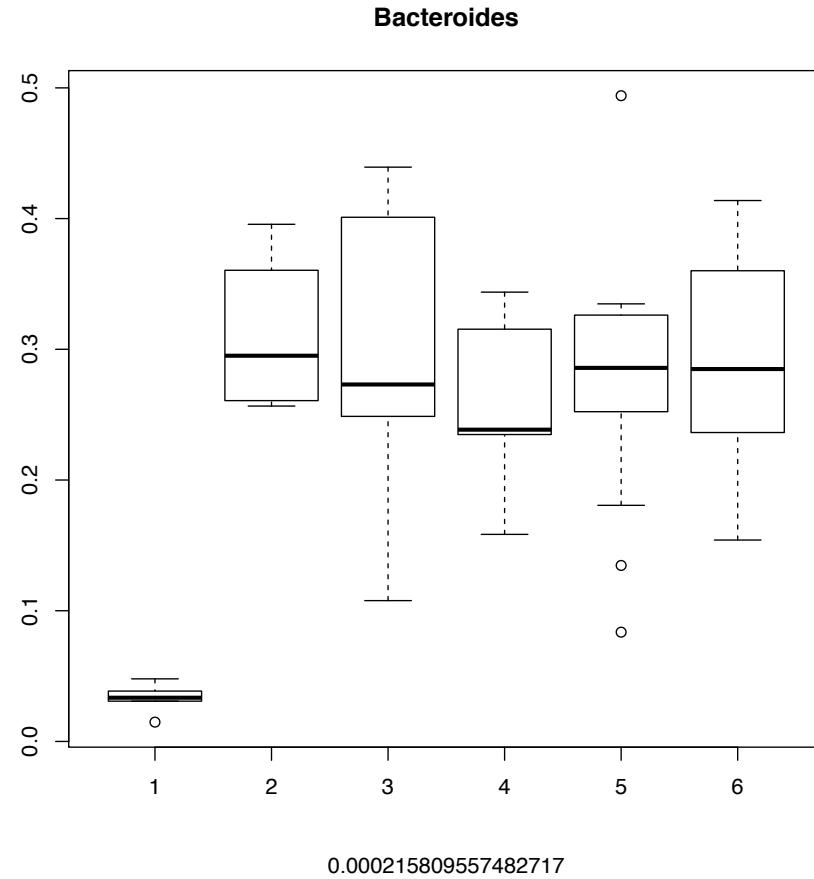
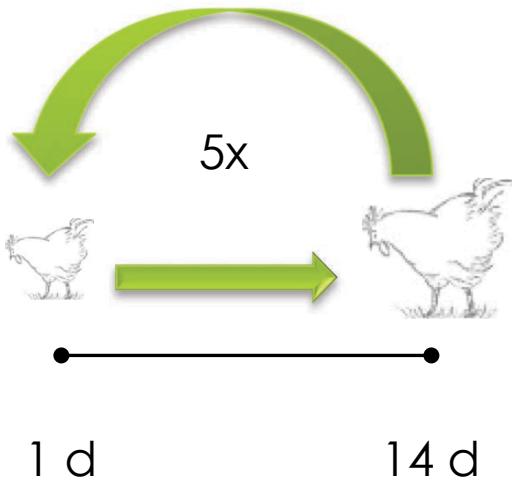
Can FMT 'lay the golden egg'?



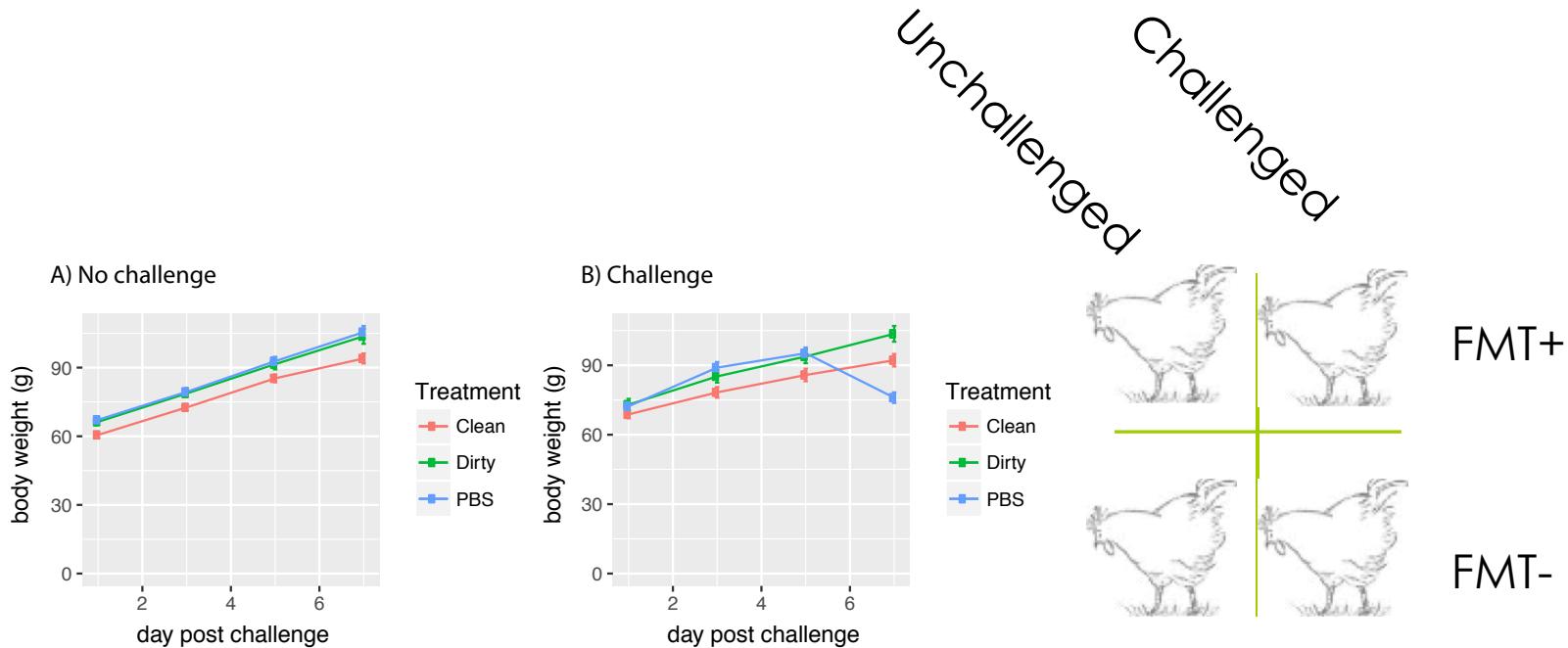
Can FMT 'lay the golden egg'?



Can FMT 'lay the golden egg'?

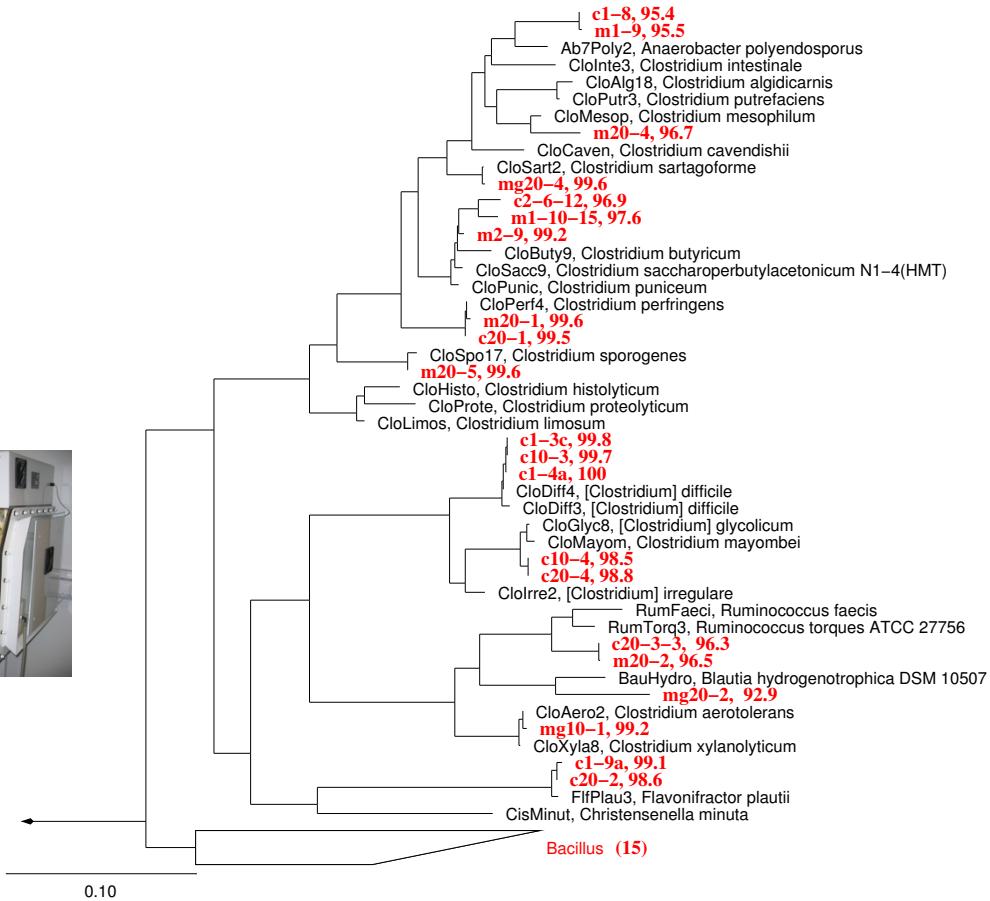


Can FMT 'lay the golden egg'?



Strain Identification

Microbiome datasets can guide targeted cultivation of potentially novel strains from chicken G-I tract:



Novel strains shown in red genome sequenced

Strain characterization

Characterization of new isolates

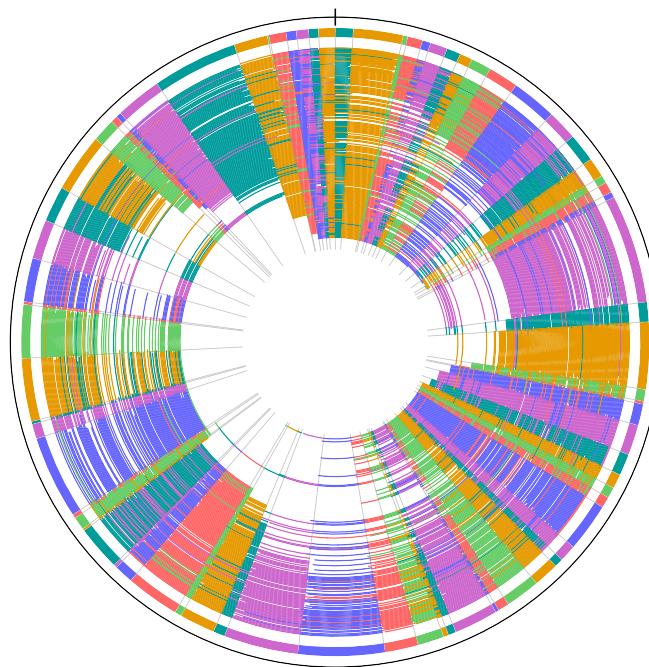
- *Genome Sequencing*
- *Optical Mapping*

Outer ring = consensus of cut sites

Fragment sizes and order reflect true arrangement in genome

Provides true barcode of genome

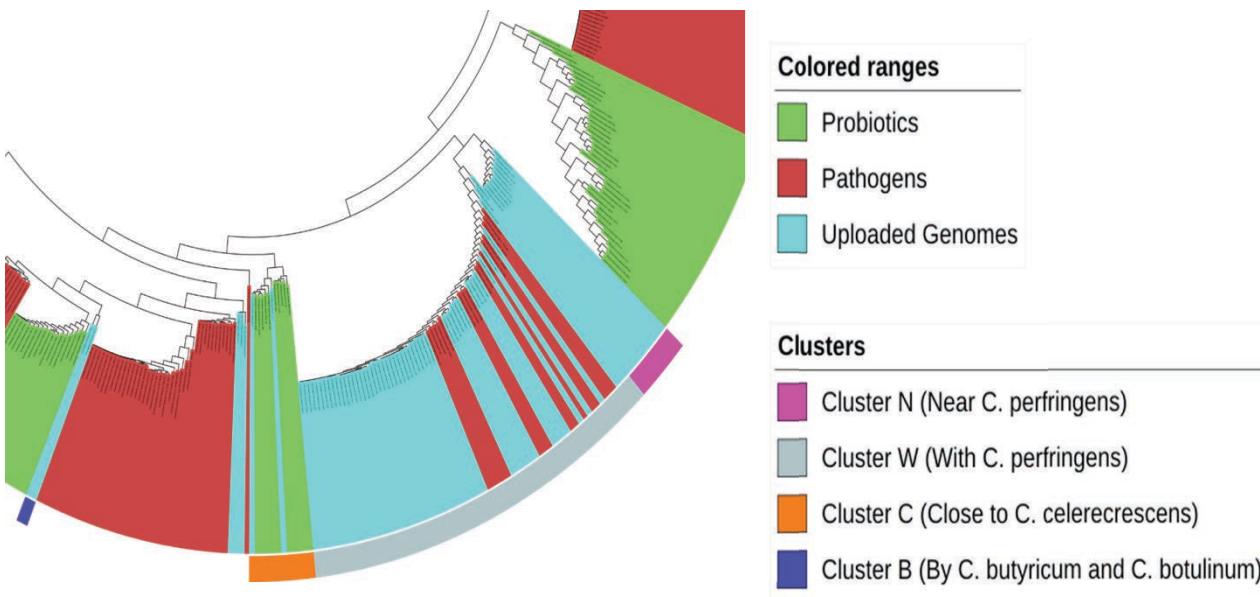
Provides template for assembly of genome sequence reads

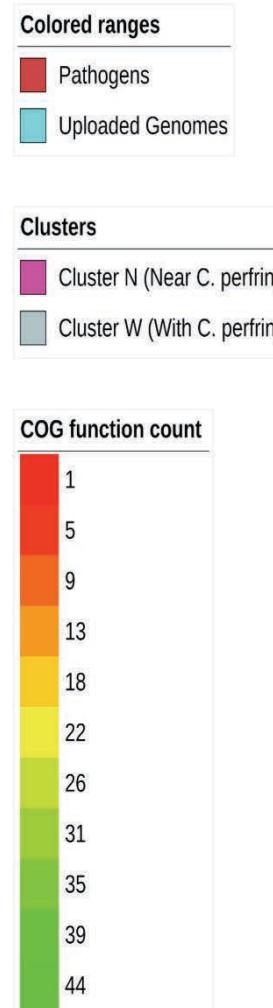
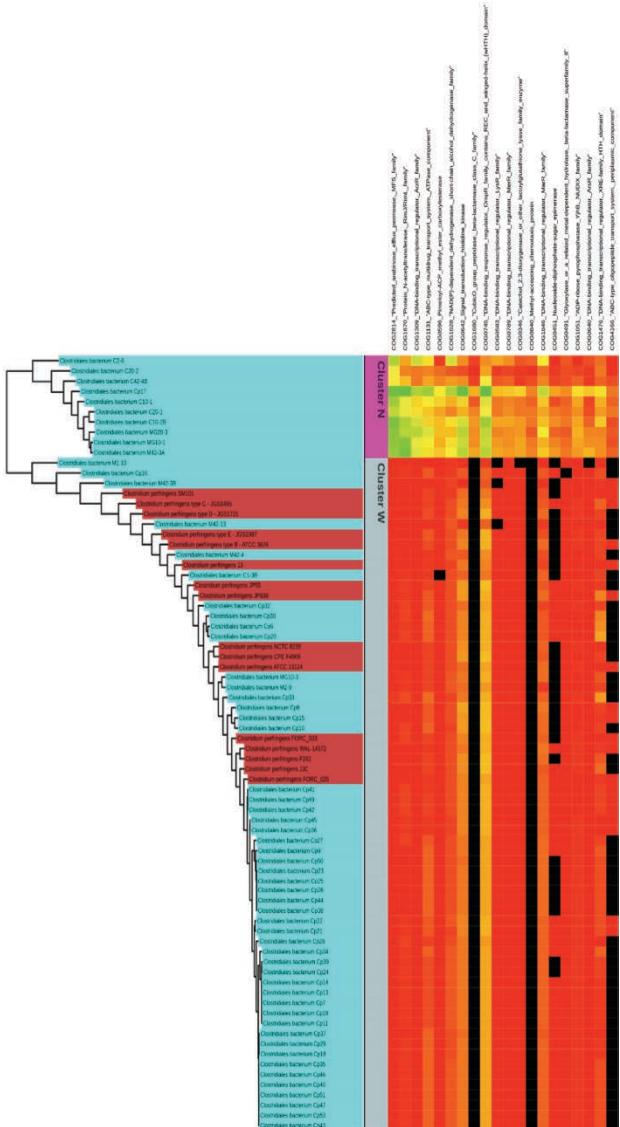


Strain characterization

Characterization of new isolates

- Genome Sequencing
- Optical Mapping





Identifying Differentially Abundant COGs between Cluster N and Cluster W)

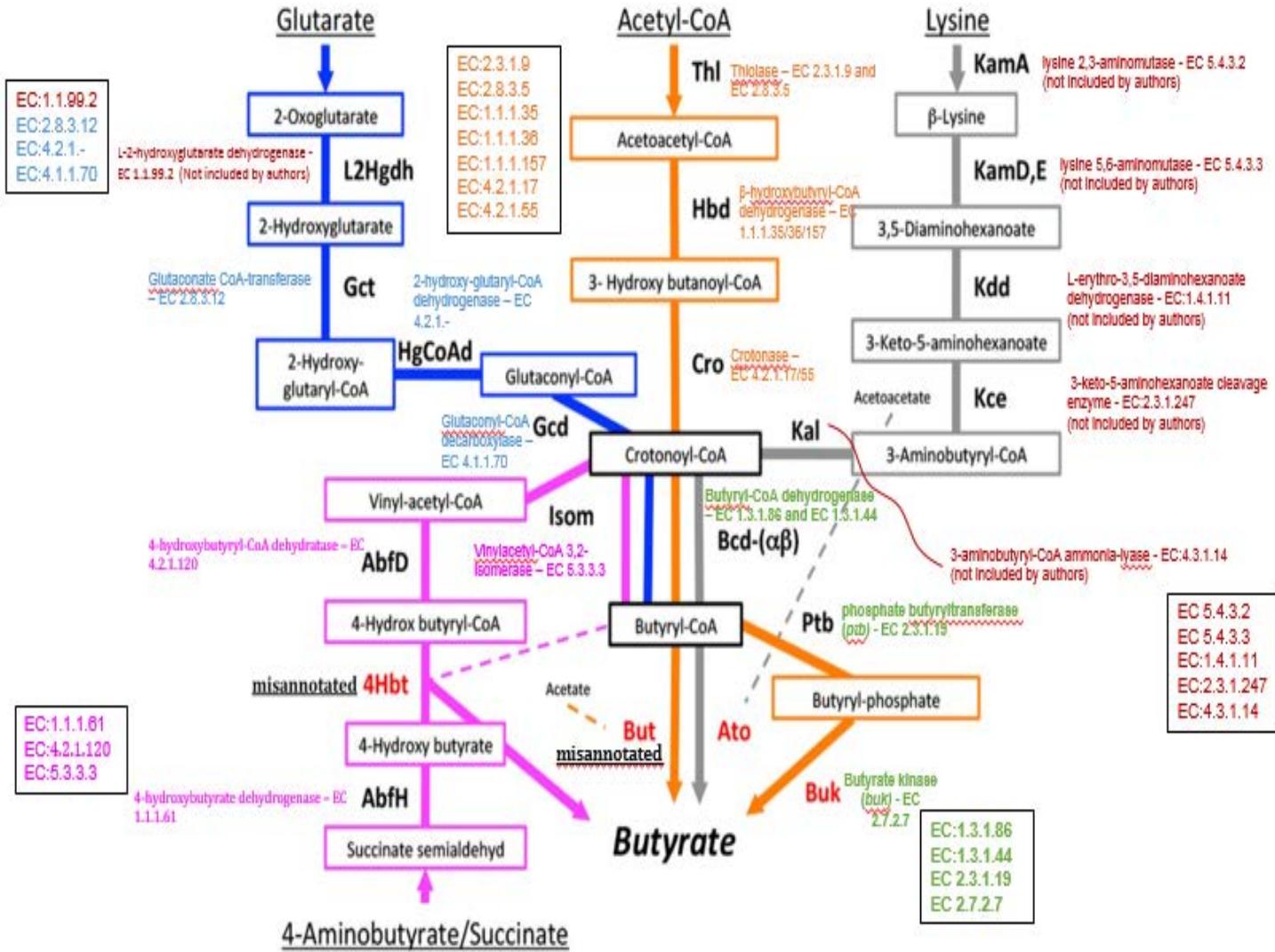
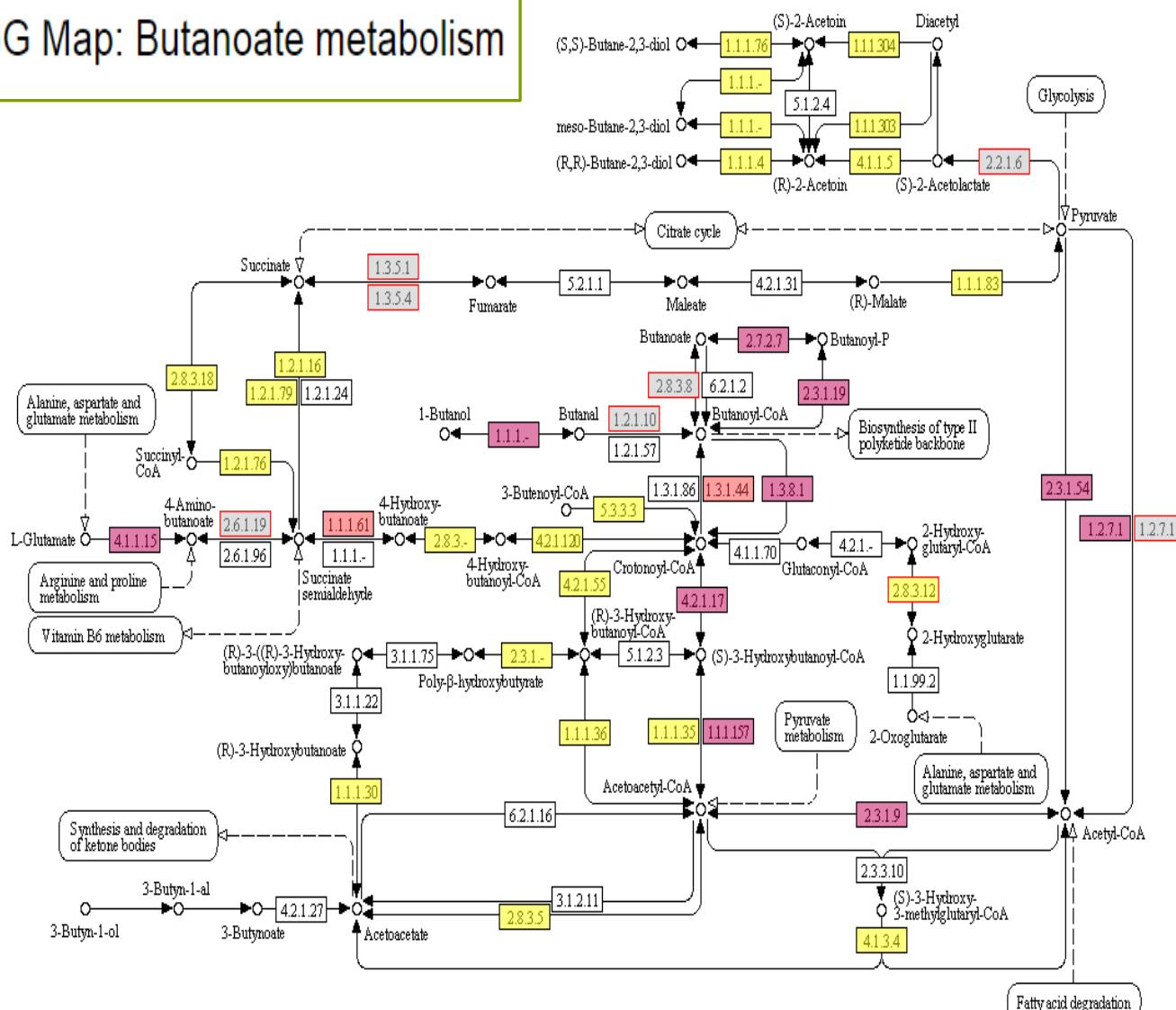


Image adapted from: doi: 10.1128/mBio.00889-14 22 April 2014 mBio vol. 5 no. 2 e00889-14

KEGG Map: Butanoate metabolism

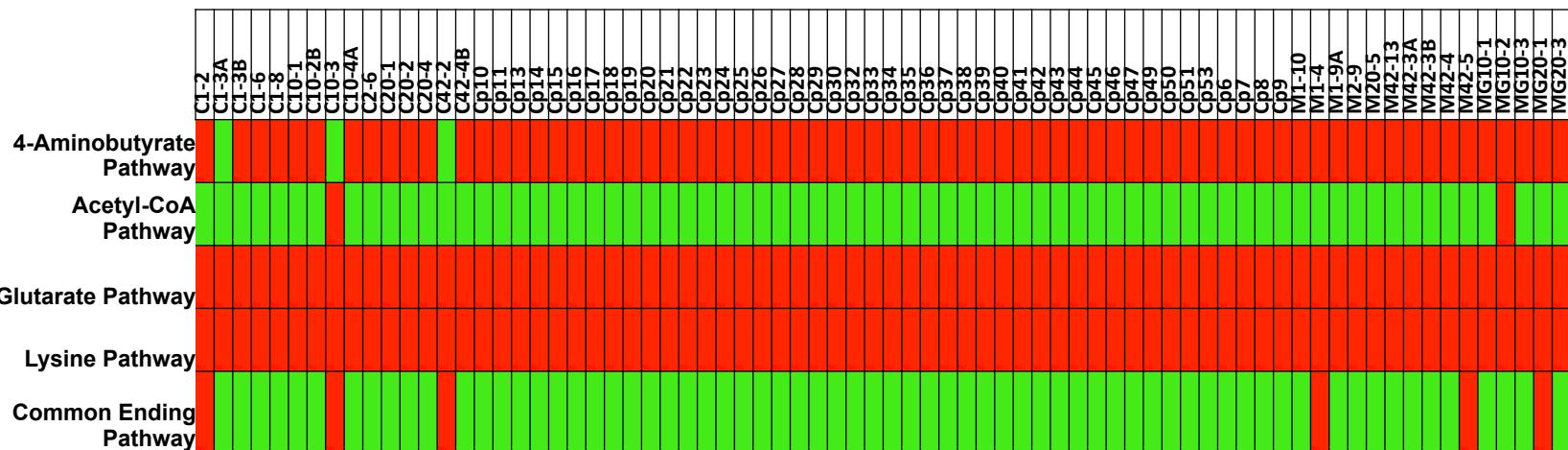


■ Genes found in all selected genomes

■ ■ ■ Genes found in some of the selected genomes [for up to 25% ■ >25% ■ >50% ■ >75% ■]

■ A red border indicates that more than one function maps to this reaction.

Butyrate Synthesis Pathways of new strains



Presence of Pathway

Absence of Pathway

***Color Represents Presence or Absence of Pathway based on KEGG EC numbers found in Genome (allowing for 1 missing enzyme)**

A Majority of Uploaded Genomes can synthesize Butyrate from Acetyl-CoA according to their functional profile

CONCLUSIONS

- ❑ Managing the microbiota can improve performance and food safety
- ❑ Next-generation sequencing is a transformative tool
- ❑ Proper management starts with understanding natural communities
- ❑ ‘Efficacy-first’ inversion of traditional strain-centric approach may be valuable

Acknowledgements

USDA ARS Athens

- Bruce Seal
- Johnna Garrish
- Mark Berrang
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- Eric Line
- Jeff Buhr
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- Rick Meinersmann
- Cesar Morales
- Susan Brooks
- Jessica Johnson
- Raja Chalghoumi (Fulbright fellow)

SEPRL

- Laszlo Zak
- Michael Day

UGA

- Sammy Aggrey
- Steve Collett
- Eldin Talundzic

ARS College Station

- Mike Kogut

Western University

- Dr. Dominique Griffon
- Dr. Santiago Aguilar
- Ella Richardson
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- Franck Carbonero
- Rex Gaskins

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- Vladimir Loparev
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