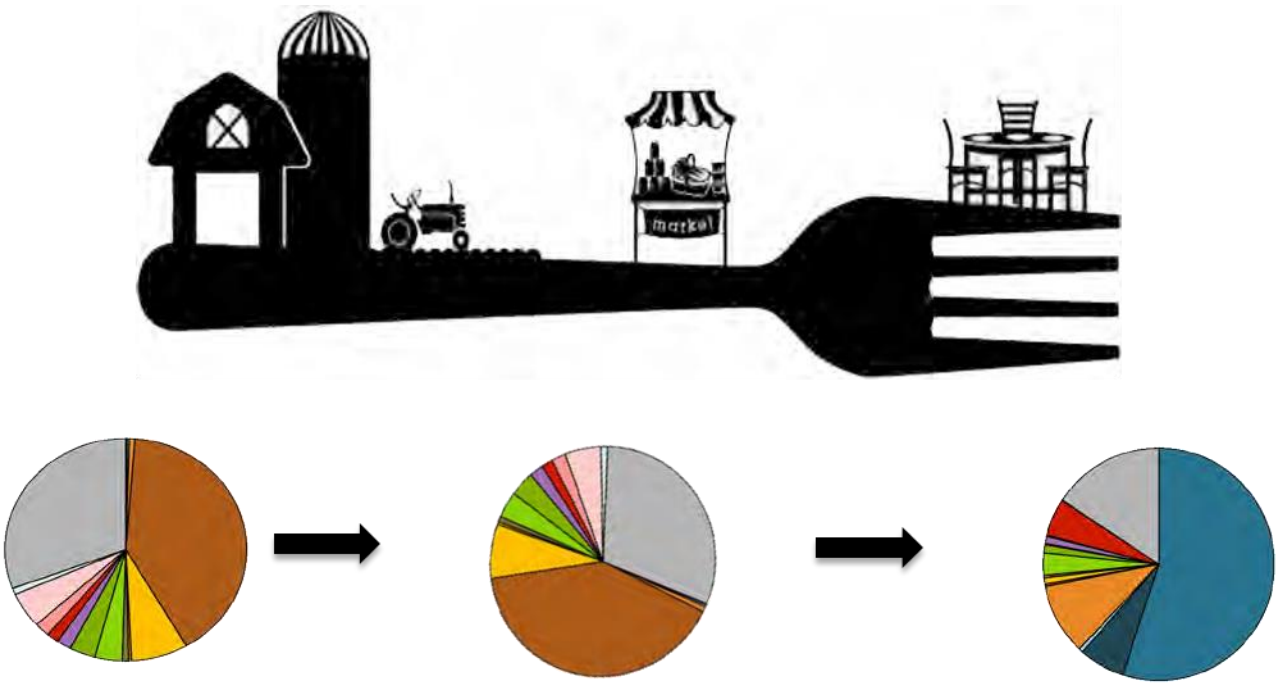
MetagenomeTRAKR:

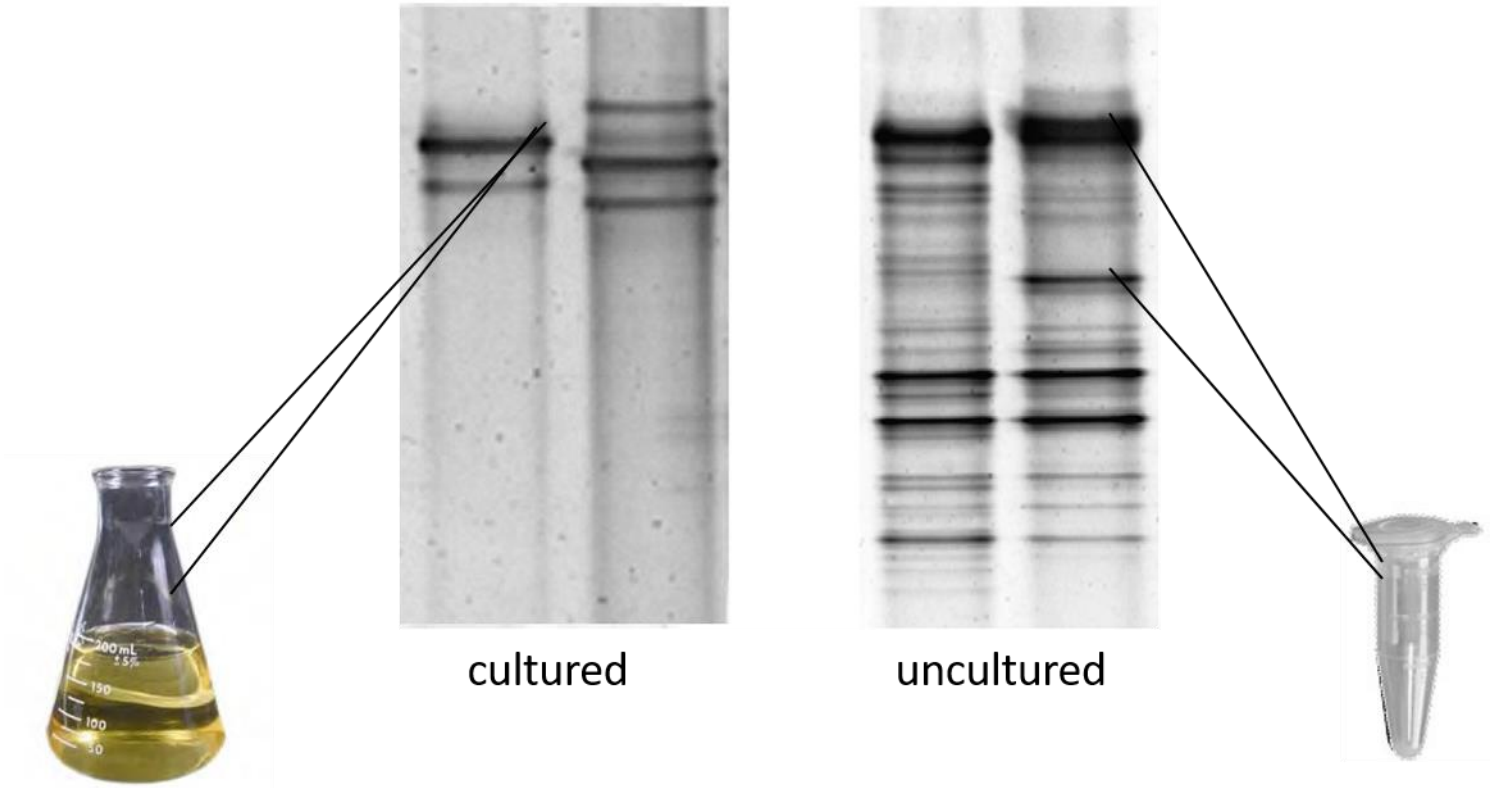
Using metagenomic approaches for pathogen detection and description of food and agricultural microbiomes



Farm to Fork Continuum – CFSAN Microbiome research

Metagenomics

Culture dependent vs. Culture Independent



DGGE (Denaturing Gradient Gel Electrophoresis) of 16S from the surfaces of apple trees

The ecology of pathogenicity and the ecology of nutrition

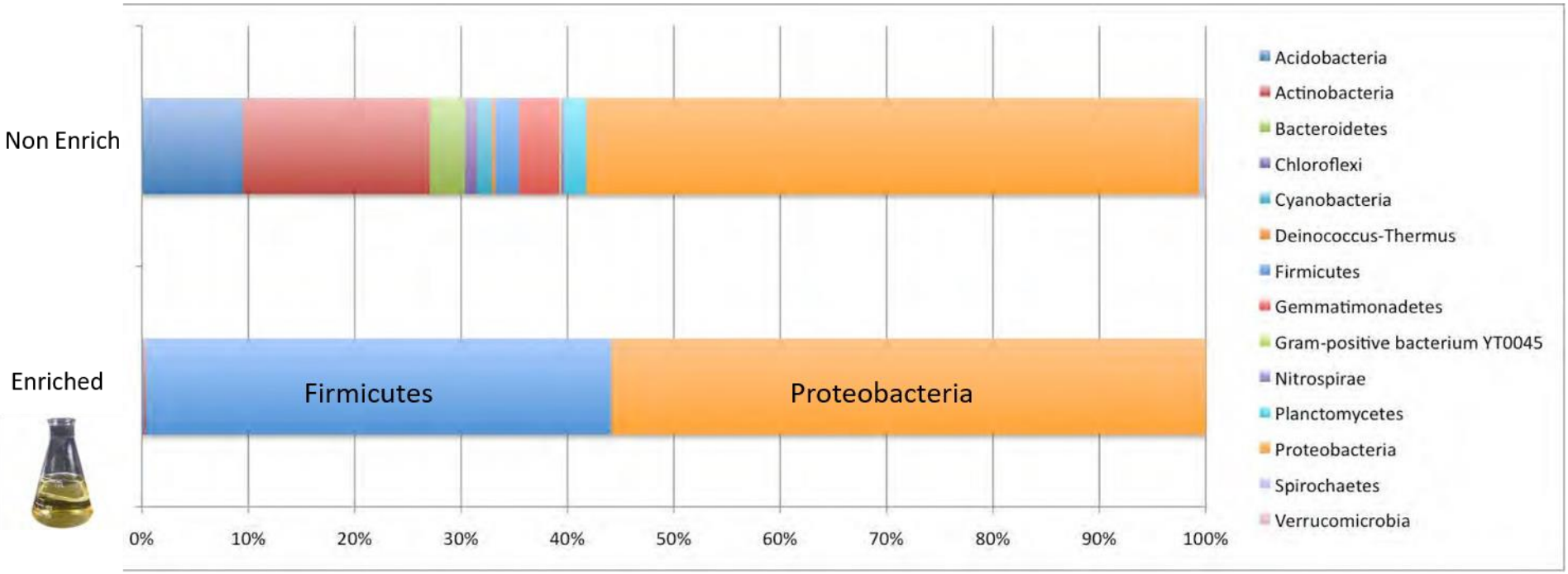
Applied MetagenomeTRAKR

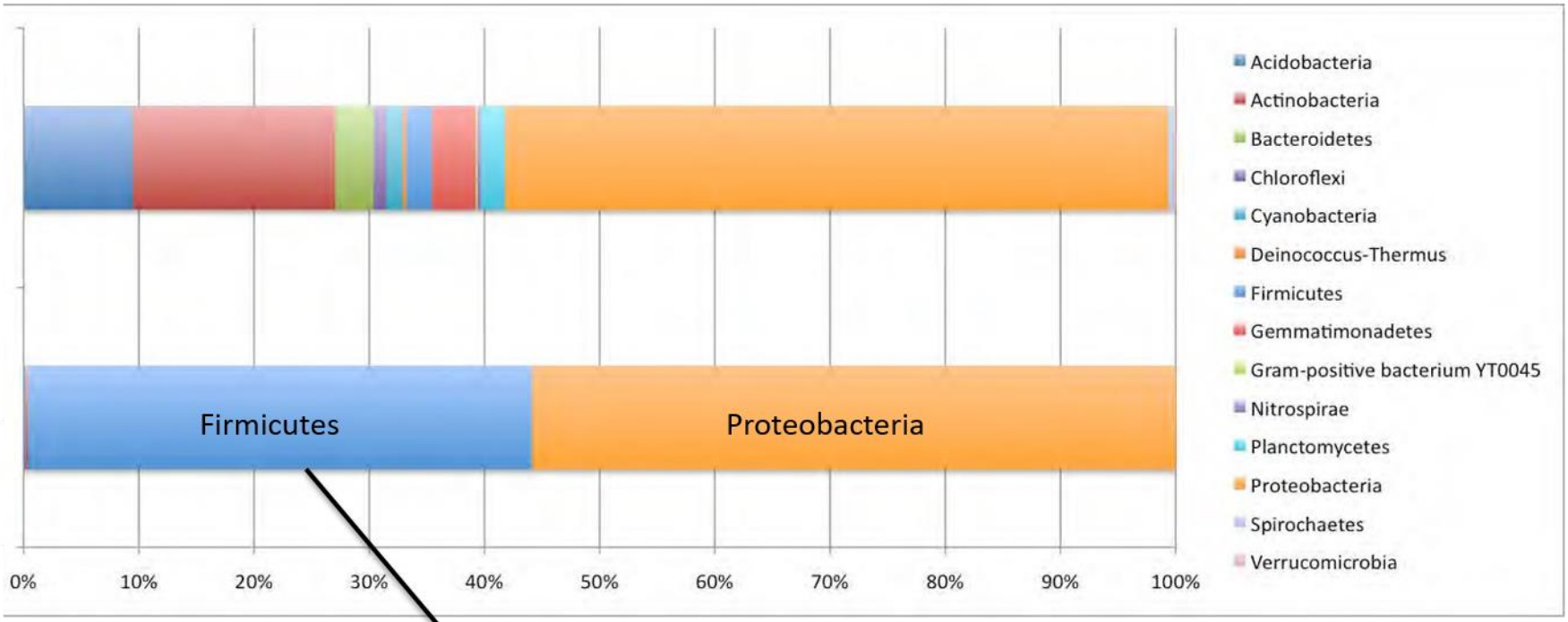
Quasi-metagenomics

Methods Development

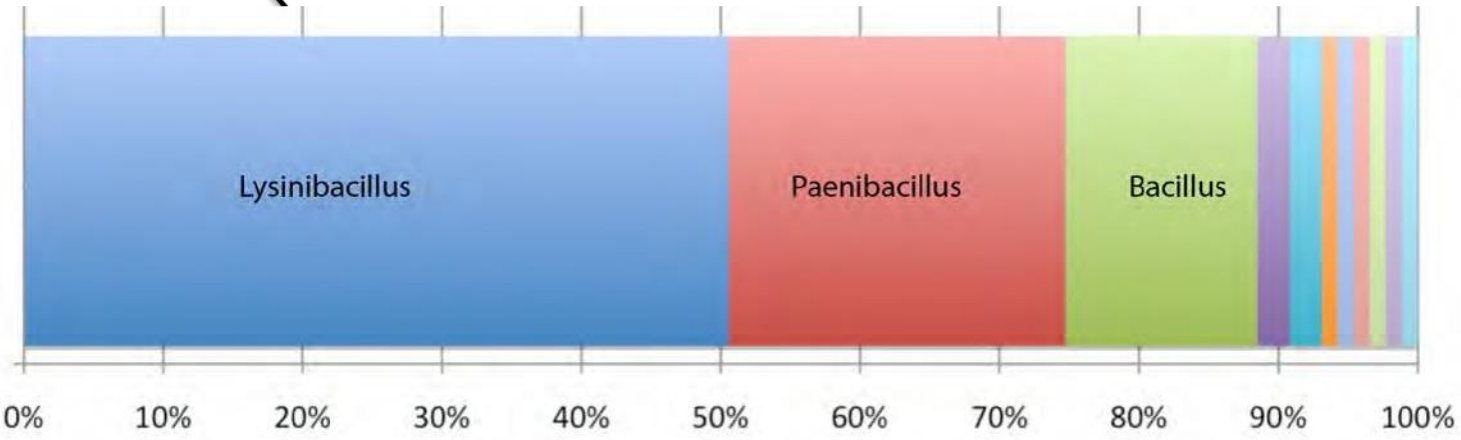
Enrichment Methods: what co-enriches?

target organism: *Salmonella*



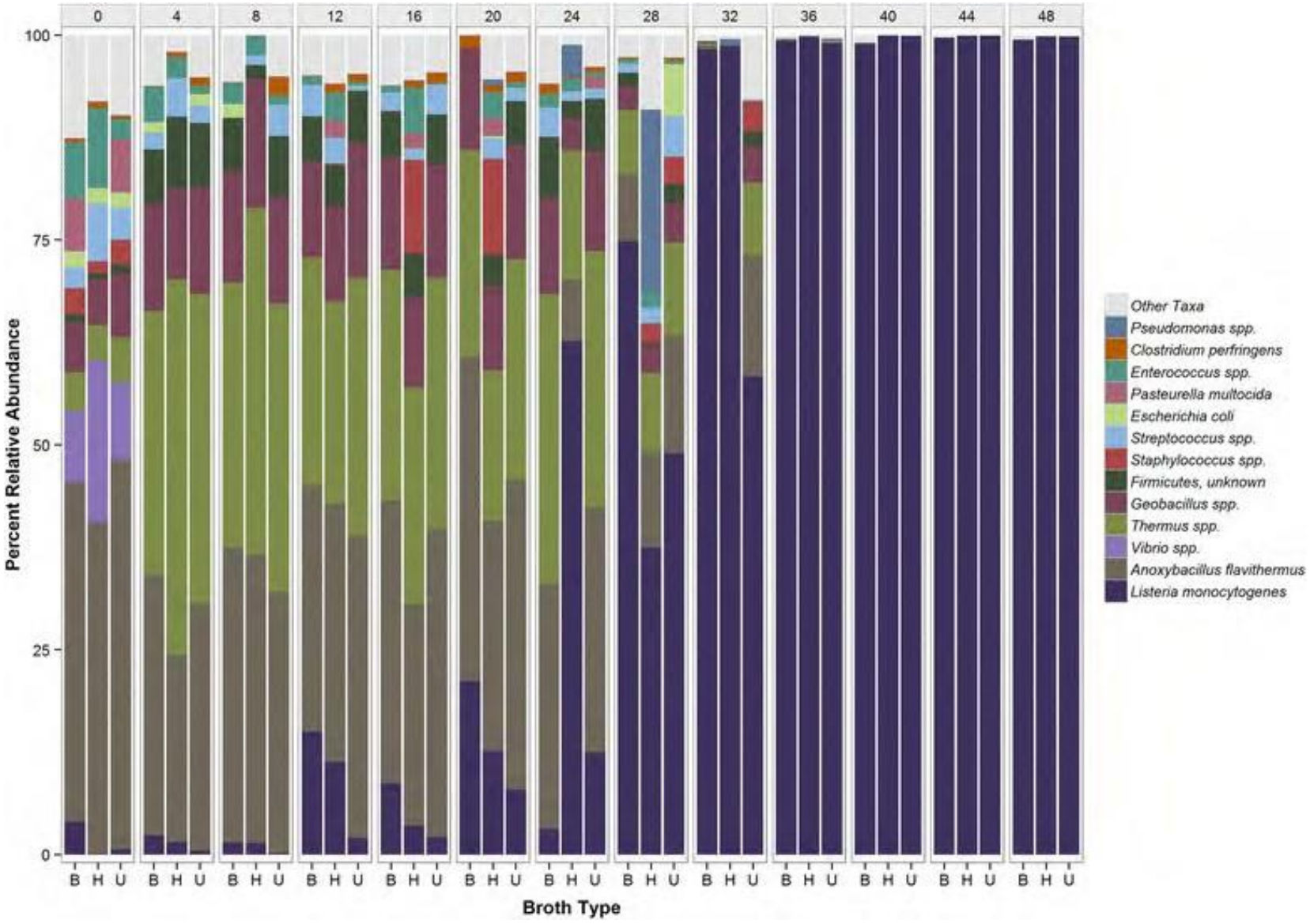


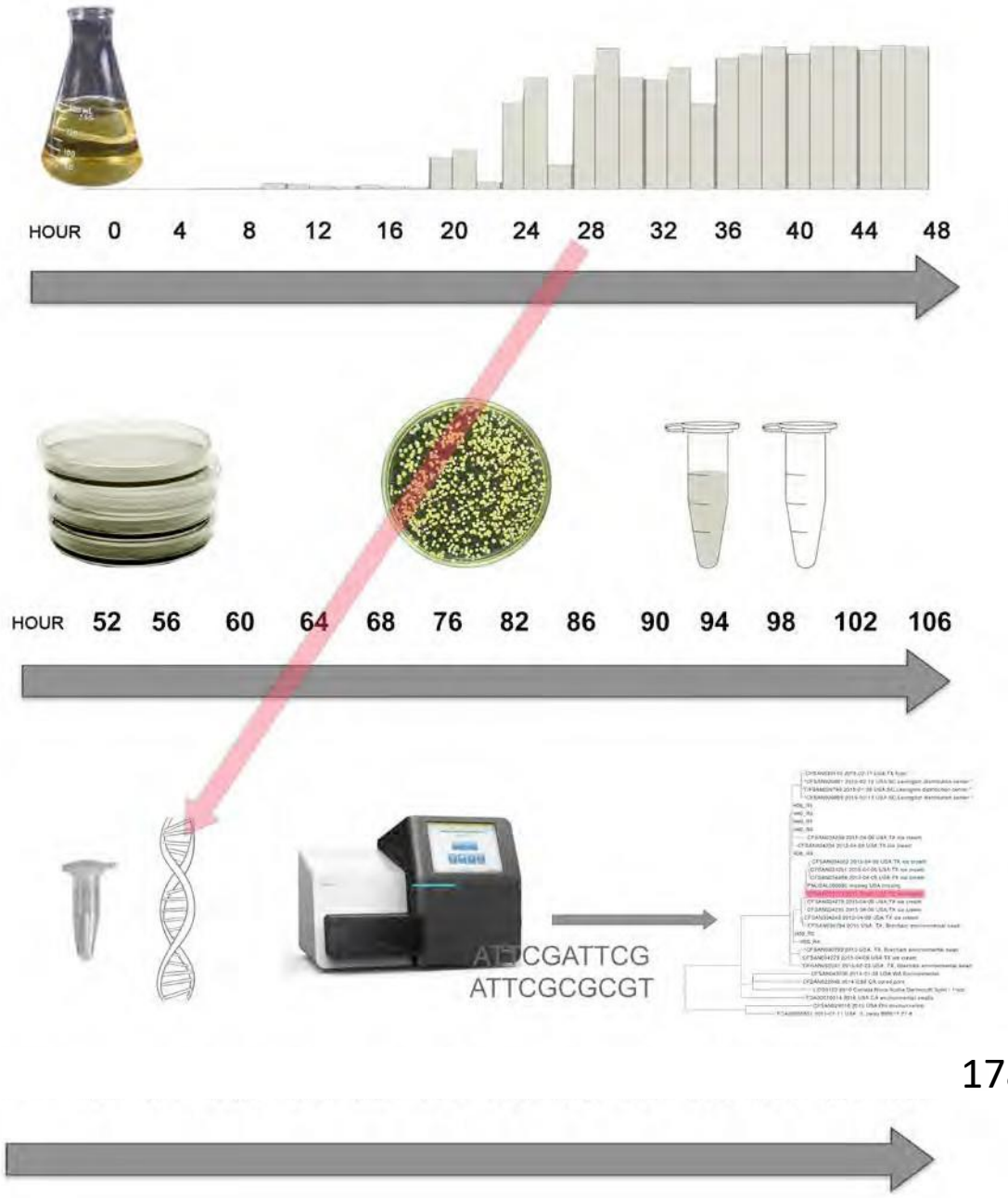
Enriched Firmicutes



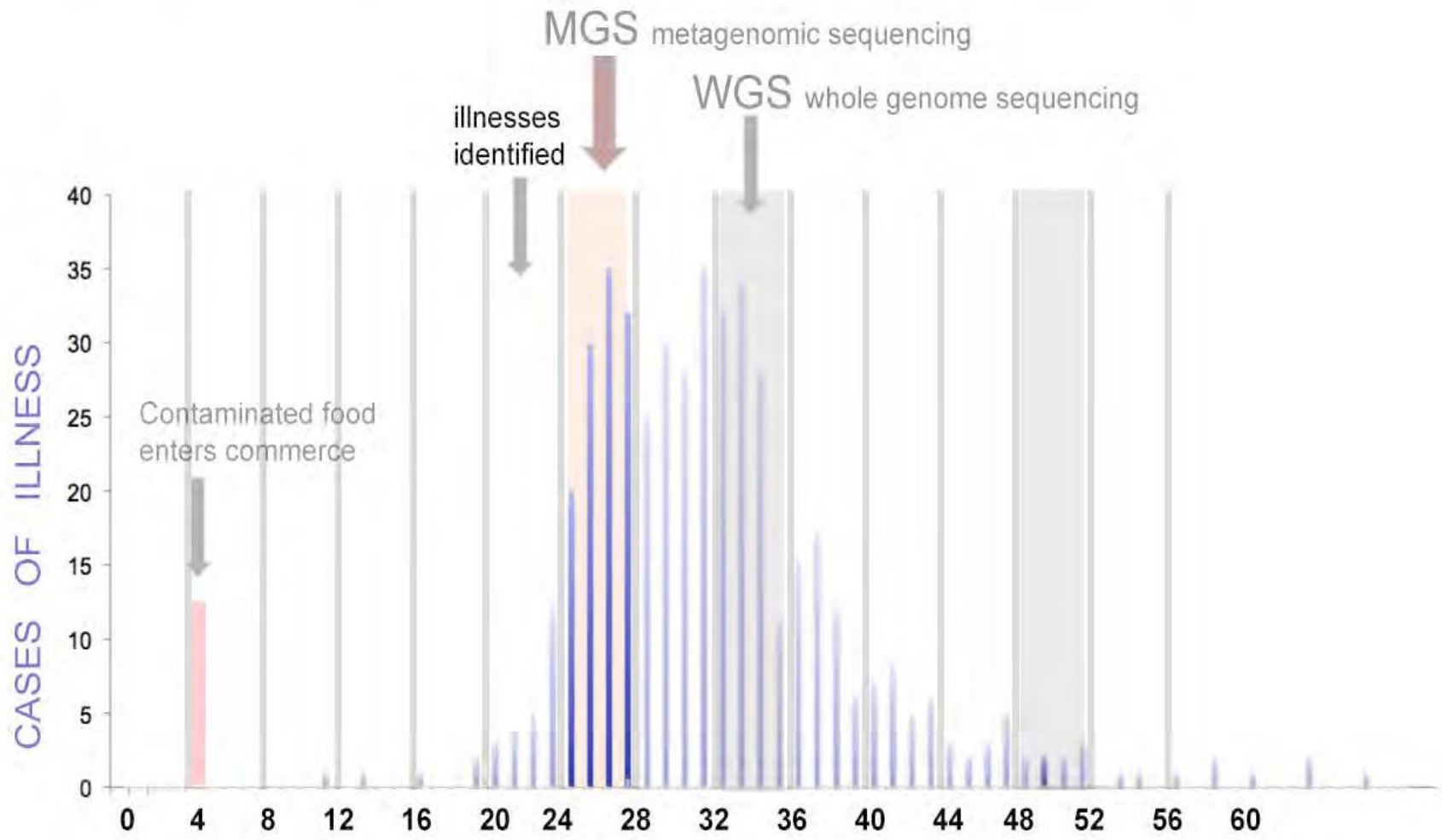
Bacillus fusiformis and *Bacillus sphaericus*

Listeria monocytogenes in Ice cream – 48 hours of enrichment

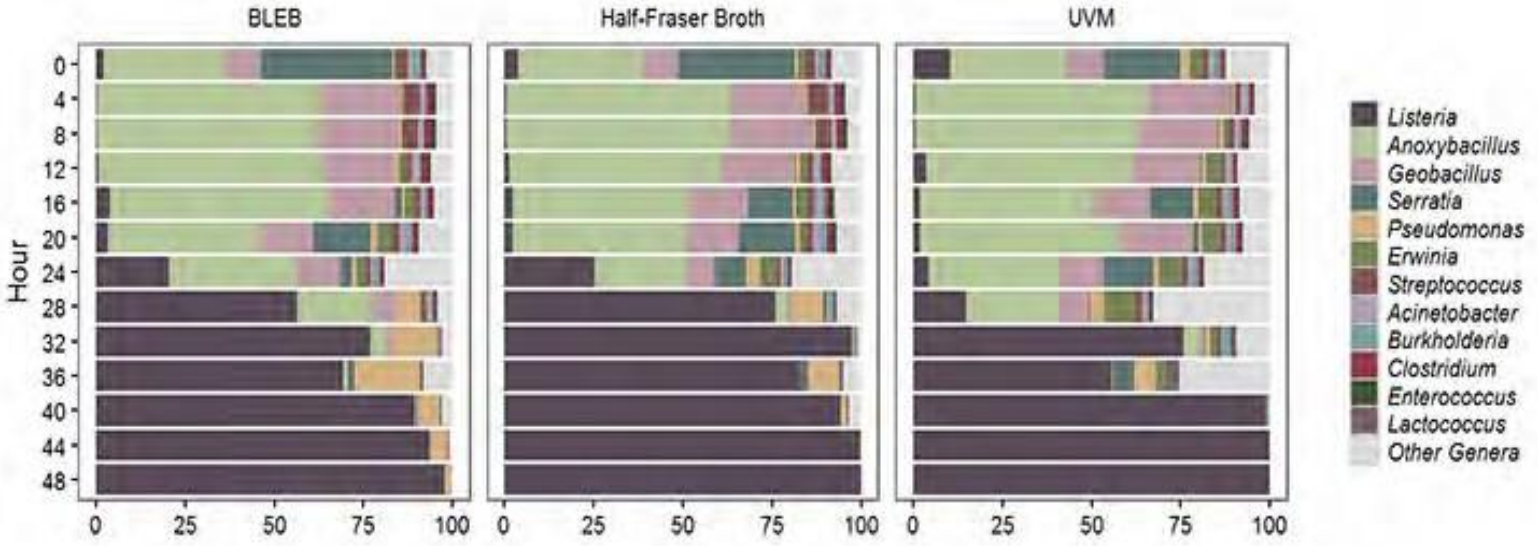



HOUR 0 4 8 12 16 20 24 28 32 36 40 44 48
HOUR 52 56 60 64 68 76 82 86 90 94 98 102 106
ATTCGATTCG
ATTCGCGCGT
178 H

DAYS TO SOURCE IDENTIFICATION



Ice Cream 2015 Outbreak – *Listeria monocytogenes*



FDA

Method as described in FDA Bacteriological Analytical Manual (BAM)

ISO

Method as described in International Organization of Standardization (ISO)

USDA

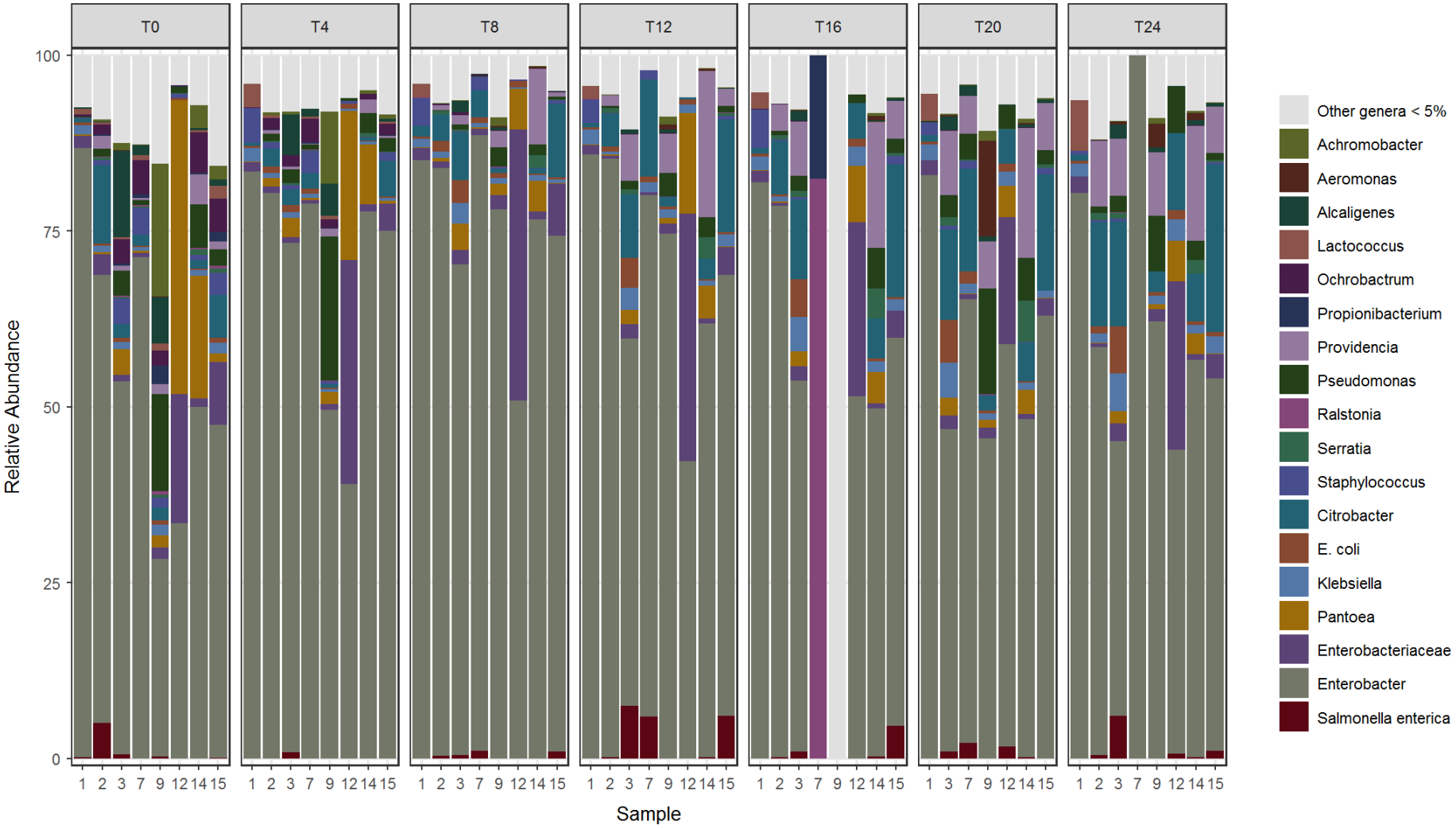
Method as described in USDA Microbiolological Guidebook (MLG)

BMC Microbiology 2016

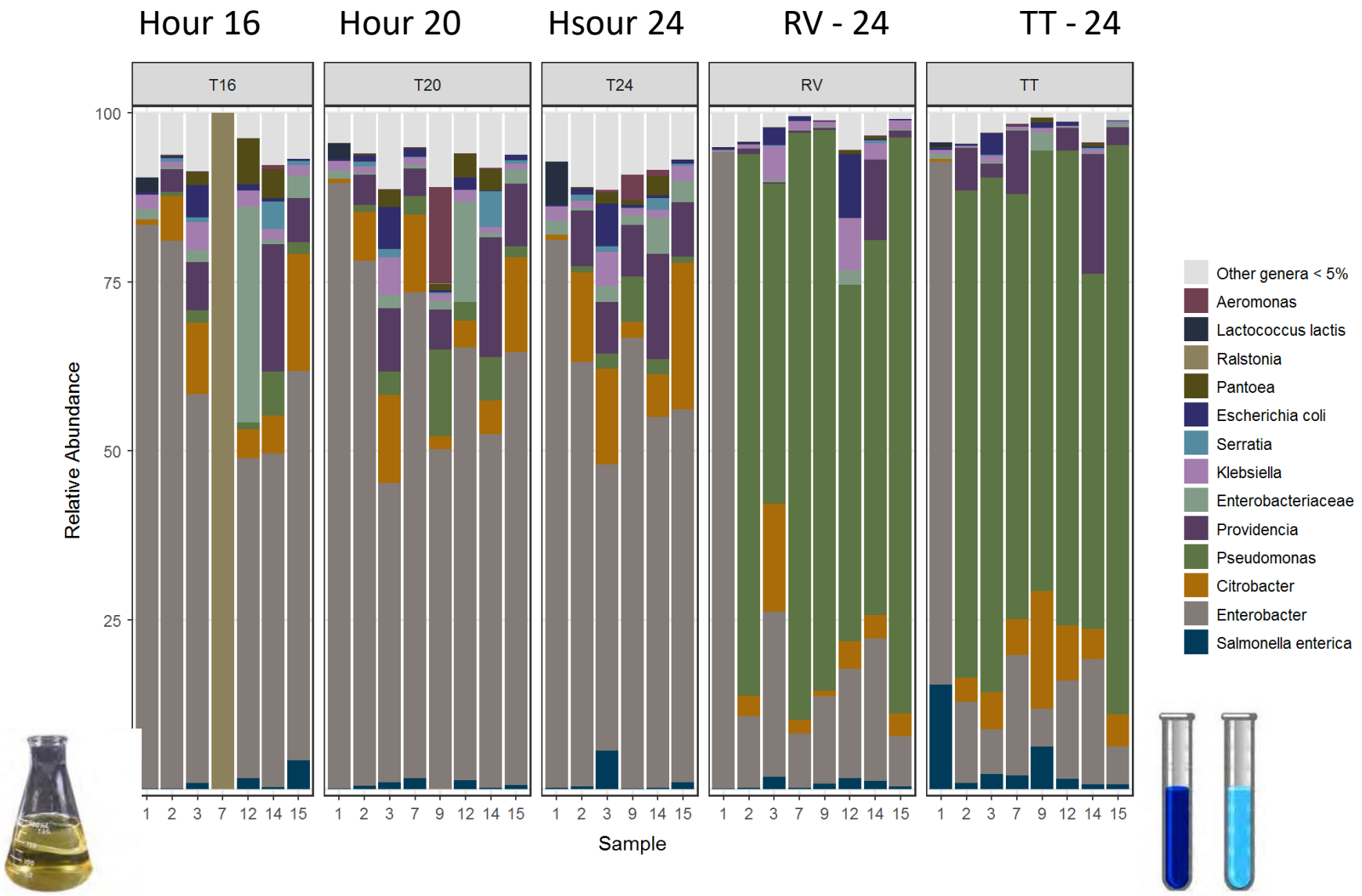
Agona, Thompson,
Kiambu, Senftenbberg

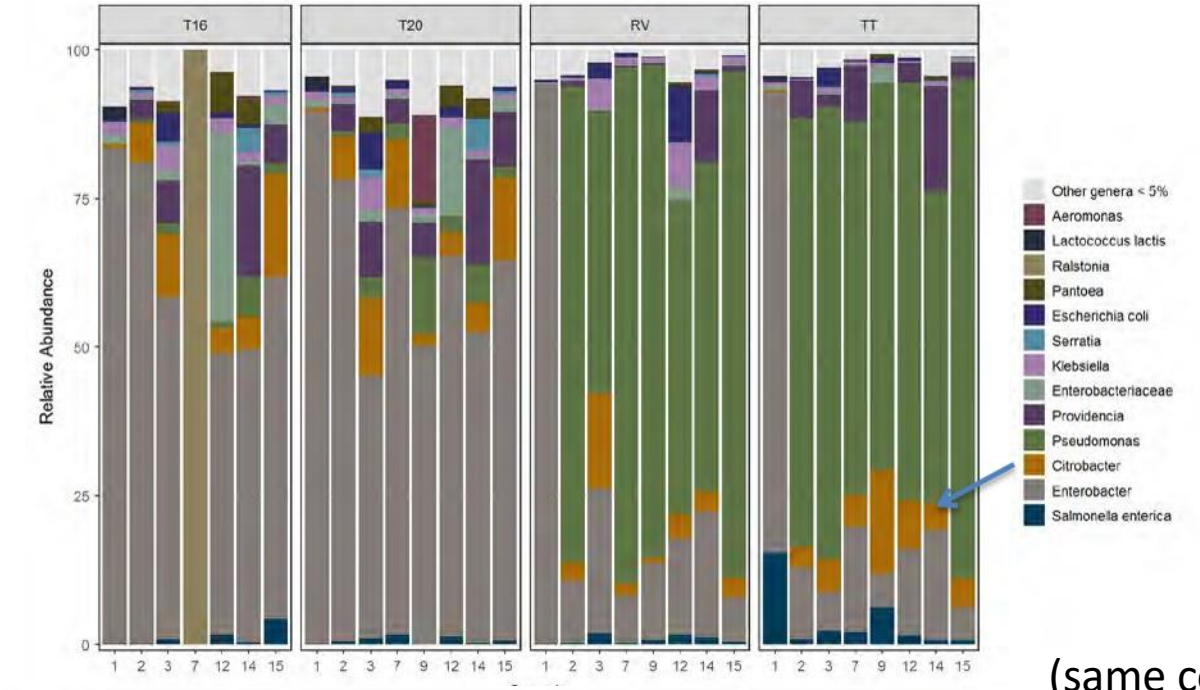
Newport,
Infantis

SUMMARY: MPBW 24 hour enrichments from Group1 papayas



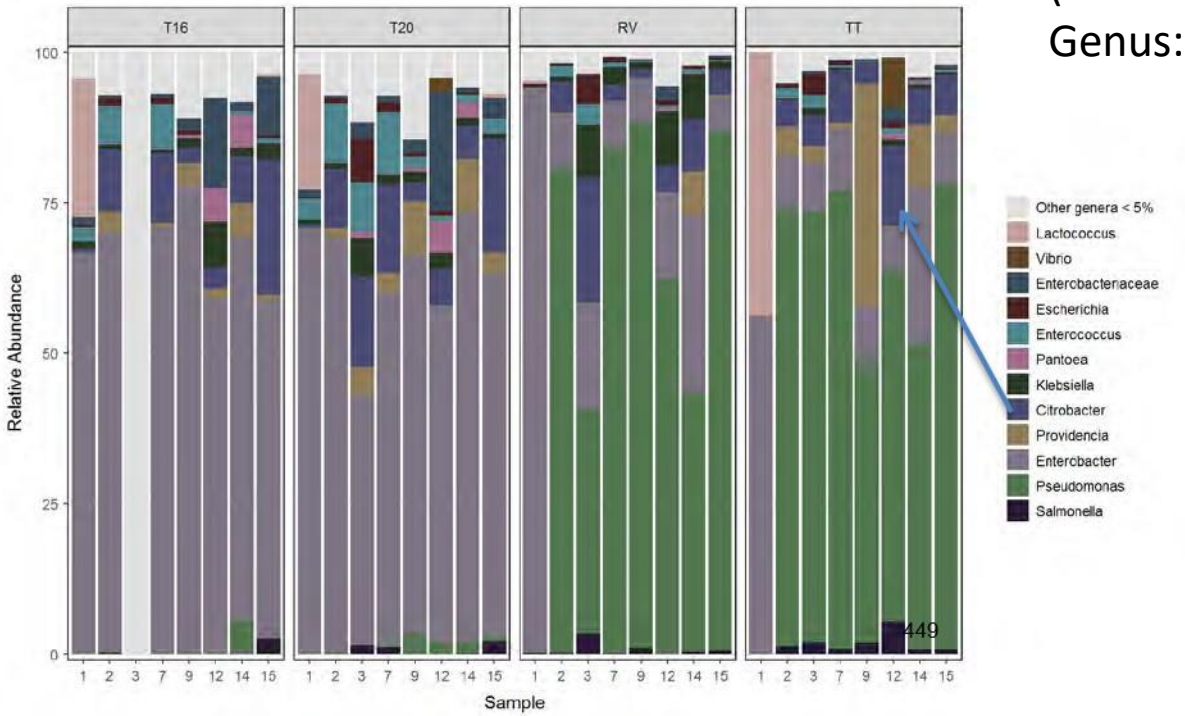
Enriching papayas for Salmonella using the BAM





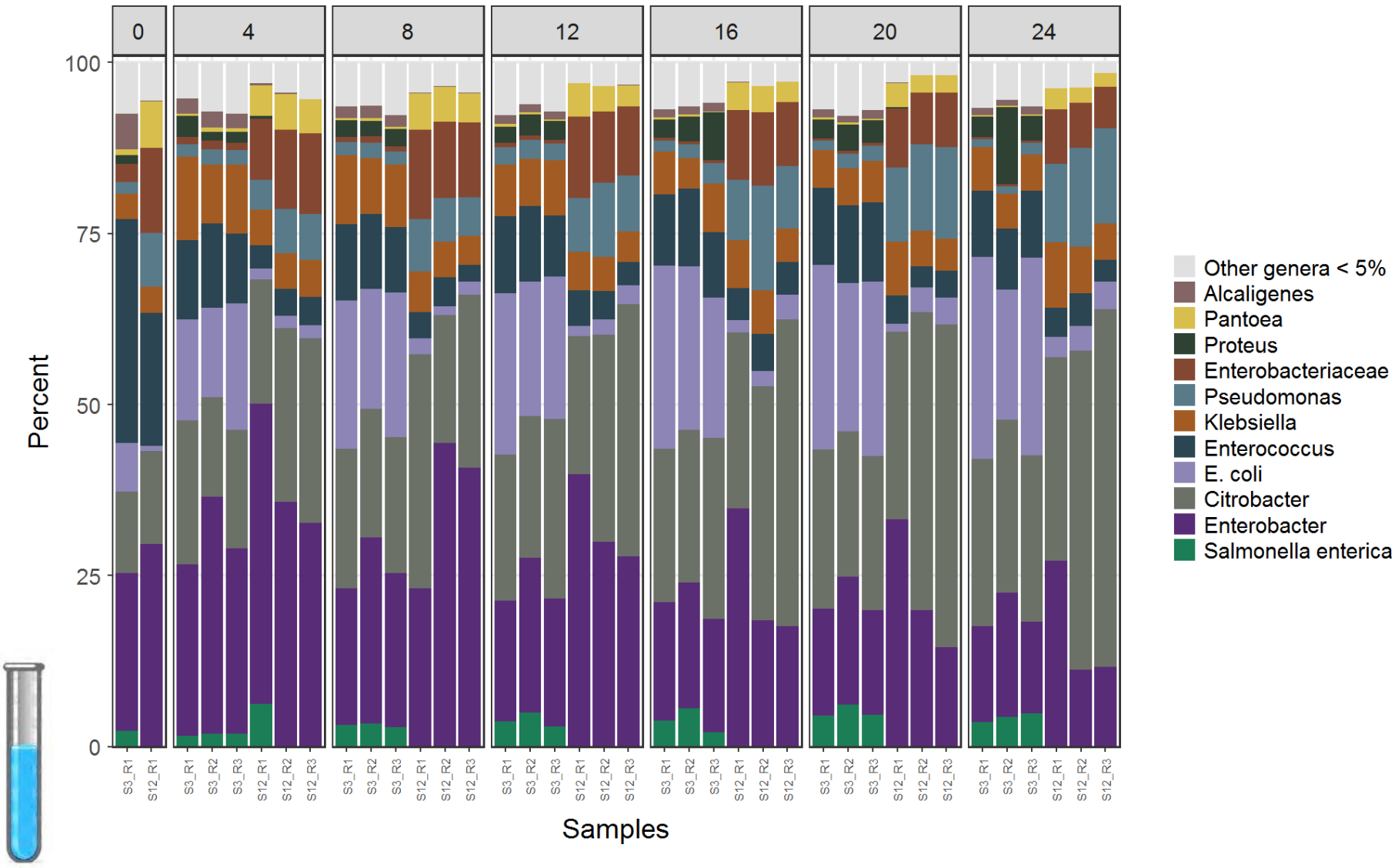
Gram positive
Extraction 35

(same color: same genera – order by Genus: next chart – for easier comparison)



Gram negative
Extraction 35

TT every 4 hours incubated at 42 degrees – positive and negative papaya samples




00 mL
± 5%
150
100
50

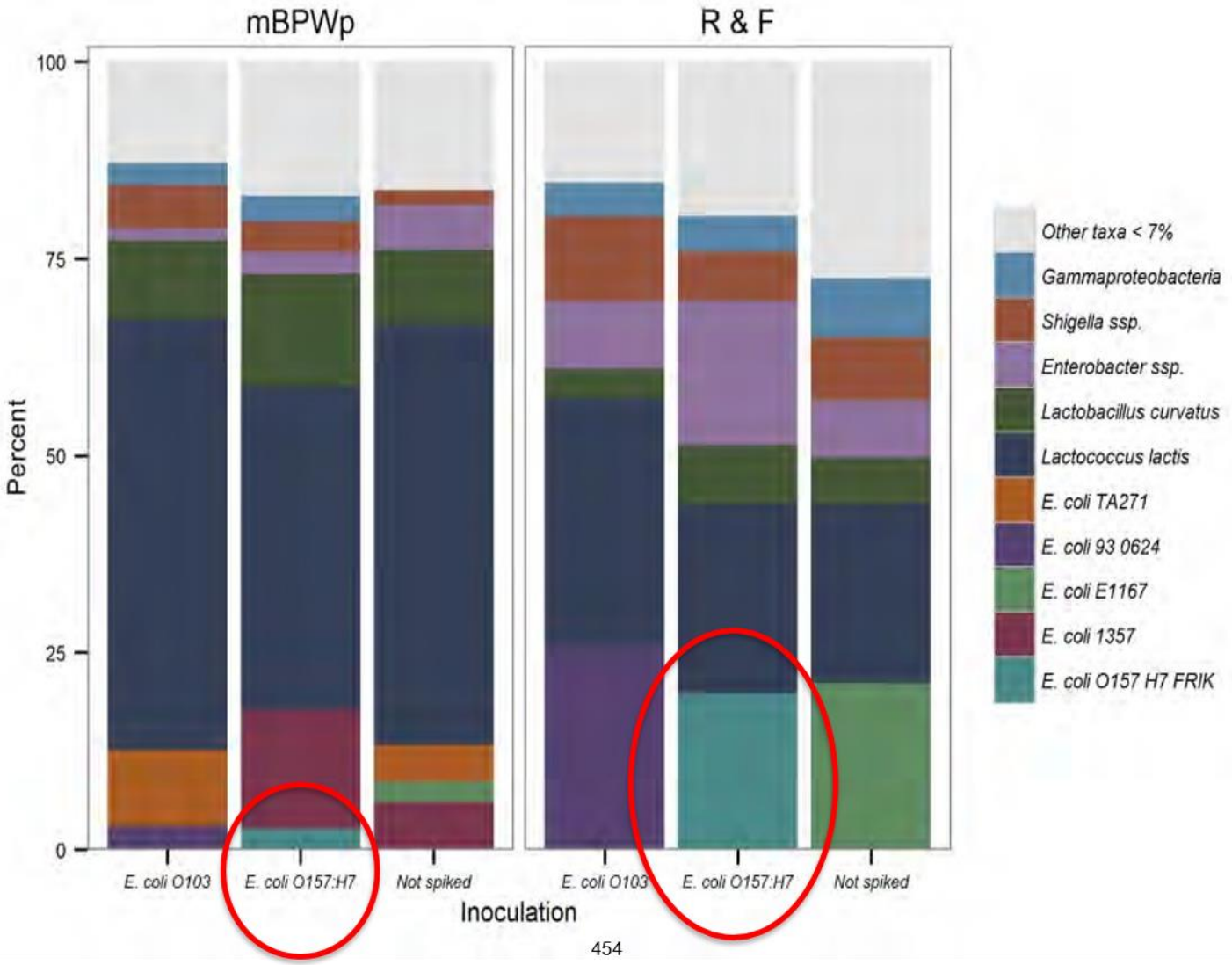
Characterization of microflora in Latin-style cheeses by next-generation sequencing technology

Lusk et al. BMC Microbiology 2012, ***12:254***

Evaluation of Enriched Microflora of Raw Milk Cheese Spiked with *E. coli* O157:H7 and *E. coli* O103

TINA LUSK PFEFER
RESEARCH MICROBIOLGIST
FDA/CFSAN

MICROBIOME RESEARCH AT THE NIH/FDA
MARCH 13, 2017

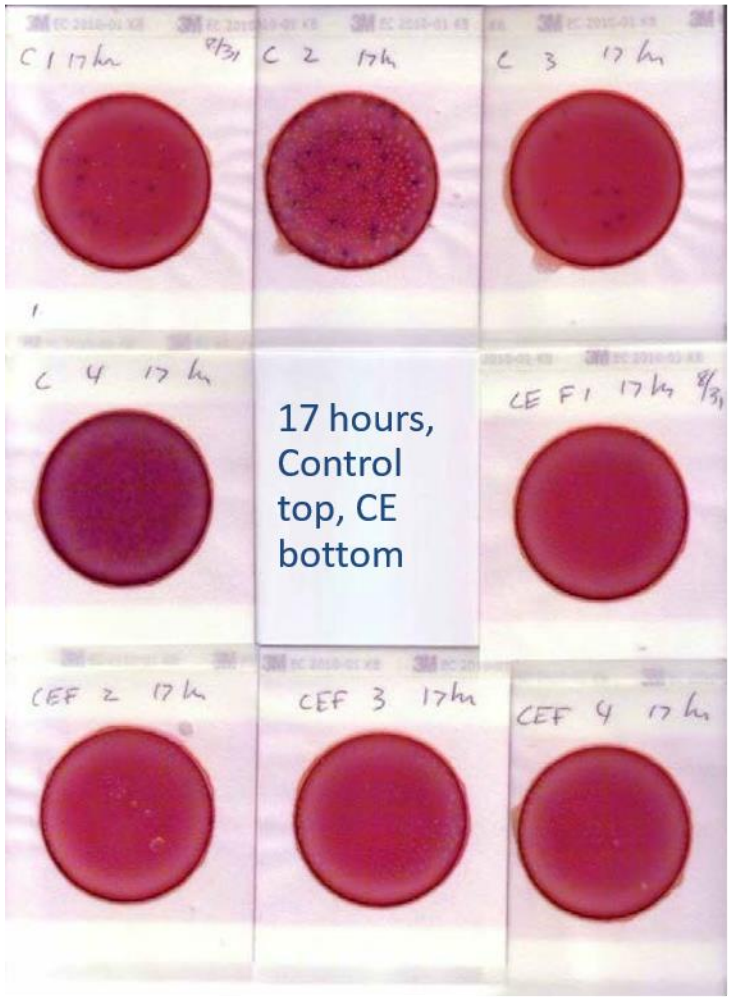

mBPWp
R & F
100
75
50
25
0
Percent
E. coli O103
E. coli O157:H7
Not spiked
E. coli O103
E. coli O157:H7
Not spiked
Inoculation
Other taxa < 7%
Gammaproteobacteria
Shigella ssp.
Enterobacter ssp.
Lactobacillus curvatus
Lactococcus lactis
E. coli TA271
E. coli 93 0624
E. coli E1167
E. coli 1357
E. coli O157 H7 FRIK

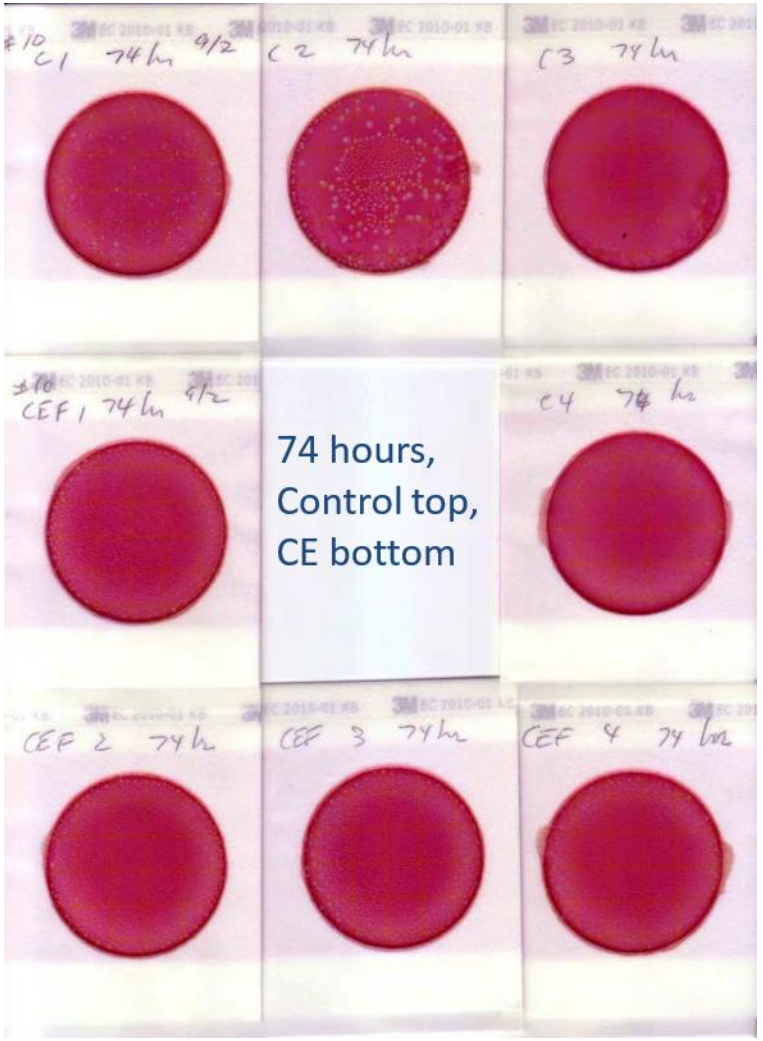
PI: Bob Sanderson

SPROUTS

T0 T1 T2 T3 T4

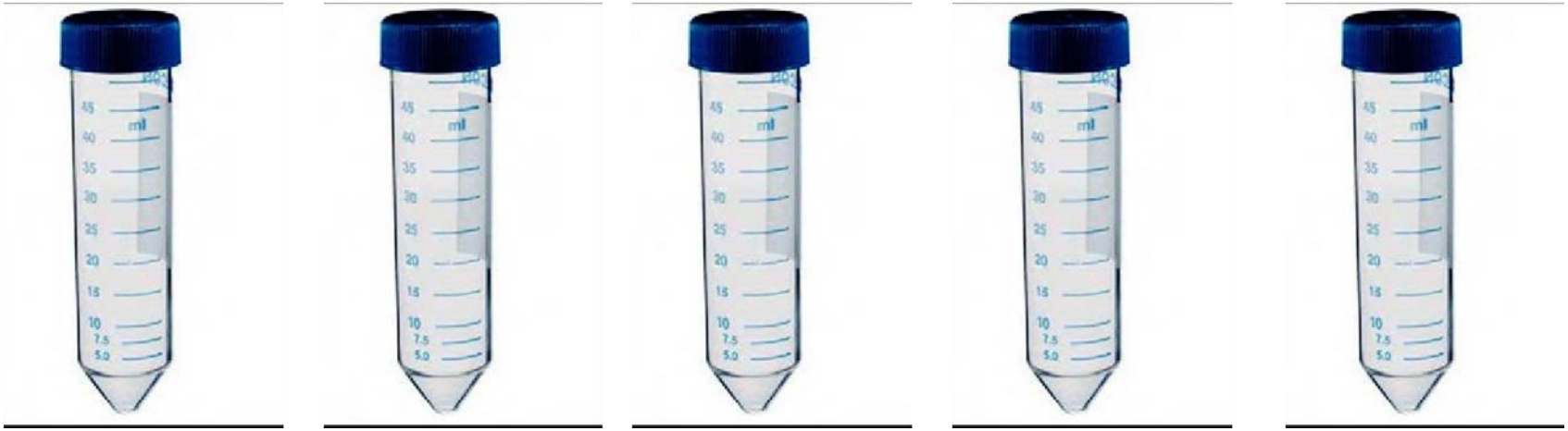
3M Petri Films – noticing a suppressive effect



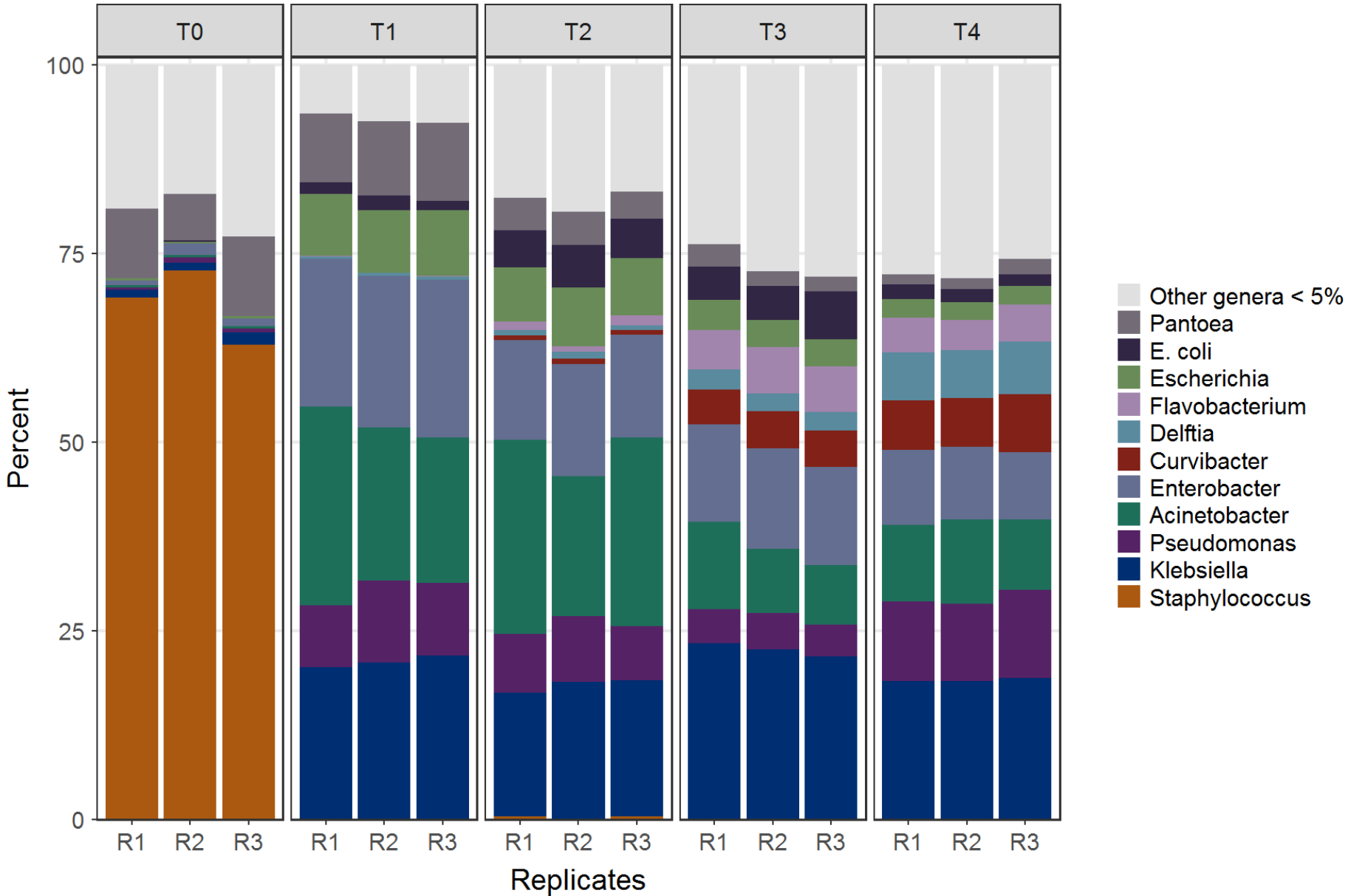


EXPERIMENTAL DESIGN

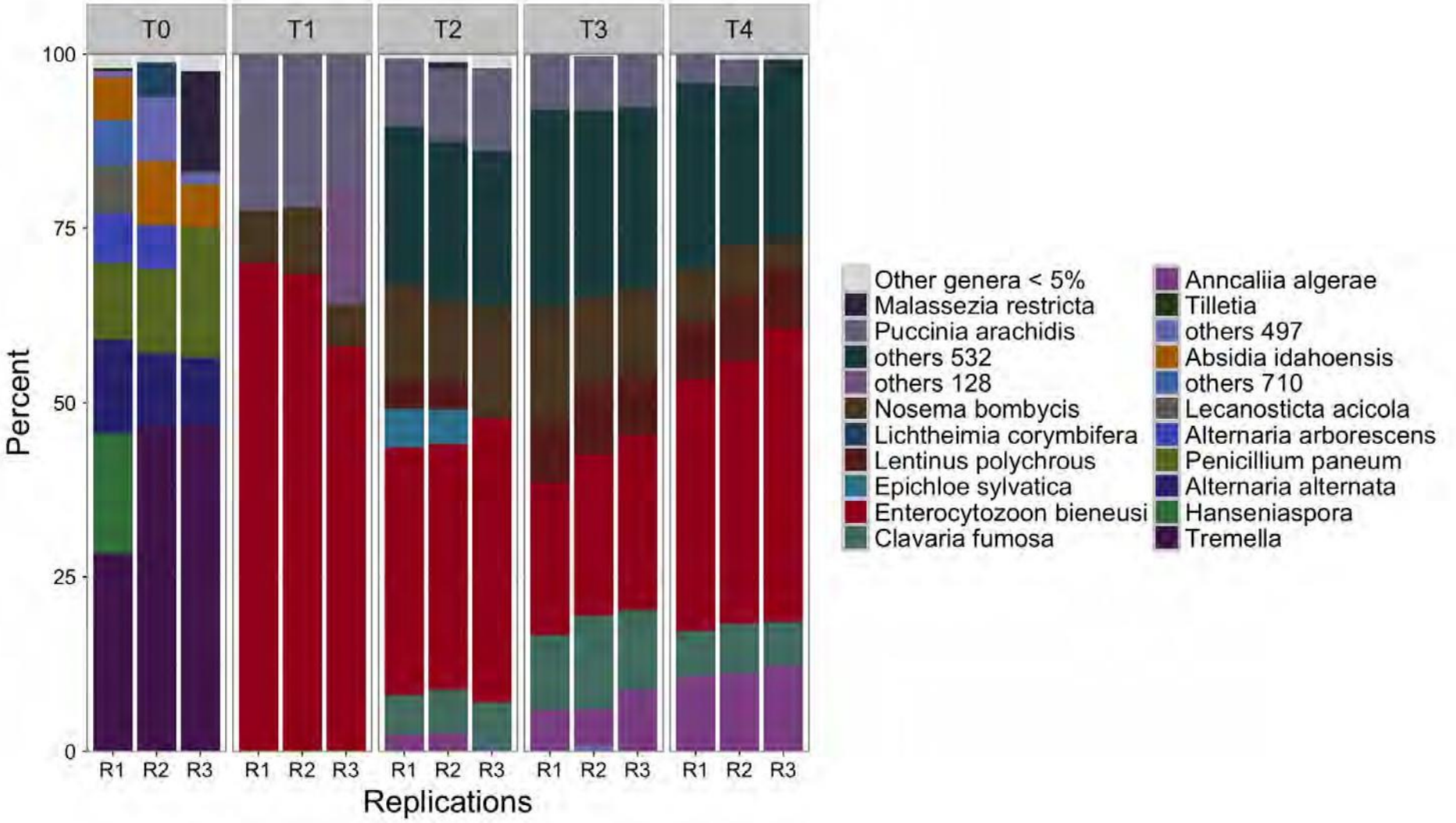
T0 T1 T2 T3 T4



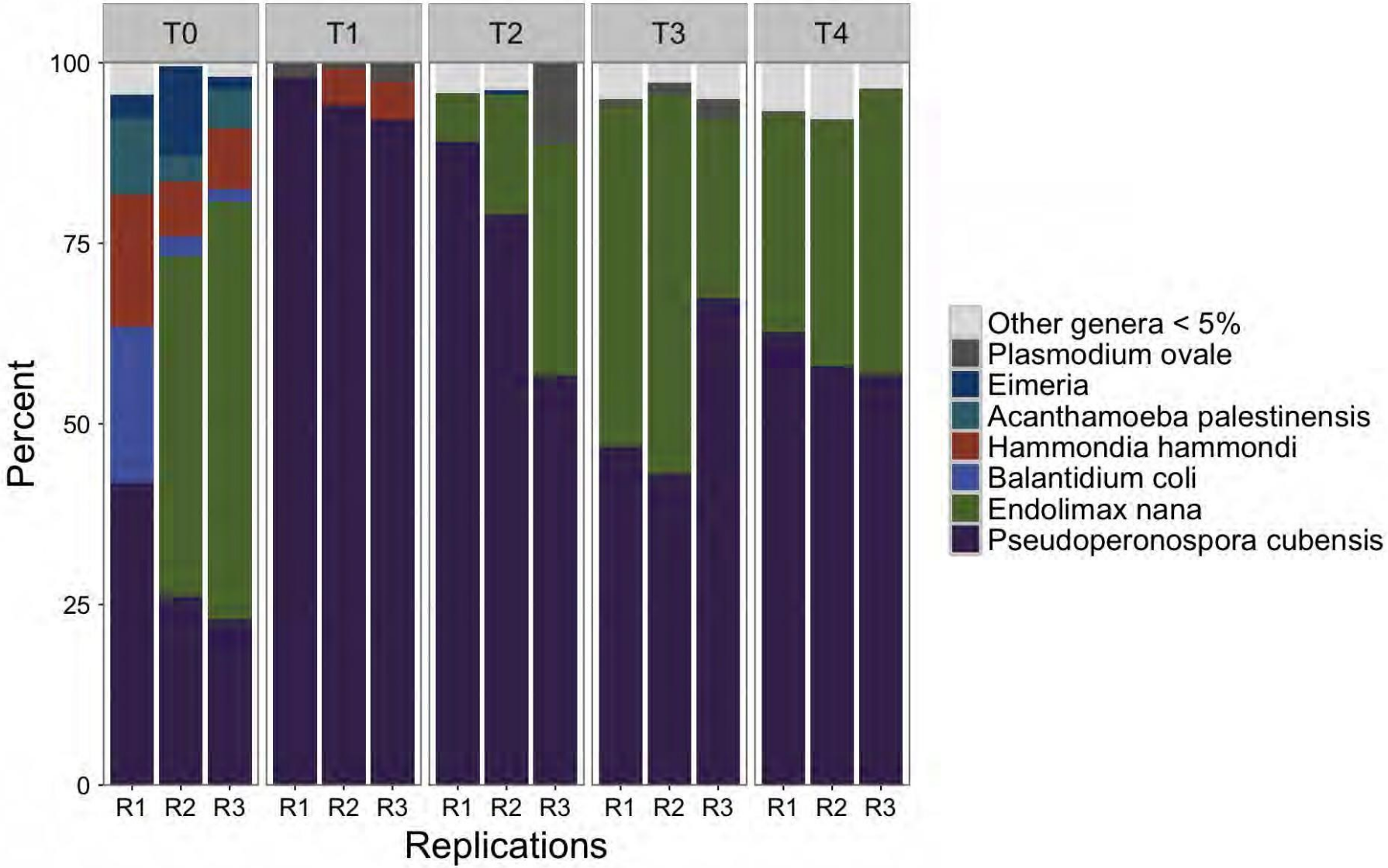
Bacteria found in sprout water over 4 days



Fungi found in sprout water over 4 days



Protists found in sprout water over 4 days



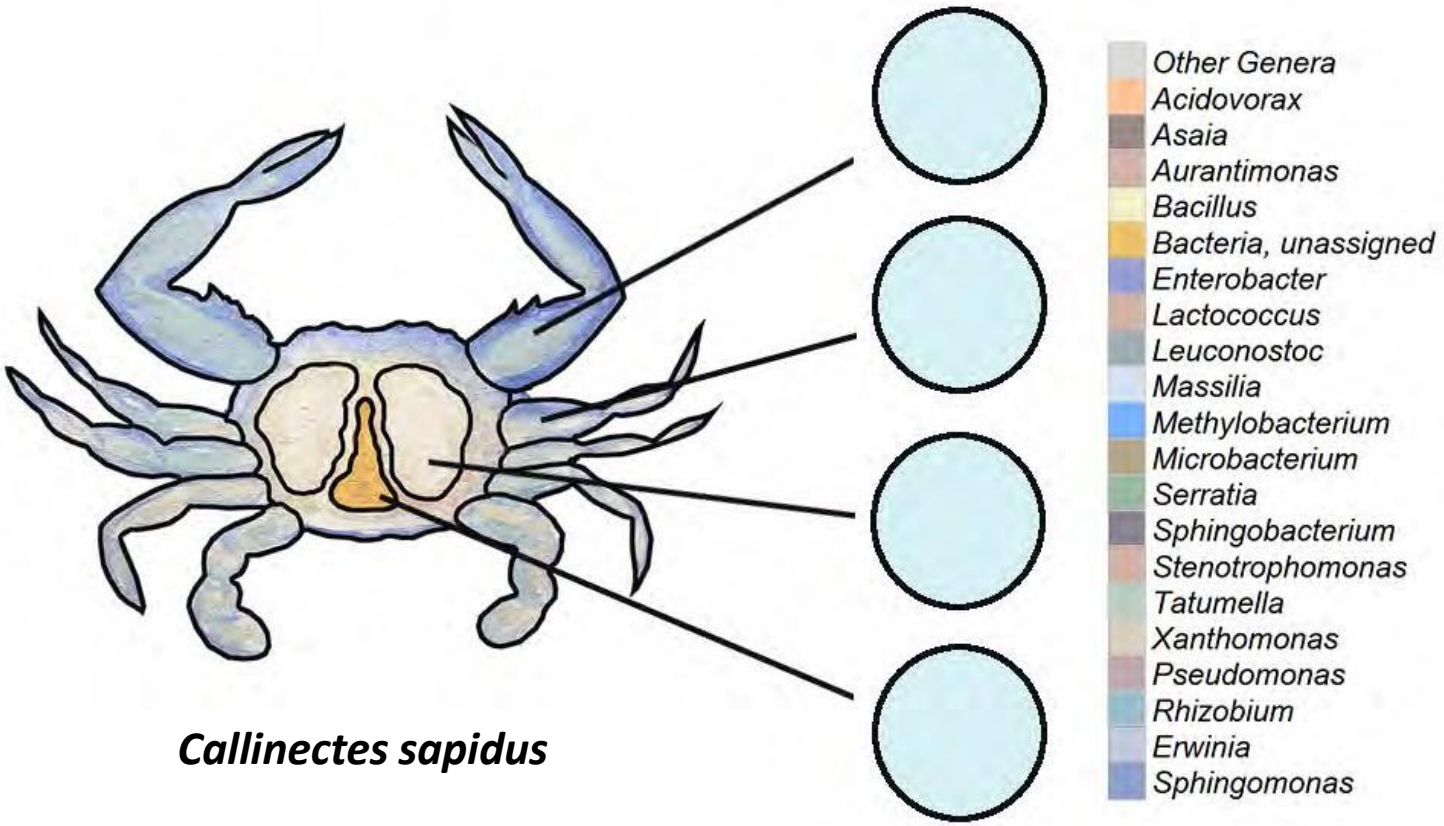
Water dna extraction

Pseudoponospora

Protist

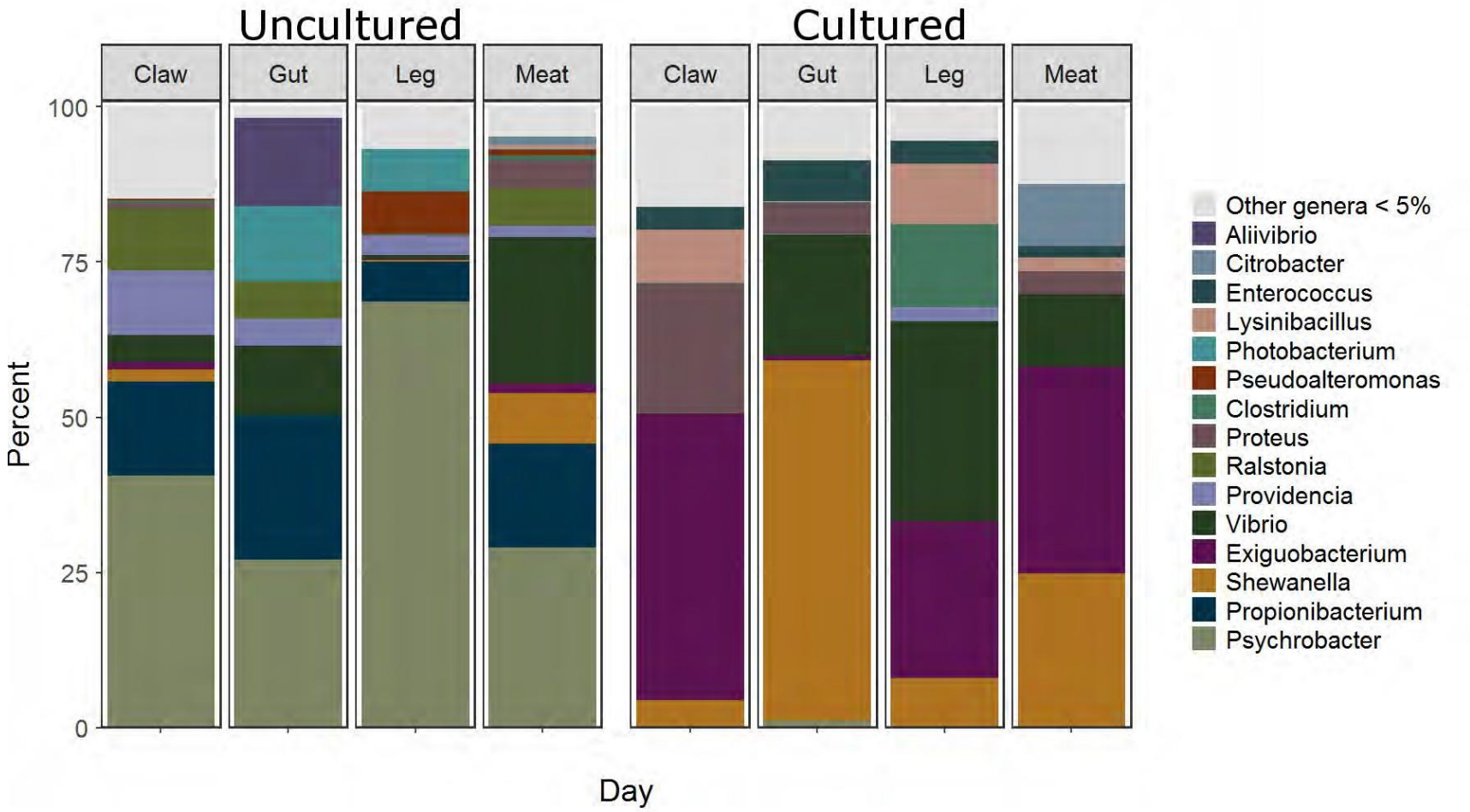
A **protist** **is any eukaryotic organism that is not an animal, plant or fungus.**

Maryland Blue Crab Microbiome

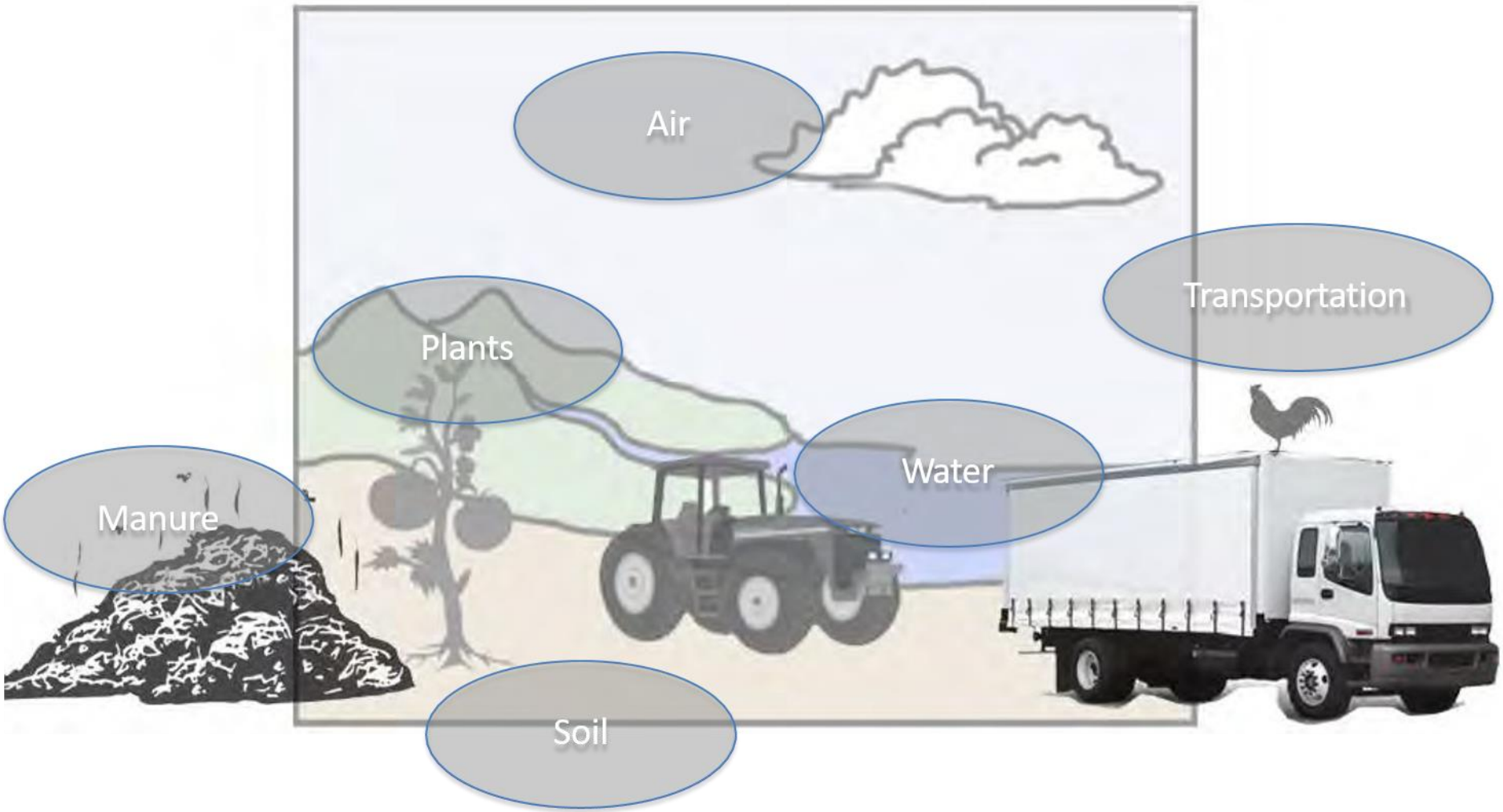


Callinectes sapidus

Maryland Blue Crab Microbiome (2)



Identifying risk factors



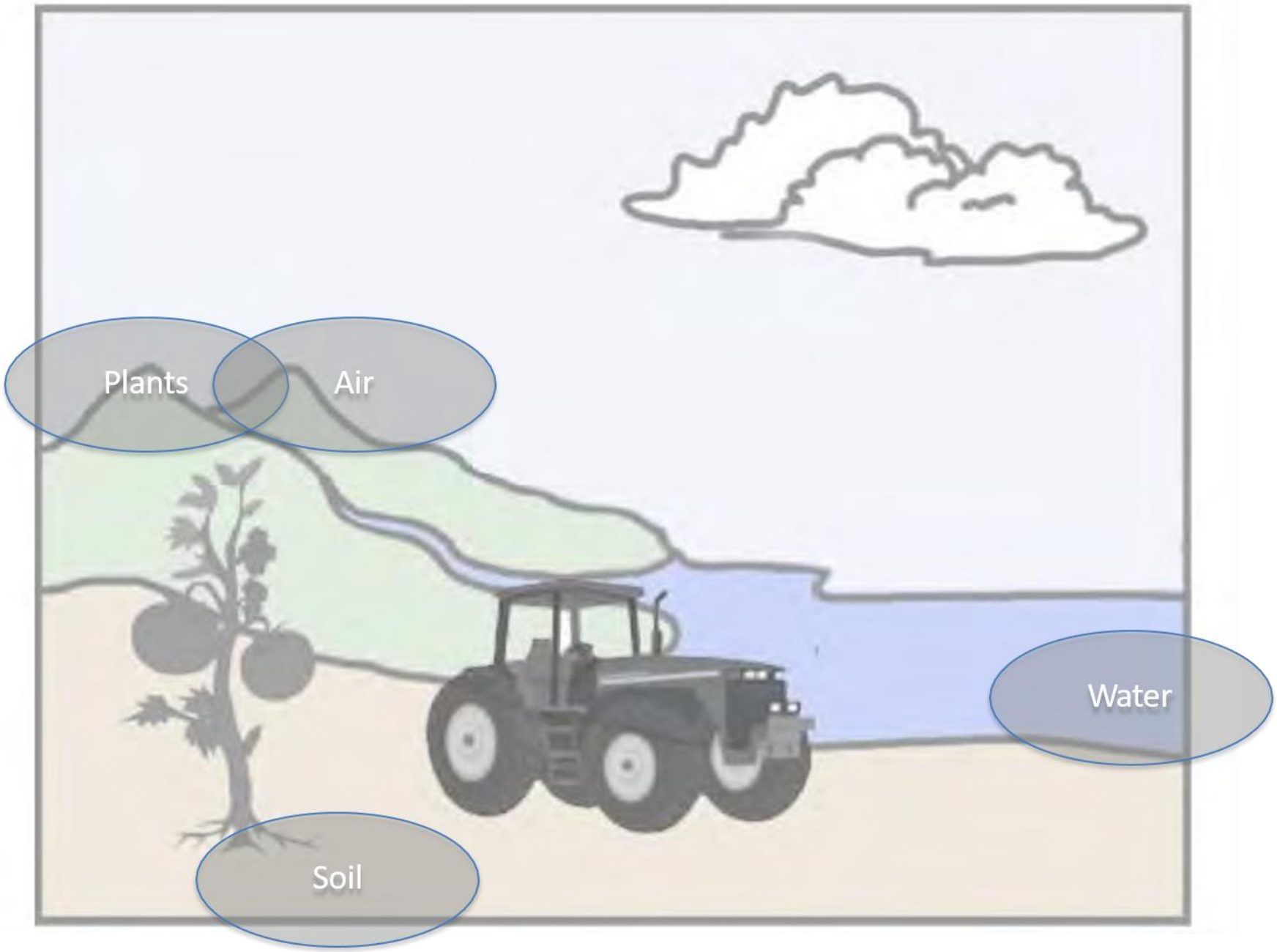
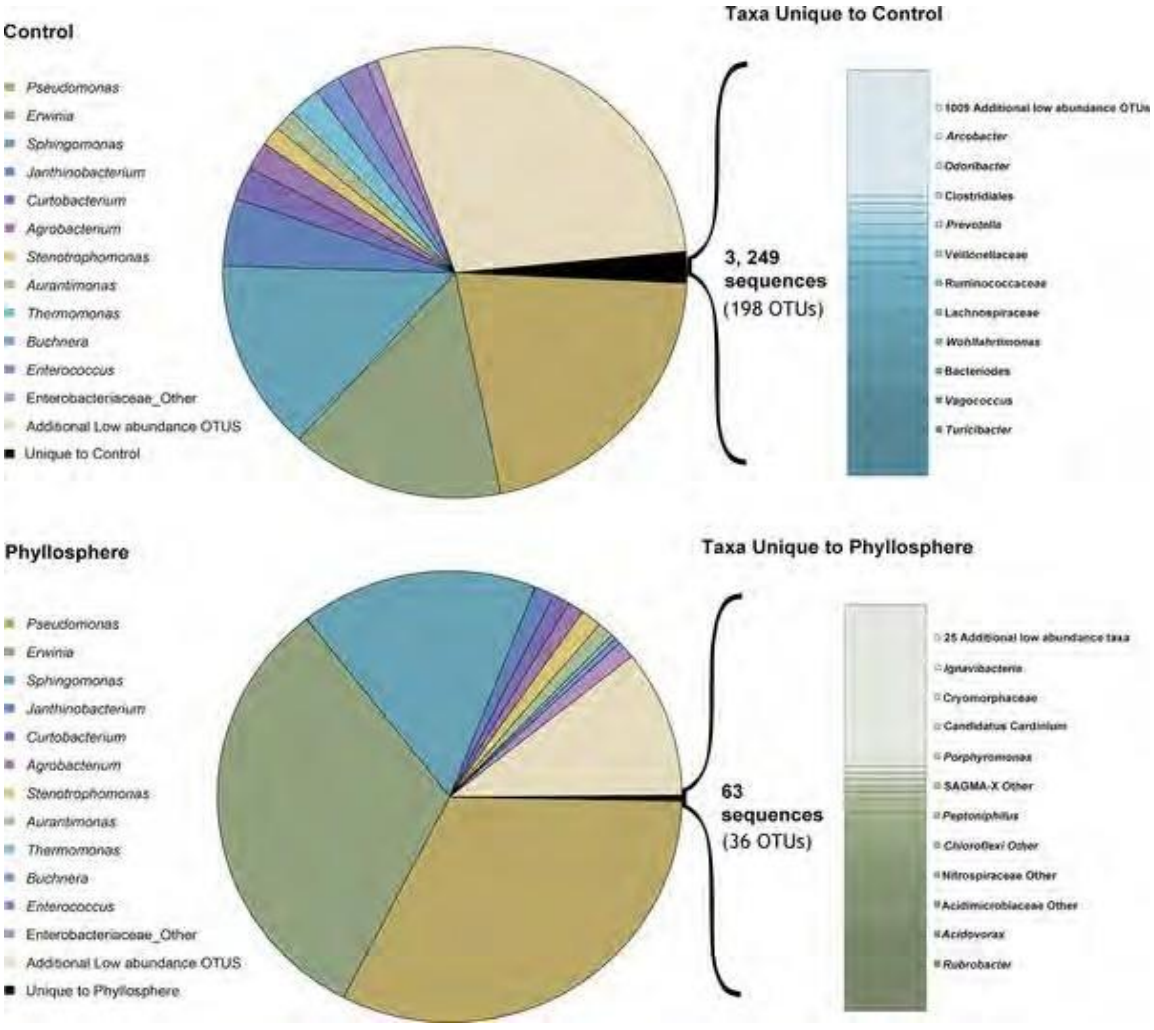
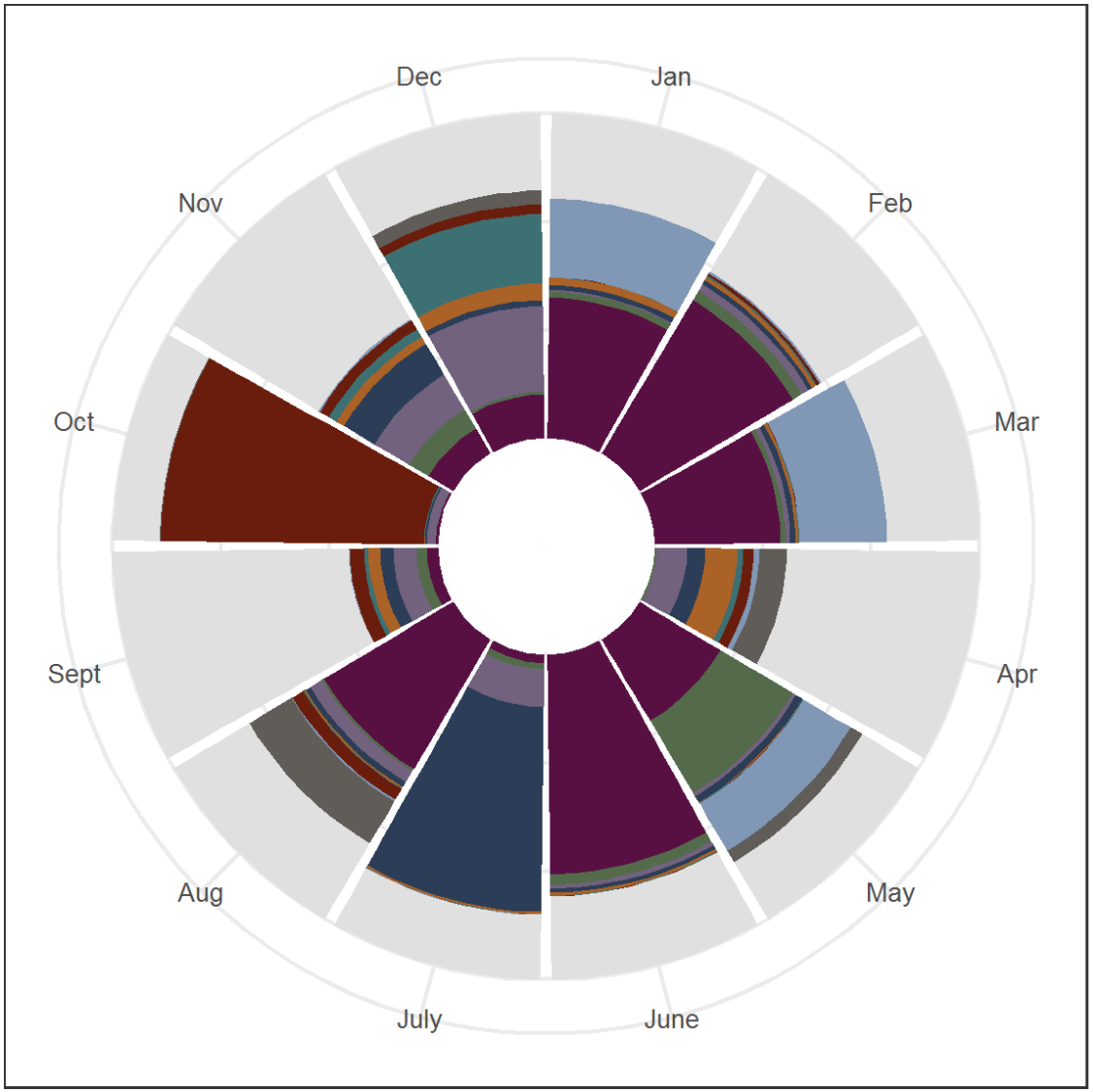

Plants
Air
Water
Soil

Fig 1. Bacterial Genera Unique to Phyllosphere and Control Samples.



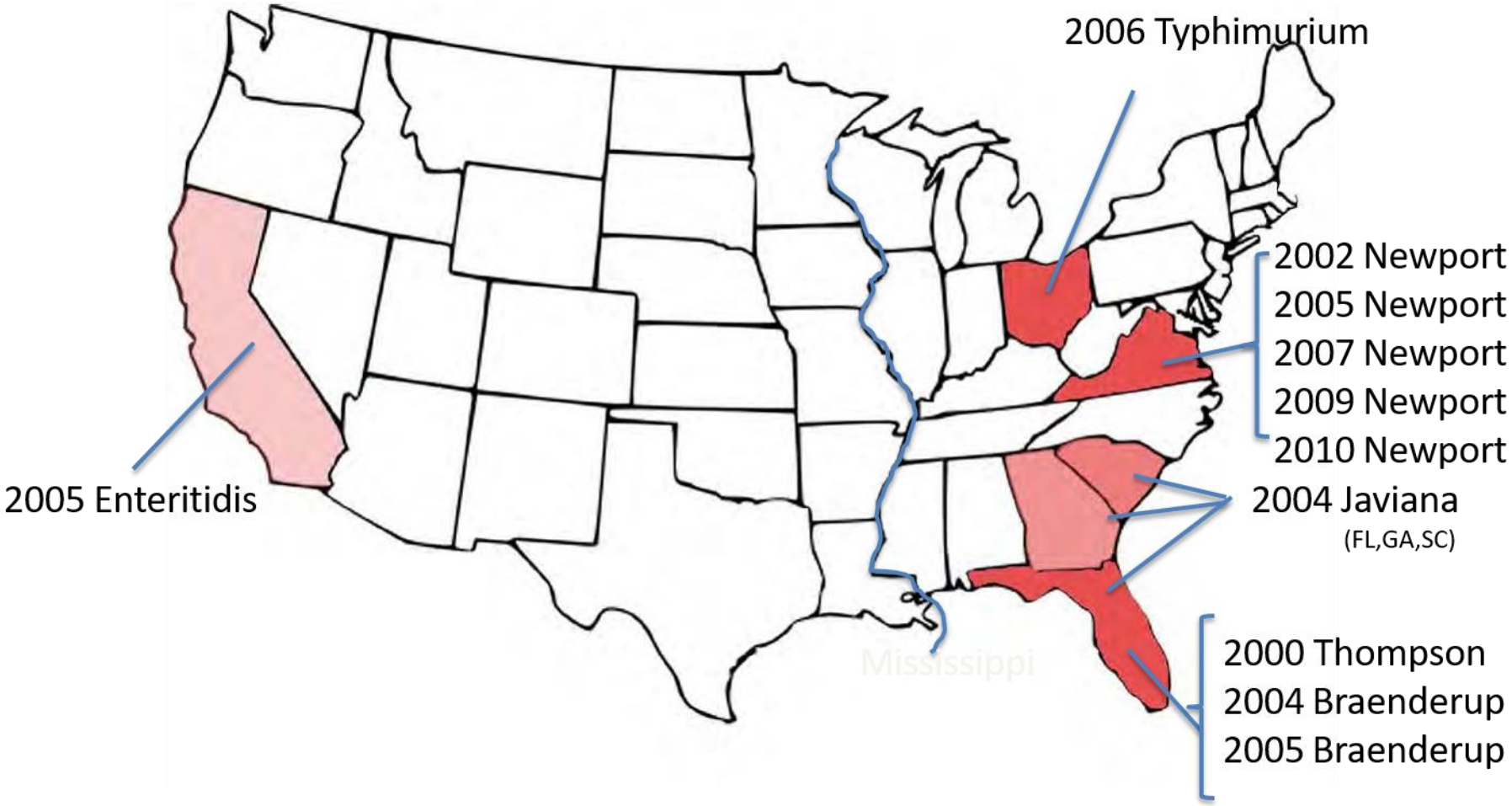
Ottesen AR, Gorham S, Reed E, Newell MJ, Ramachandran P, et al. (2016) Using a Control to Better Understand Phyllosphere Microbiota. PLOS ONE 11(9): e0163482. https://doi.org/10.1371/journal.pone.0163482
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0163482

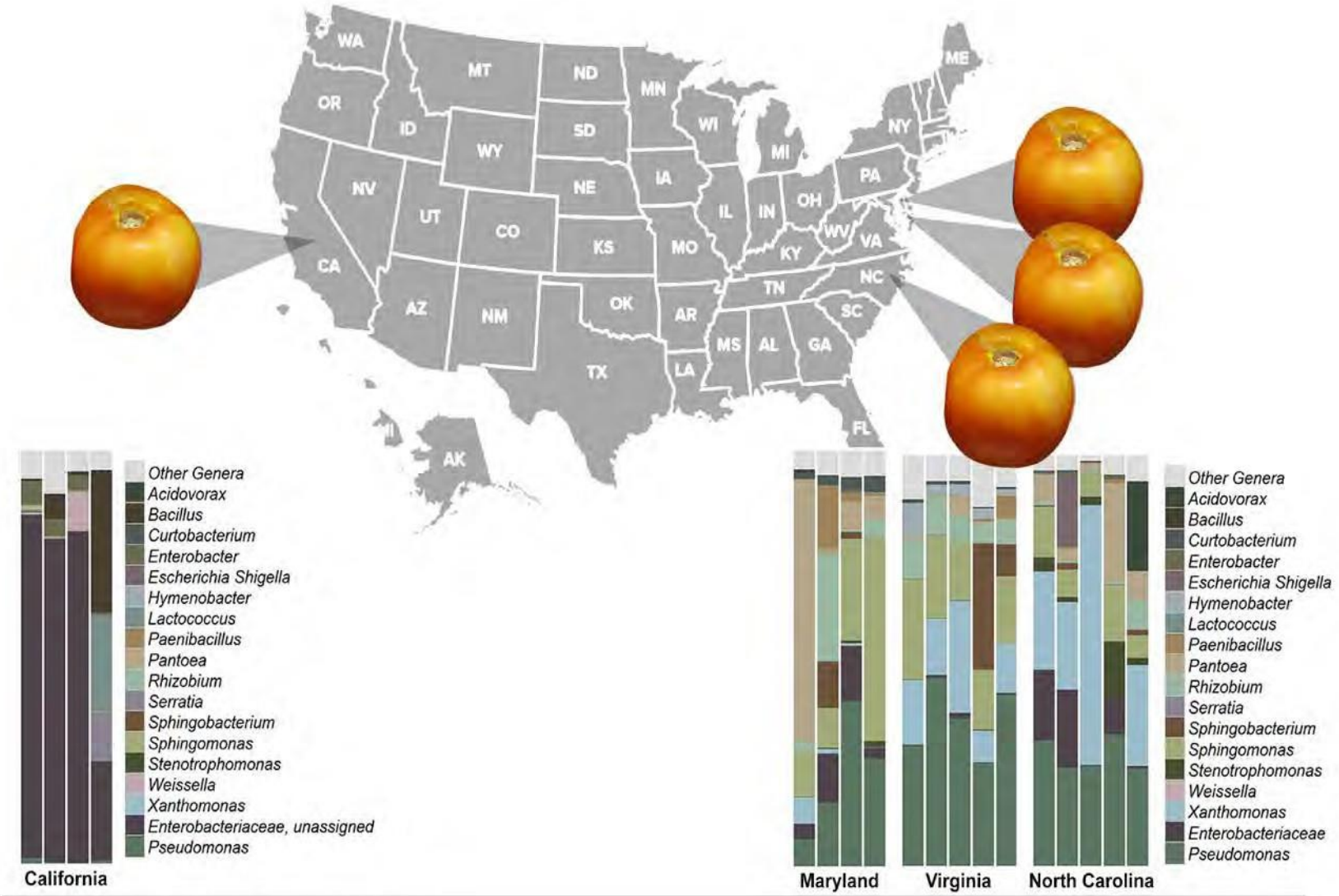
Agricultural Well water January - December



Salmonella – Tomato Associated Outbreaks

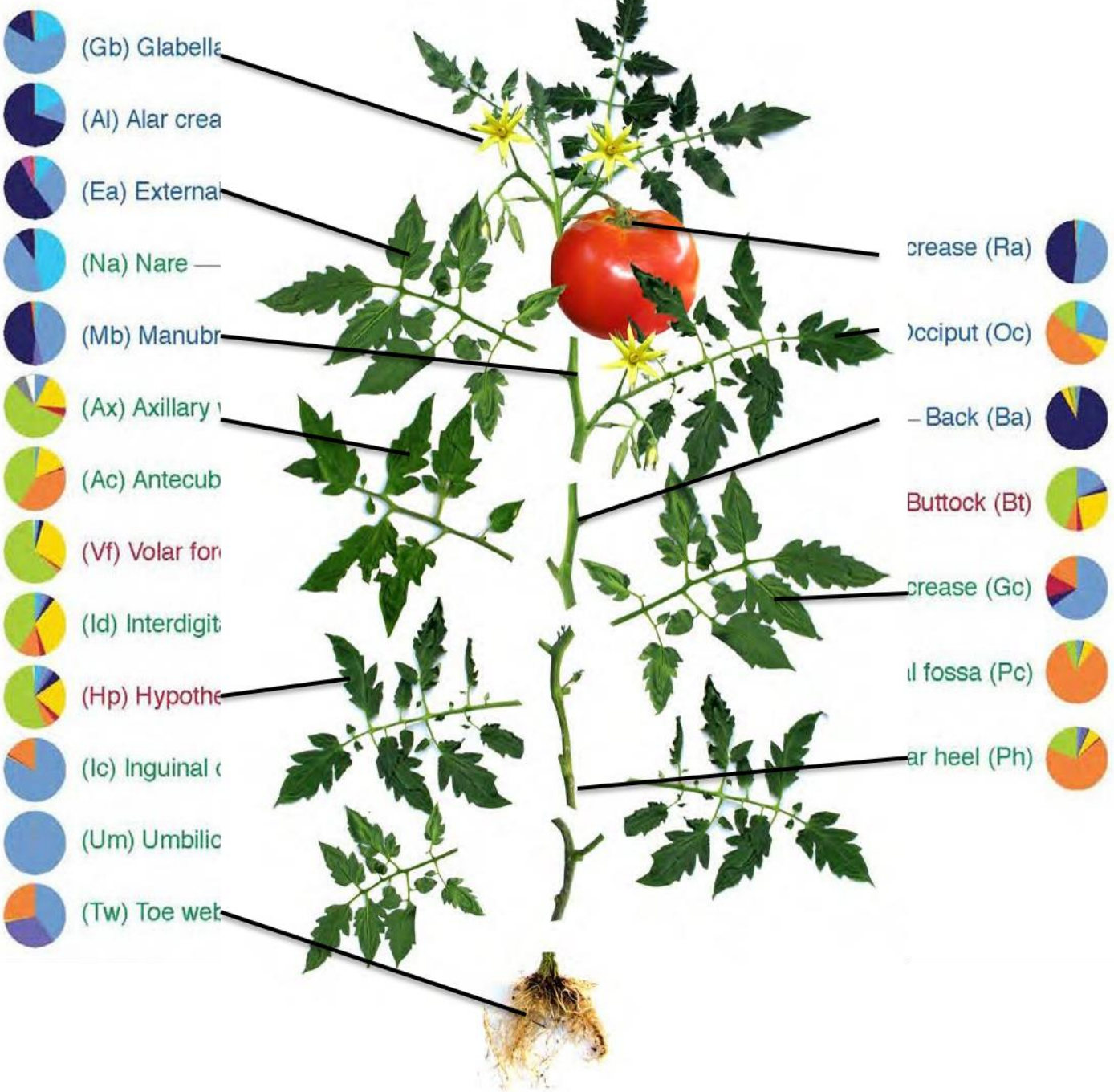
occurring between 2000 and 2008 – with definitive geographic trace-back



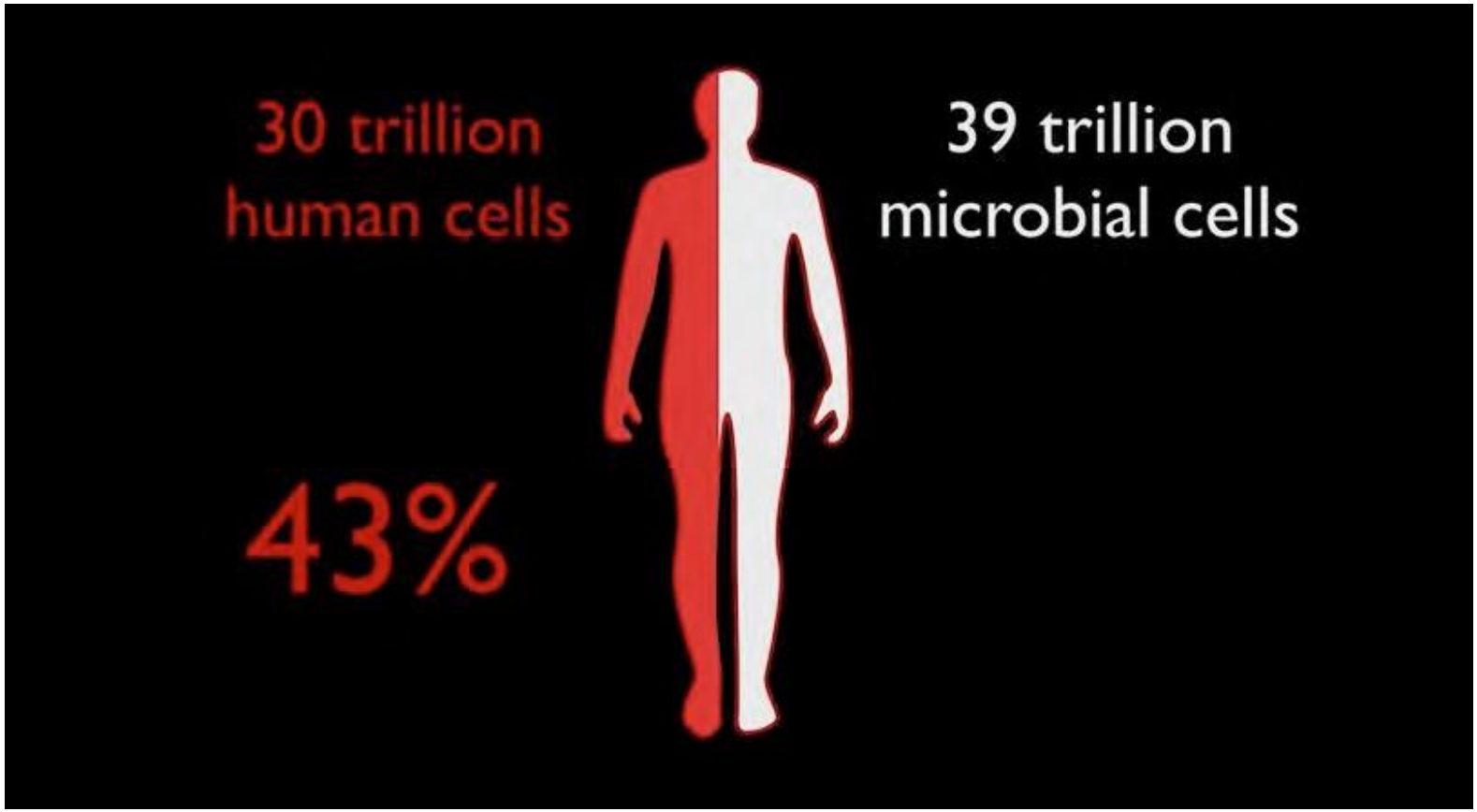


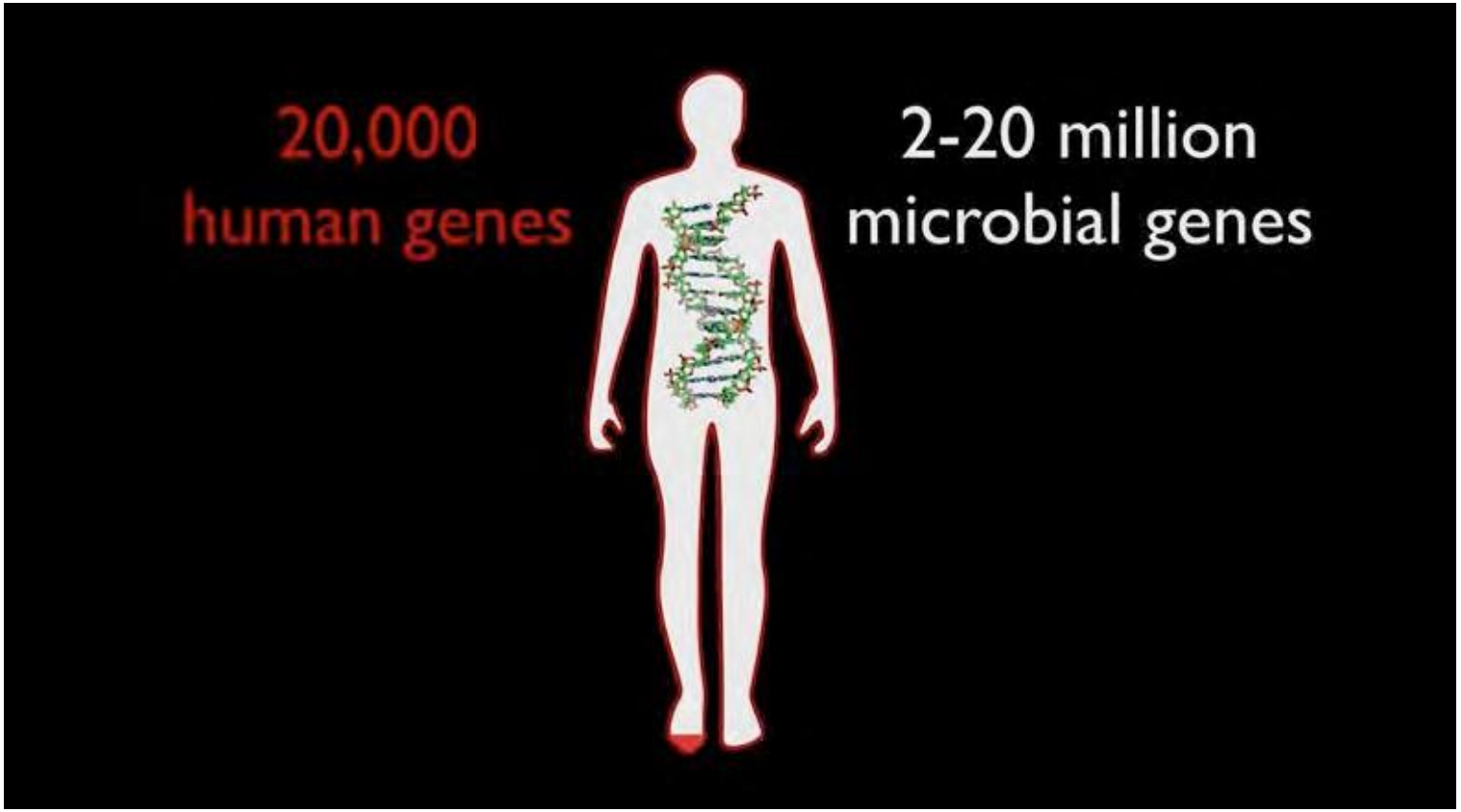
Bacterial Familes in CA tomato carposphere
16S rRNA gene V1-V2

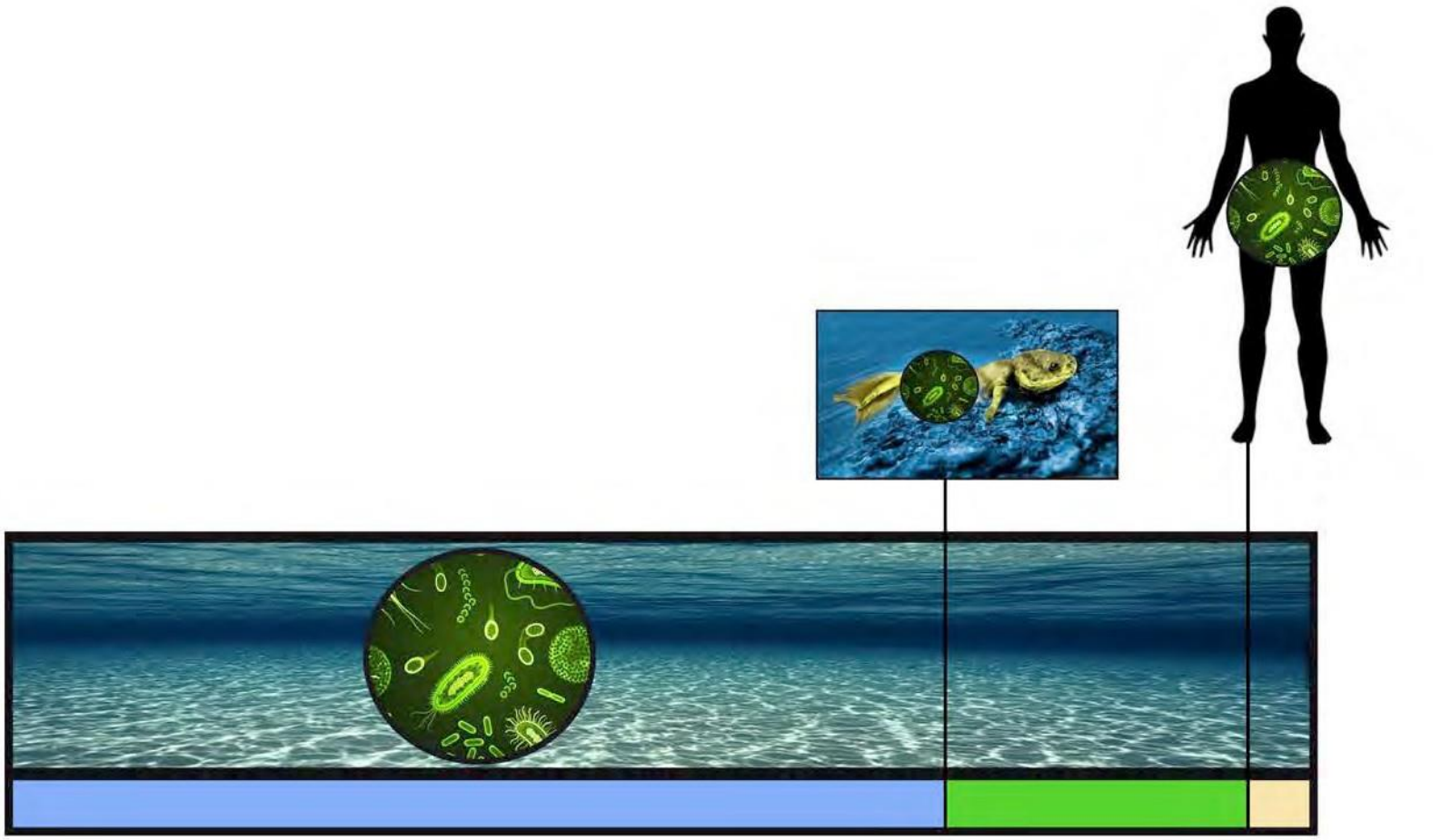
Bacterial Familes in MD, VA and NC tomato carposphere
16S rRNA gene


(Gb) Glabella
(Al) Alar crea
(Ea) External
(Na) Nare —
(Mb) Manubr
(Ax) Axillary
(Ac) Antecub
(Vf) Volar for
(Id) Interdigit
(Hp) Hypothe
(Ic) Inguinal
(Um) Umbilic
(Tw) Toe web
crease (Ra)
Occiput (Oc)
– Back (Ba)
Buttock (Bt)
crease (Gc)
l fossa (Pc)
ar heel (Ph)

Let food be thy medicine and medicine be thy food
All disease begins in the gut – Hippocrates (~2500 years ago)

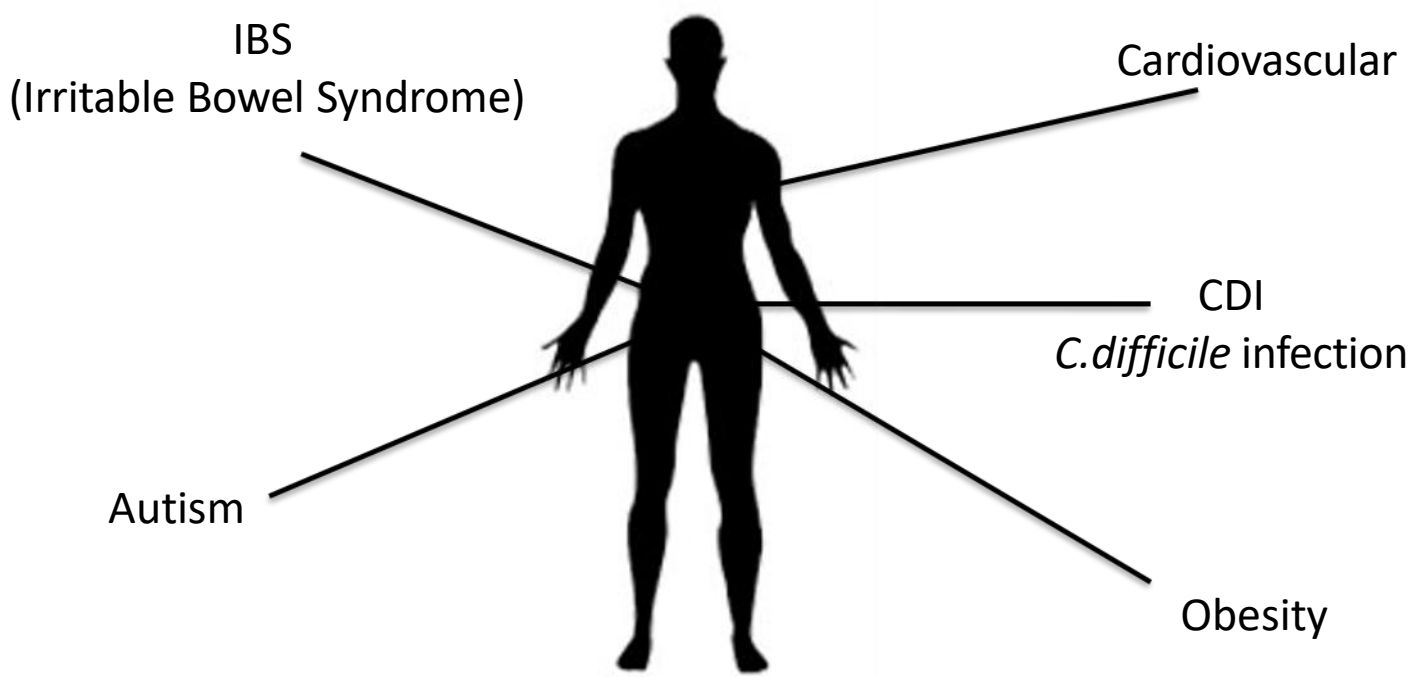

30 trillion
human cells
43%
39 trillion
microbial cells


20,000
human genes
2-20 million
microbial genes

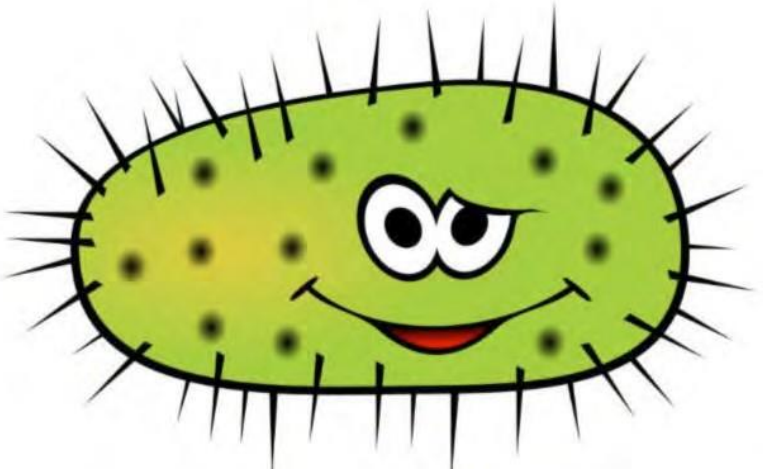

3.5 billion
500 million
1 million

The human microbiome is now correlated with many

STATES OF HEALTH AND DISEASE…



Vitamin D



AUTO IMMUNE DISEASES AND THE MICROBIOME

Intracellular microbes slow innate immune defenses by dysregulating the vitamin D nuclear receptor, allowing pathogens to accumulate in tissue and blood.

Molecular mimicry between pathogen and host causes further dysfunction by interfering with human protein interactions.

Asthma, Octopus

Loss of Diversity

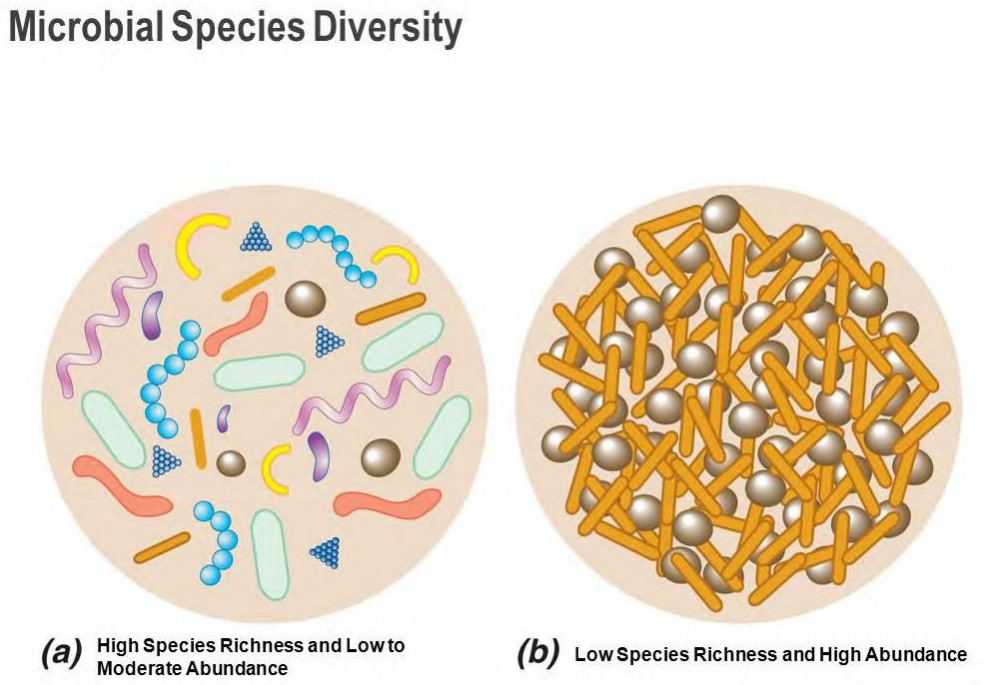
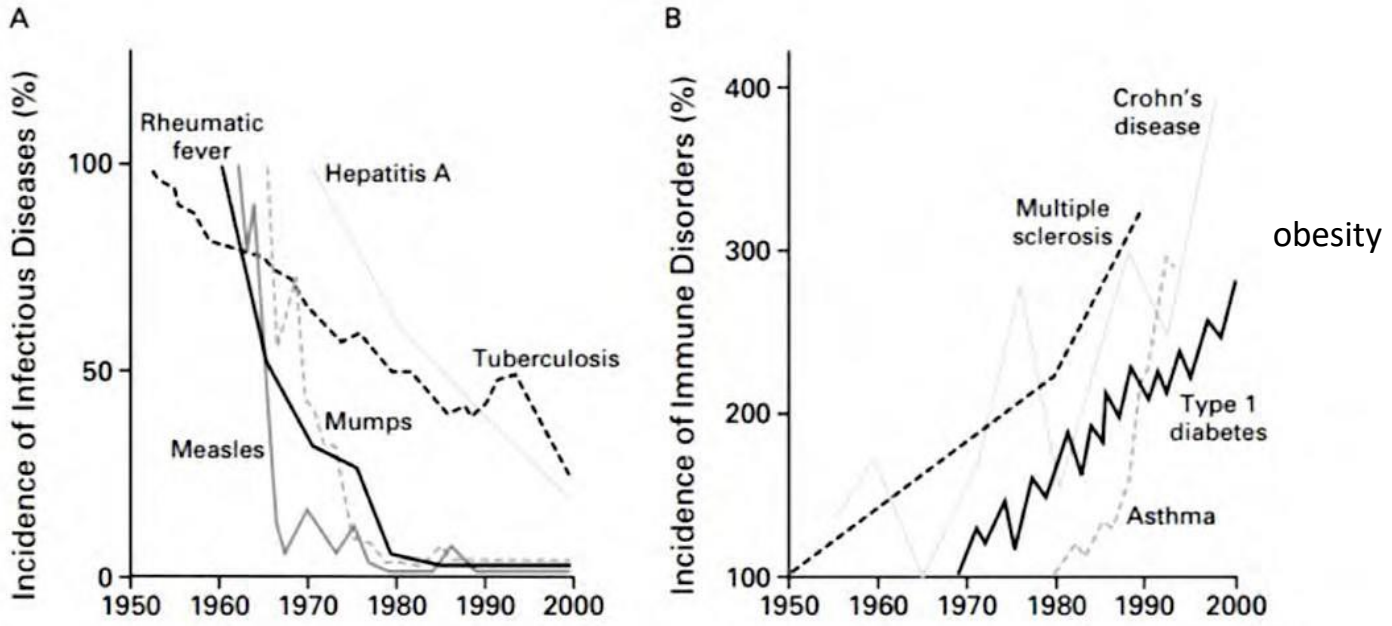


Figure 23.1

The effect of infections on susceptibility to autoimmune and allergic diseases

N Engl J Med. 2002



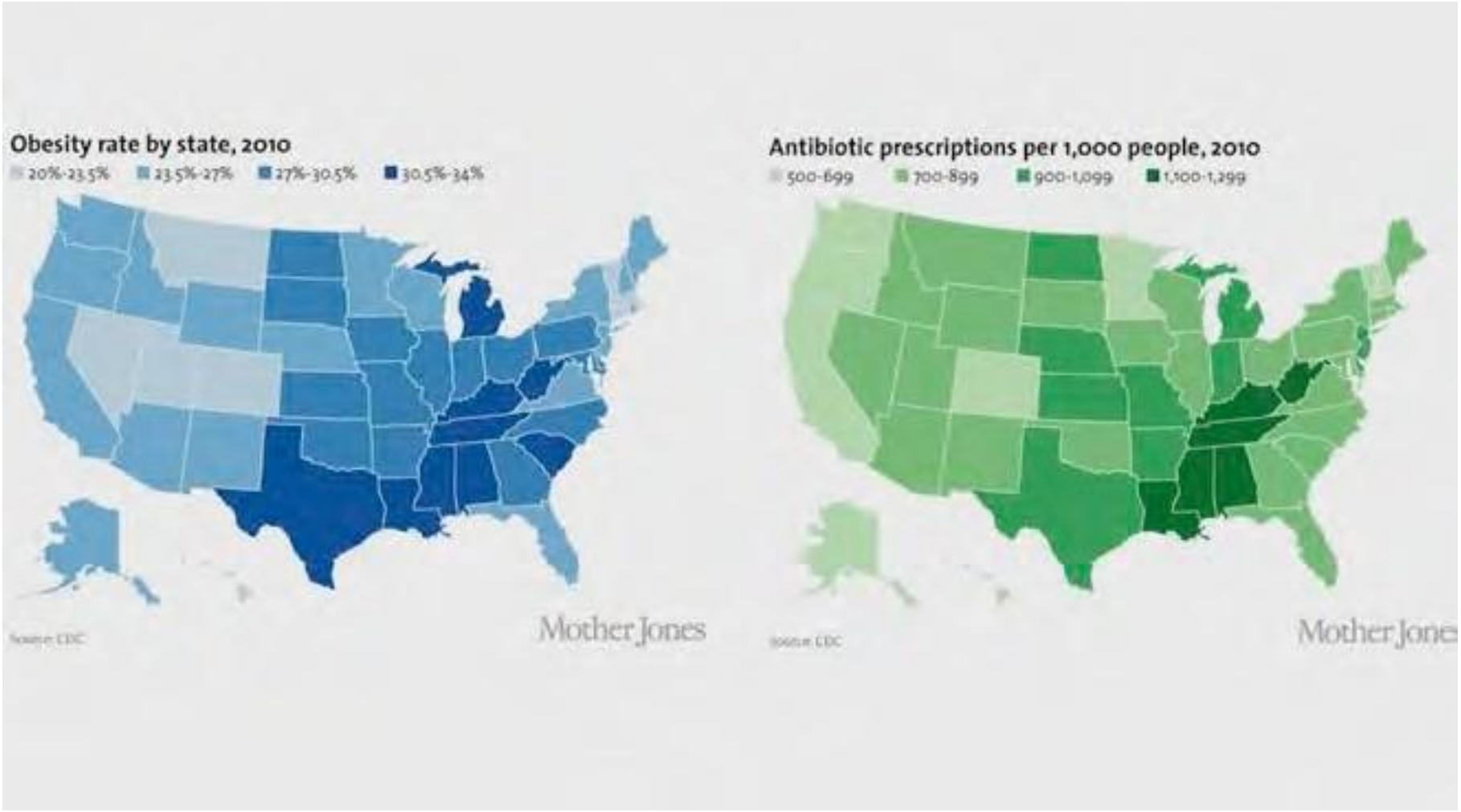
obesity

Figure 1. Inverse Relation between the Incidence of Prototypical Infectious Diseases (Panel A) and the Incidence of Immune Disorders (Panel B) from 1950 to 2000.

In Panel A, data concerning infectious diseases are derived from reports of the Centers for Disease Control and Prevention, except for the data on hepatitis A, which are derived from Joussemet et al.[12] In Panel B, data on immune disorders are derived from Swarbrick et al.,[10] Dubois et al.,[13] Tuomilehto et al.,[14] and Pugliatti et al.[15]

Bach (2002) N Engl J Med, Vol. 347, 911-920

Antibiotic use

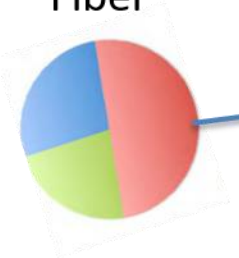



Welch's
Fruit Snacks
Jolly Rancher
Hard Candy
Jolly Rancher
Chews
Lifesavers
Gummies
Exotics
Lifesavers
Gummies
5 Flavors
Sour Patch
Trolli
Sour Brite Crawlers
Trolli
Sour Brite Bites
Lifesavers
Gummies
Neons
Sour Jacks
Mrs. Freshley's
Twinkies
Creme Filled
Pop-Tarts
Nutri-Grain
Chocolate
Cinnamon Rolls
Chocolate Chip Muffins
Soft Croissant
Mike and Ike
Sour Patch
Pistachios

NEW LABEL / WHAT'S DIFFERENT

Carbs

Fiber



Servings: larger, bolder type

Nutrition Facts

8 servings per container
Serving size **2/3 cup (55g)**

Amount per serving
Calories **230**

	% Daily Value*
Total Fat 8g	**10%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 160mg	**7%**
Total Carbohydrate 37g	**13%**
Dietary Fiber 4g	**14%**
Total Sugars 12g	
Includes 10g Added Sugars	**20%**
Protein 3g	
Vitamin D 2mcg	10%
Calcium 260mg	20%
Iron 8mg	45%
Potassium 235mg	6%

* The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

New: added sugars

Change in nutrients required

Serving sizes updated

Calories: larger type

Updated daily values

Actual amounts declared

New footnote

Fiber

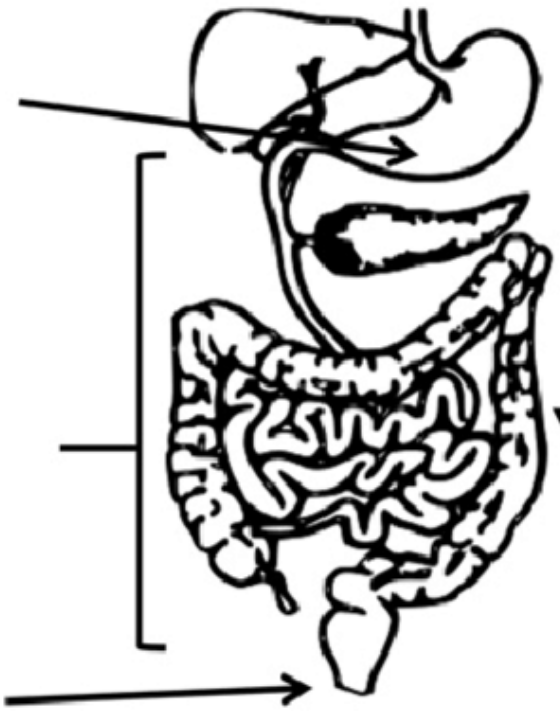
INSOLUBLE FIBER

Increase through water binding the volume of the stomach content
↓
Satiety ↑

Decreased transit time through gastro-intestinal tract

NON FERMENTABLE

Stool weight ↑
"bulking effect"



SOLUBLE FIBER

Soluble in water

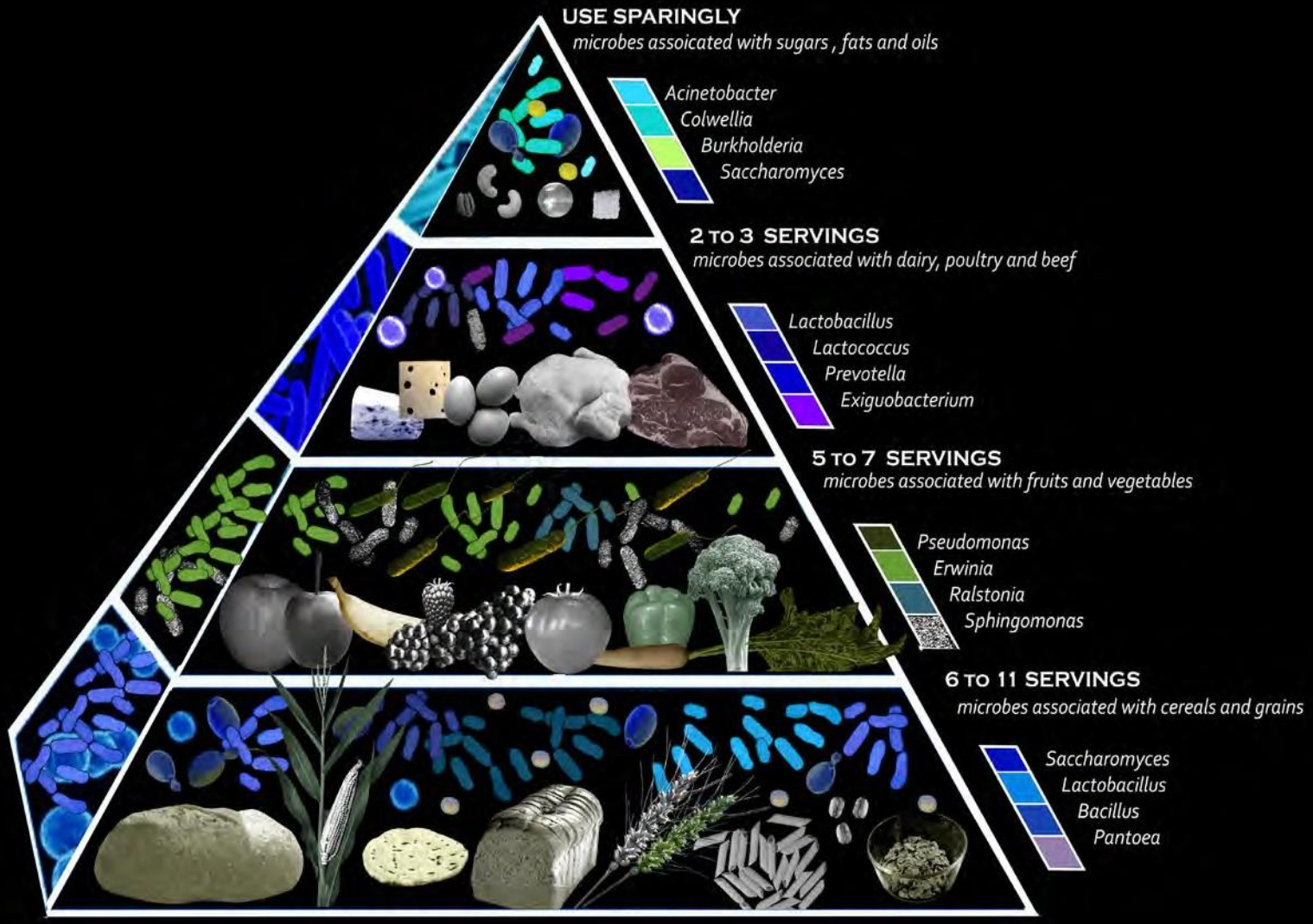
Passage through stomach and small intestine
-No influence: unchanged arrival in colon
-If very viscous: delayed rate of digestion

FERMENTABLE

Dependent on:
- Substrate : physical and chemical properties
- Microbiota composition
- Transit time

Micriobial mass ↑
↓
Stool weight ↑

Microbial Food Pyramid



rabbit hole

Microbiome Research at the NIH/FDA

"Sponsored by the NIH intramural Microbiome Working Group"

March 13, 2017
Lipsett Auditorium, NIH Main Campus

SAVE THE DATE!

Overview:

The purpose of this meeting is to bring together labs across the NIH and FDA intramural programs that are working on microbiome related projects. It is hoped that this meeting will lead to more communication, interactions and new collaborations between laboratories interested in how the microbiome impacts health and disease.

"Graphic courtesy of Hyperbiotics"

General Information

For conference related questions please contact:
Howard Young
301-846-5743

Location

Lipsett Auditorium
NIH Main Campus
Building 10
Bethesda, Md. 20892

Microbiome Research at the NIH/FDA

"Sponsored by the NIH Microbiome Interest Group"

March 13, 2017

Overview | Agenda | Visitor Information | Registration | WebEx Invitation

General Information

For conference related questions please contact:
Howard Young
301-846-5743

Location

Lipsett Auditorium
NIH Main Campus
Building 10
Bethesda, Md. 20892

Agenda

"Graphic courtesy of Hyperbiotics"

Opening remarks:

08:30 - 08:40 **Howard Young** *(Chair)*

Morning Session:

08:40 - 08:50 **Introduction to microbiome research at the FDA**
Andrea Ottesen, Chair

08:50 - 09:05 **Thoughts on the microbiome medicine /nutrition intersect for FDA**
Robert Califf

09:05 - 09:20 **Microbiome of oysters**
Padmini Ramachandran

09:20 - 09:35 **Role of MAIT cells in resistance to *Clostridium difficile* infection**
Paul Carlson

09:35 - 09:50 **Strain-Level Discrimination of Shiga Toxin-Producing Escherichia coli in Spinach Microbiome**
Susan Leonard

09:50 - 10:05 **High level overview of data gaps in assessing the risk to human health of antimicrobial residues in food animal edible tissues on the human intestinal microbiome** *Heather Harbottle*

10:05 - 10:20 **BREAK**

10:20 - 10:32 **Impact of antibiotic exposure on microbiome and resistome**
Daniel Tadesse

10:32 - 10:44 **NGS Toolbox for Probiotic Analysis**
Carmen Tartera

10:44 - 10:56 **Microbiome of pasteurized and unpasteurized Gouda cheeses**
Joelle Salazar

10:56 - 11:08 **Evaluation of Enriched Microflora of Raw Milk Cheese Spiked with *E. coli* O157:H7 and *E. coli* O103**
Tina Pfefer

11:08 - 11:20 **Food microbiomes: profiling food commodities**
Karen Jarvis

11:20 - 11:32 **High-Resolution Taxonomic Profiling to Enhance Translational Microbiome Research**
James Robert White

11:32 - 11:44 **Impact of the gut microbiome on the response to TNF antagonist treatment; *In vivo* pilot study**
Odile Gabay Engel

11:44 - 11:56 **Models to assess effects of nanoparticles on intestinal microbiome**
Sangeeta Khare

Food microbiomes, oysters
MAIT cells *C. diff*
Detection, *E. Coli*

AMR
Probiotics, Nutrition

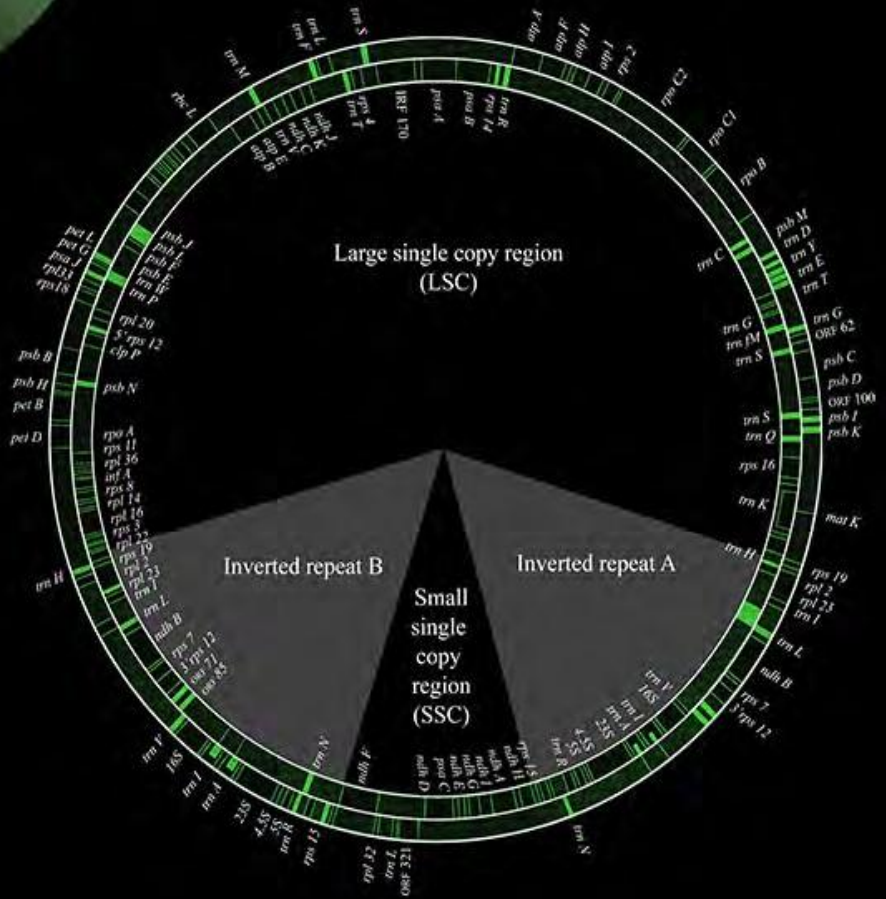
Food microbiomes, cheeses

Food microbiomes, cilantro, masala, cucumber, sprouts

TNF antagonist
Nanoparticles

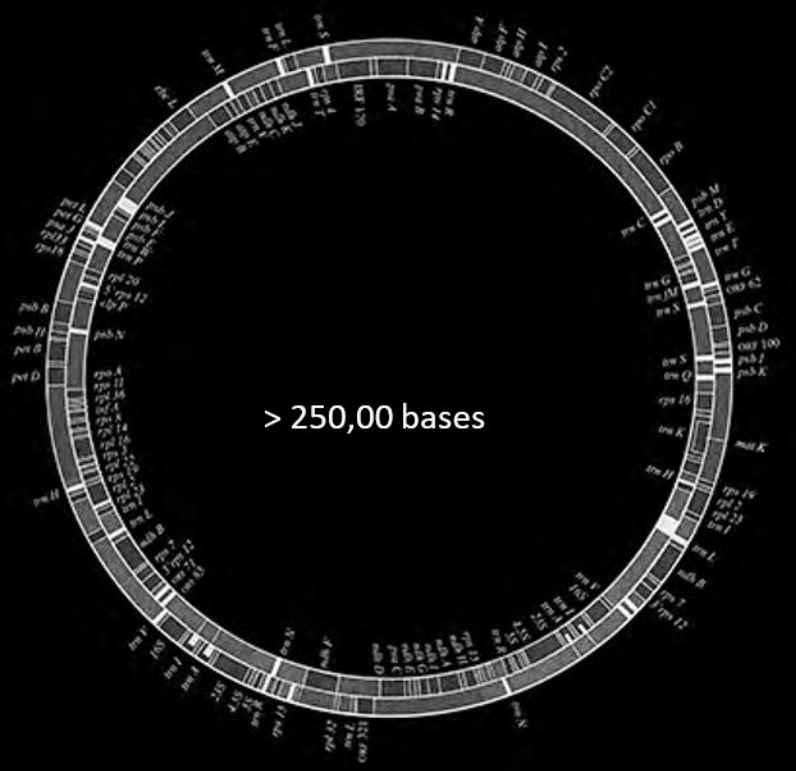
MITOchonTRAKR

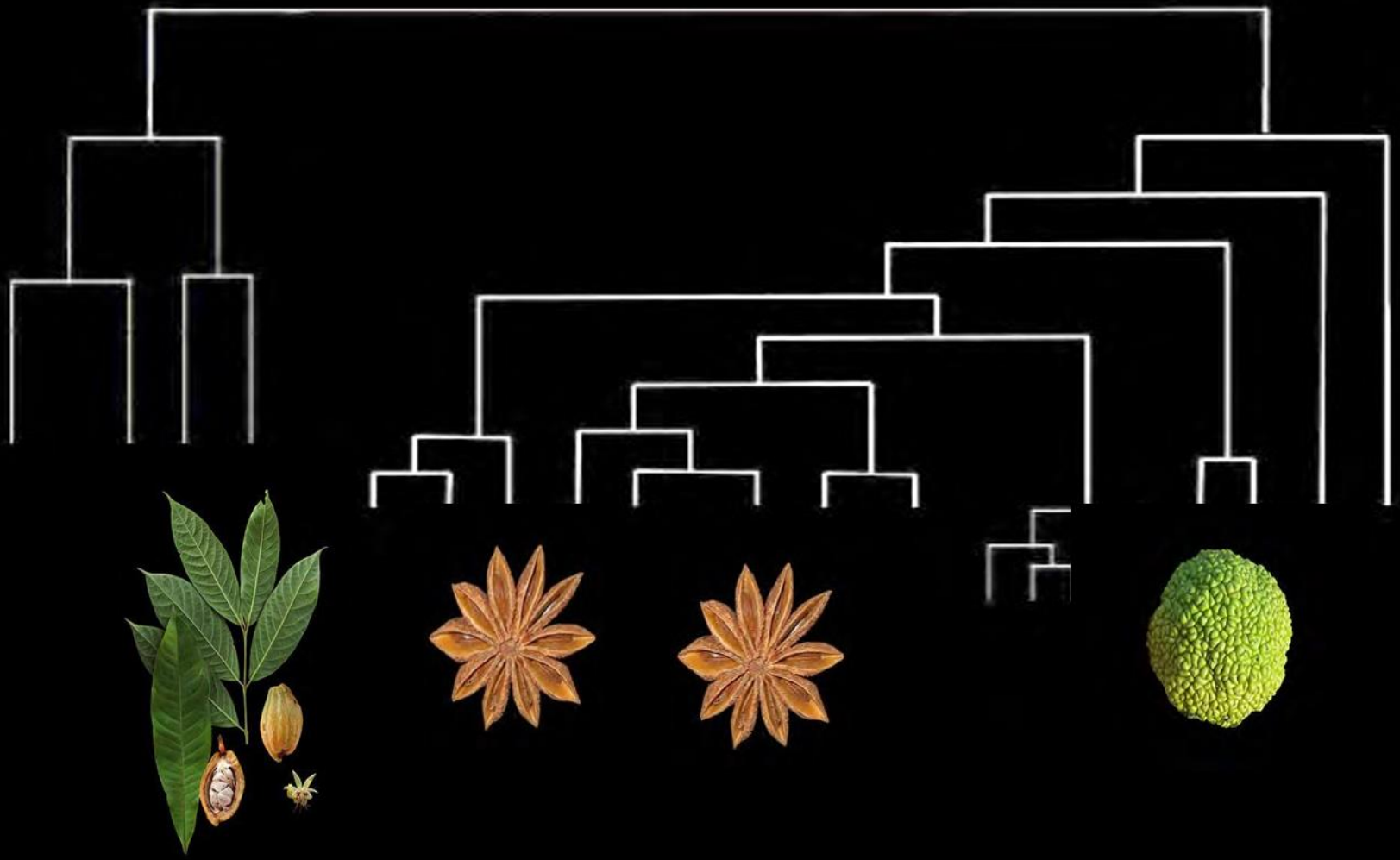
GenomeTRAKRcp

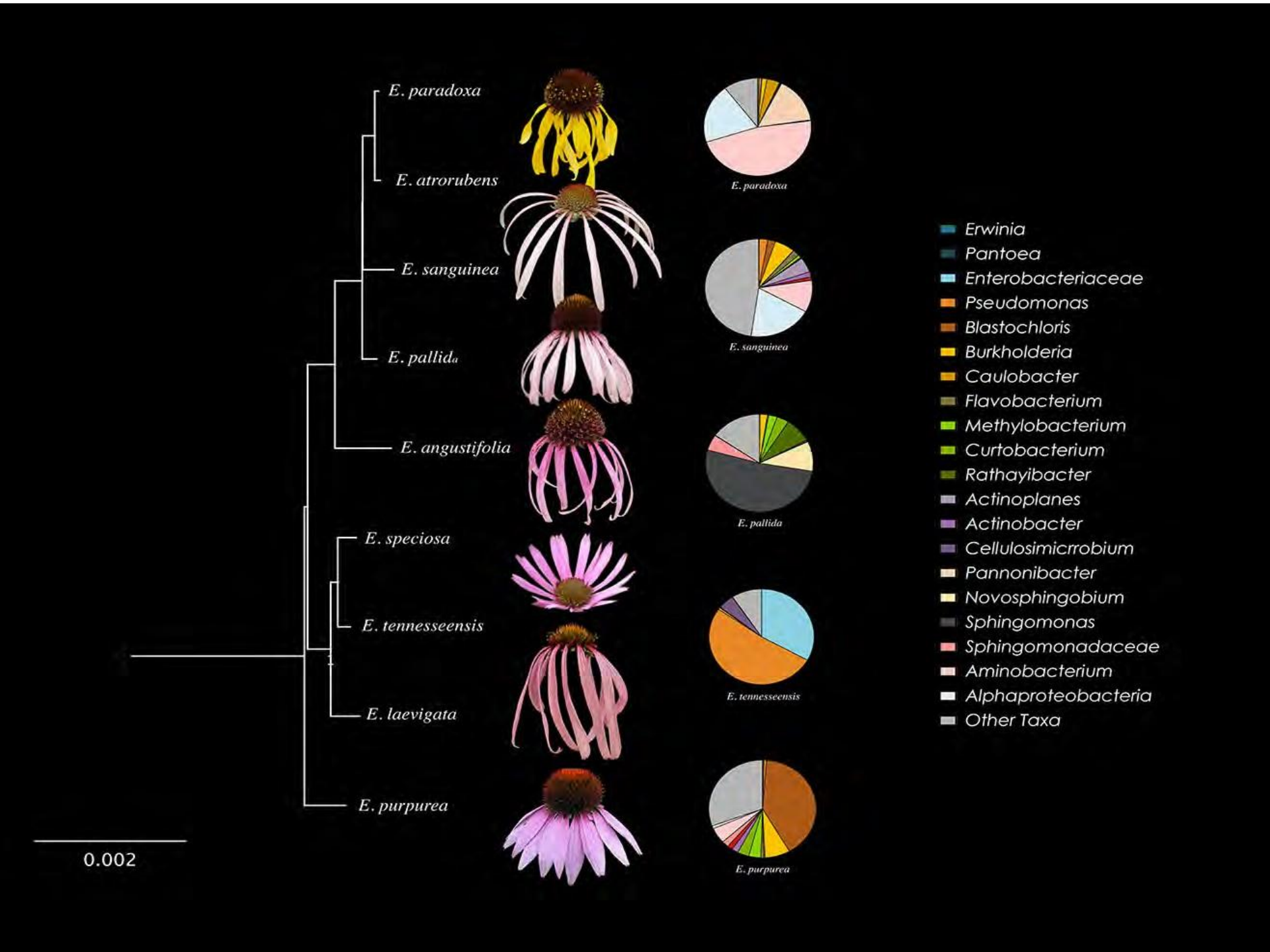


Sara Handy, Ning Zhang, Padmini Ramachandran, Yan Luo, Ruth Timme, Andrea Ottesen

GenomeTrakrCP (Chloroplasts)





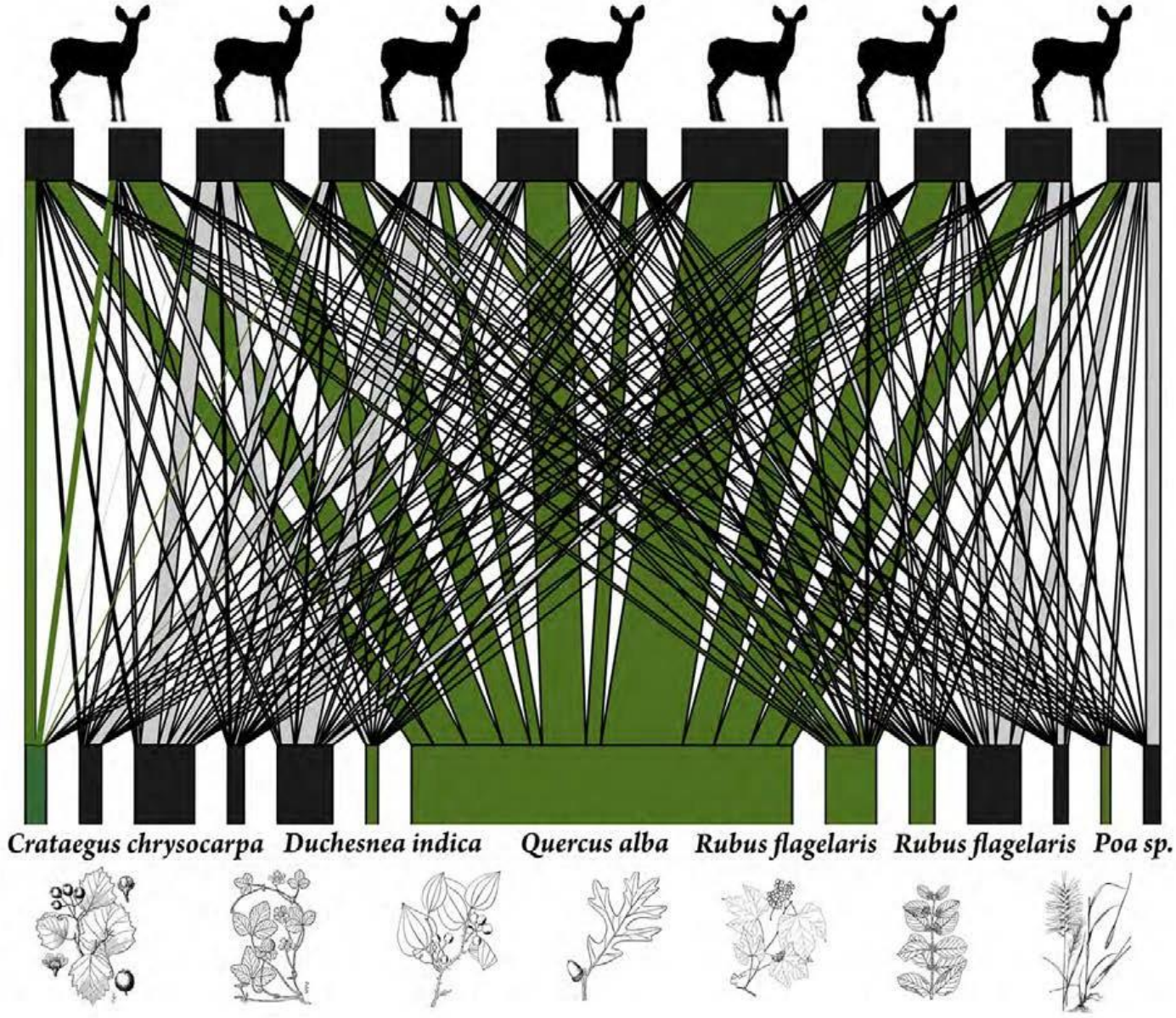

E. paradoxa
E. atrorubens
E. sanguinea
E. pallida
E. angustifolia
E. speciosa
E. tennesseensis
E. laevigata
E. purpurea
0.002
E. paradoxa
E. sanguinea
E. pallida
E. tennesseensis
E. purpurea
Erwinia
Pantoea
Enterobacteriaceae
Pseudomonas
Blastochloris
Burkholderia
Caulobacter
Flavobacterium
Methylobacterium
Curtobacterium
Rathayibacter
Actinoplanes
Actinobacter
Cellulosimicrrobium
Pannonibacter
Novosphingobium
Sphingomonas
Sphingomonadaceae
Aminobacterium
Alphaproteobacteria
Other Taxa

bacterial fingerprints of foods and dietary supplements

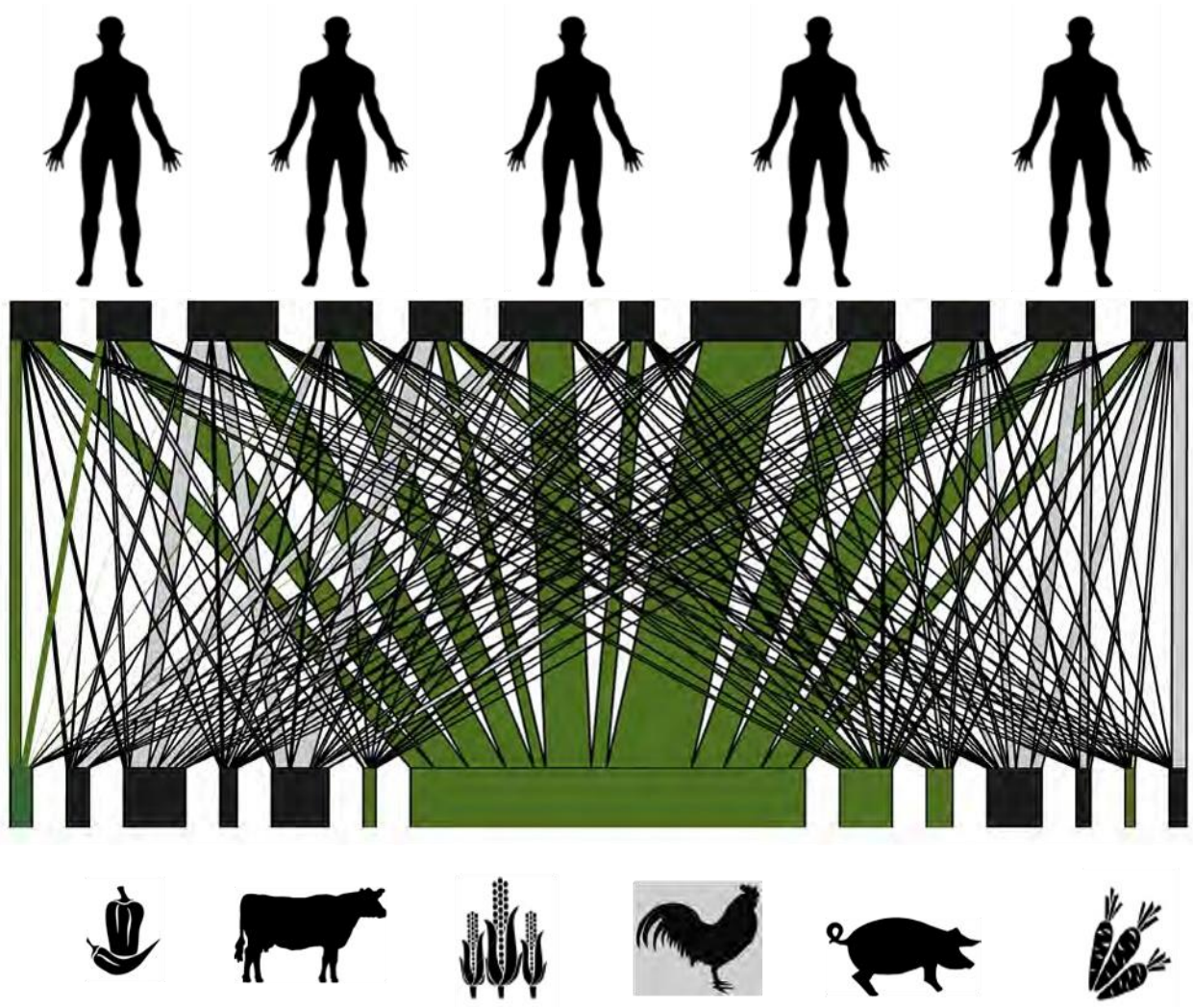
capsule tea

- *Erwinia*
- *Pantoea*
- *Enterobacteriaceae*
- *Pseudomonas*
- *Blastochloris*
- *Burkholderia*
- *Caulobacter*
- *Flavobacterium*
- *Methylobacterium*
- *Curtobacterium*
- *Rathayibacter*
- *Actinoplanes*
- *Actinobacter*
- *Cellulosimicrrobium*
- *Pannonibacter*
- *Novosphingobium*
- *Sphingomonas*
- *Sphingomonadaceae*
- *Aminobacterium*
- *Alphaproteobacteria*
- *Other Taxa*

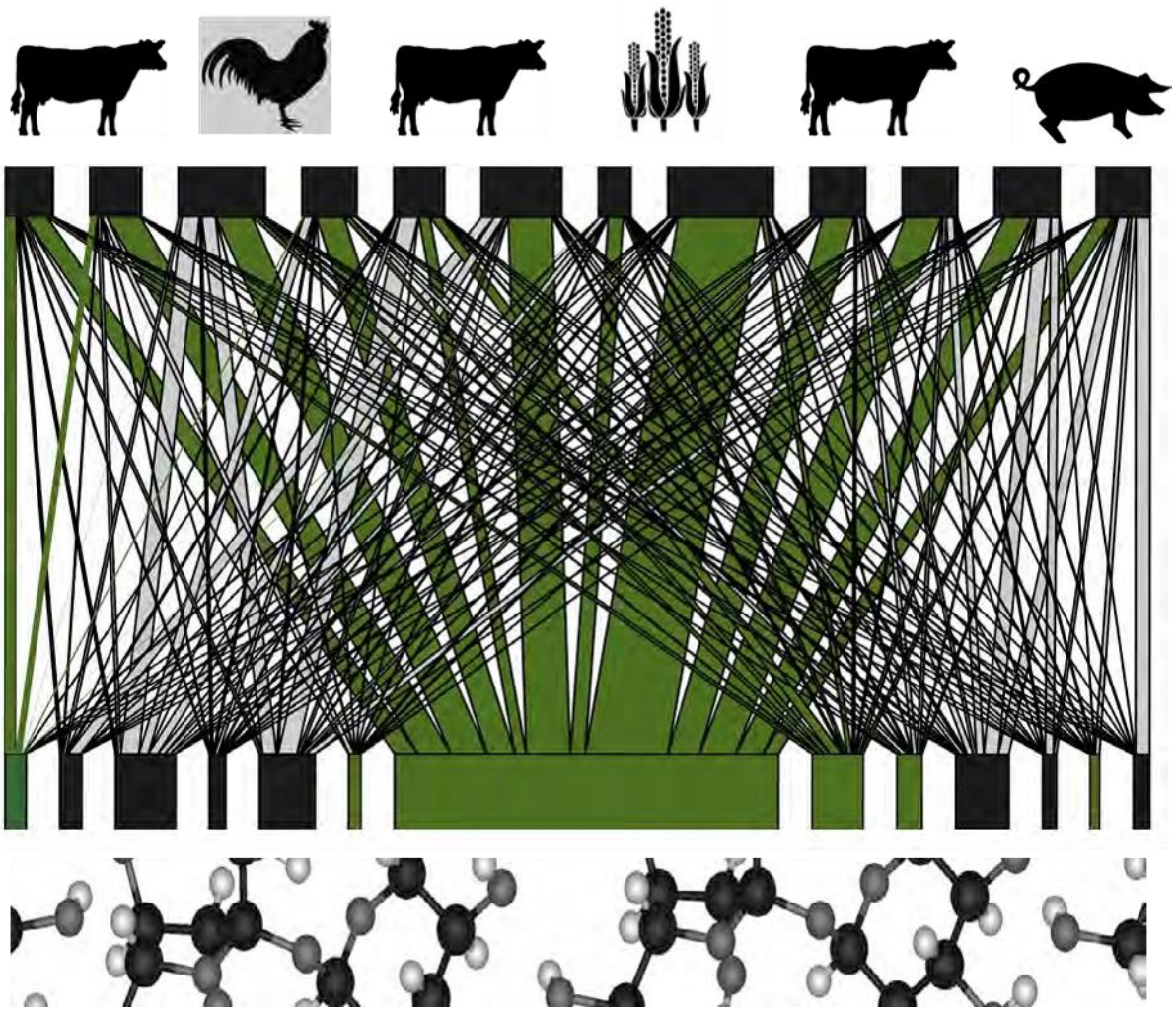
Deer Diet


Crataegus chrysocarpa
Duchesnea indica
Quercus alba
Rubus flagelaris
Rubus flagelaris
Poa sp.


Introduced
Native



and what was our food fed?



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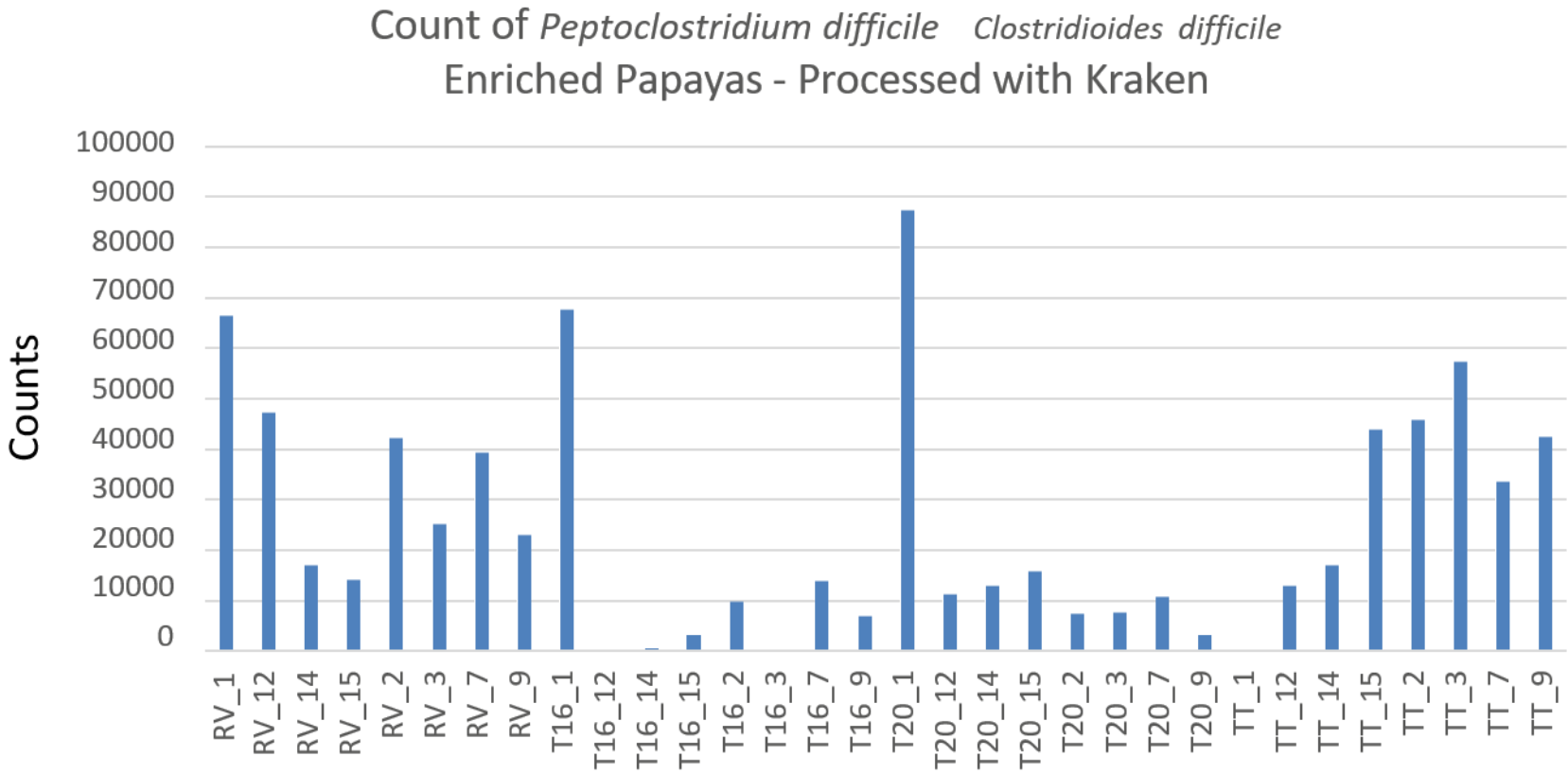
Marc Allard
Steven Musser
Kelly Bunning
John Callahan

Jennifer Beal
Brooke Whitney
David Ingram
April Hill
Paul Morin

C. diff in Papaya's from Mexico



Dr. Michael Bazaco and Dr. Beverly Wolpert Office of Analytics and Outreach