Microbe-managing: Manipulating the Human Gut Microbial Ecosystem to Enhance Health

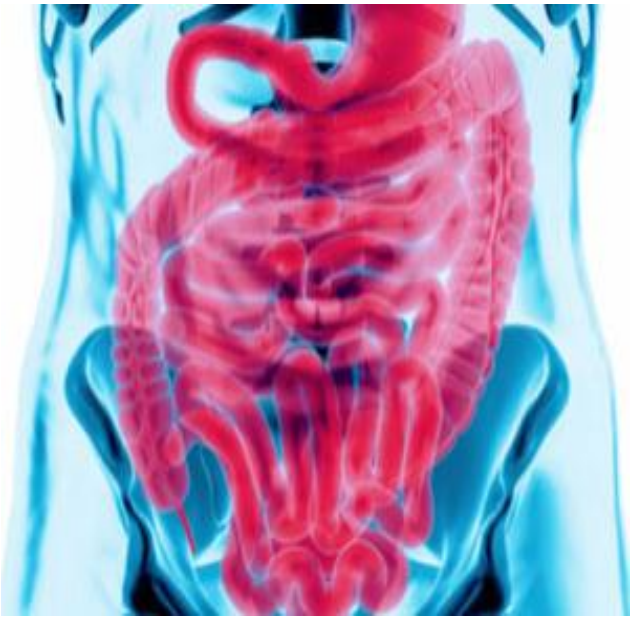
UNIVERSITY of GUELPH

CHANGING LIVES IMPROVING LIFE

Emma Allen-Vercoe

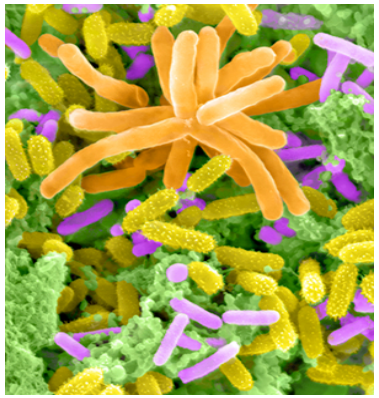
AMMI CANADA – CACMID ANNUAL CONFERENCE

April 5th 2014



Conflict of Interest Disclosure Slide

In the past 2 years I have been an employee of	The University of Guelph
In the past 2 years I have been a consultant for	N/A
In the past 2 years I have held investments in the following pharmaceutical organizations, medical devices companies or communications firms	NuBiyota
In the past 2 years I have been a member of the Scientific advisory board for	N/A
In the past 2 years I have been a speaker for	N/A
In the past 2 years I have received research support (grants) from	National Institutes of Health, National Science and Engineering Research Council, US Department of Defense, Autism Research Institute, Physician's Services Incorporated, Ontario Ministry of Agriculture and Food, Crohn's and Colitis Canada, Canada Foundation for Innovation
In the past 2 years I have received honoraria from	University of Toronto, NYU, Western University
I agree to disclose approved and non-approved indications for medications in this presentation.	N/A
I agree to use generic names of medications in this presentation.	Yes

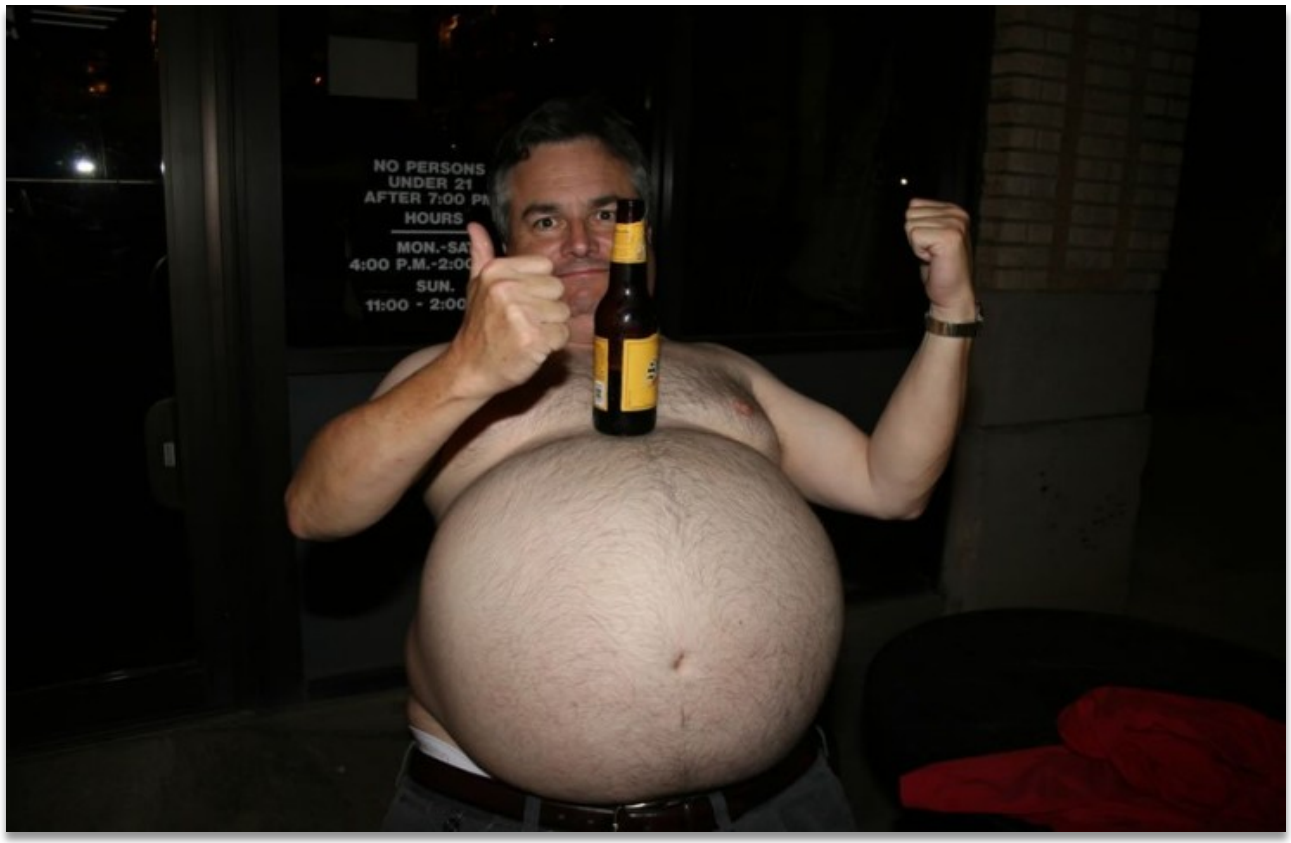


From: Joint Genome Institute

Microbes – on us, in us and all around us

- We are each colonized by millions of microbes
- Every surface of our bodies is a niche for an organized community of bugs
- Humans are 90% bacteria, 10% human!
- At least 100x more microbial genes associated with us than our own human genes
- Humans are the ‘spaceships’ operated by their microbes

There are more bacteria in your gut than there are people on the planet…



Gut bug diversity

- We each have around 500-1000 different bacterial species living in our guts
- Just as we each have unique DNA, fingerprints and iris patterns, we all have unique collections of microbial species in our guts

fingerprint

pooprint

Remarkably…

The bacterial community in your gut remains stable from

- weaning…

- …to old age

And we are only just starting to understand this homeostasis

It’s all about Balance!

http://www.gbposters.com

Maintaining the equilibrium

High diversity of species:
- Healthy ecosystem
- Functional redundancy
- Resistance to disease

Low diversity of species:
- Sick ecosystem
- Functional disability
- Susceptibility to disease

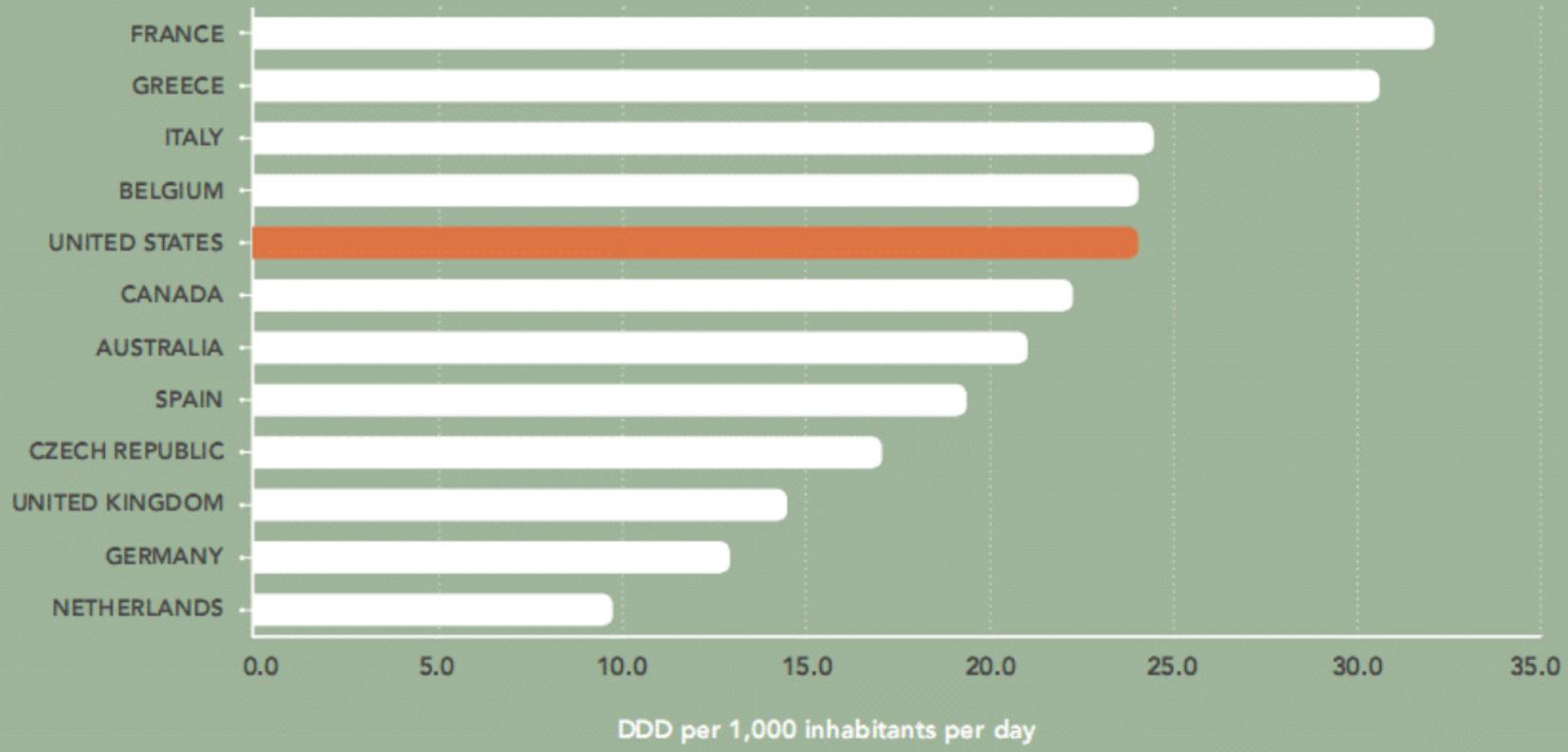
Our microbes are vitally important…

- But we are working very hard to exterminate them!

‘Extinction events’ may impact health

- Hygiene hypothesis
  - We are preventing proper colonization by being too clean
- Missing microbiota hypothesis
  - We are disturbing proper colonization across generations through e.g. antibiotic use, & poor diet
- Antibiotic use (especially in early childhood) may be particularly problematic

The United States is among the most intensive users of antibiotics in the world



Sources: United States and Canada (McManus, Hammond et al. 1997), Australia (National Prescribing Service 2005), European countries (Goossens, Ferech et al. 2003).

Note: DDD = defined daily doses, a standardized measure of antibiotic consumption.

CDDEP THE CENTER FOR Disease Dynamics, Economics & Policy
RESOURCES FOR THE FUTURE


P. DUREUIL/PHOTOALTO/ALAMY

Dosed up: could excessive prescription of antibiotics be hampering children's ability to fight disease?

Stop the killing of beneficial bacteria

Concerns about antibiotics focus on bacterial resistance — but permanent changes to our protective flora could have more serious consequences, says **Martin Blaser**.

Nature Volume:476, Pages:393–394 Date published:25 August 2011

Consequences of collateral damage

- Several studies have shown:
  - Gut microbial flora changes significantly with antibiotic use
  - Takes a long time afterwards to return to baseline
  - Sometimes does not return to baseline at all
  - Repeated ‘hits’ cause vast changes from which the ecosystem does not recover

Antunes LC, Finlay BB. Gut Microbes. 2011; Robinson CJ, Young VB. Gut Microbes. 2010; Jakobsson HE, *et al*. PLoS One. 2010; Antonopoulos DA *et al*., Infect Immun. 2009; Dethlefsen *et al*. PLoS Biol. 2008.

The additional impact of the Western diet

- Average Western diet – rich in refined foods, low in fermented foods, complex carbohydrates, fibre
- Refined foods are easily broken down in the upper GI tract
  - Thus very little left-over food makes it to the colon
- Colon is the site of most beneficial gut microbial activity
  - Starvation of this community can lead to ecosystem damage
  - ‘extinction events’ and reduced diversity


Save our Rainforests


Save our gut microbiota

Examples of diseases associated with reduced gut microbiota diversity

(published research)

Inflammatory bowel diseases

Infant colic

Eczema

Autism

Colorectal cancer

Allergic asthma

Celiac disease

Obesity

Neonatal necrotizing enterocolitis

Irritable Bowel Syndrome

***Clostridium difficile* infection**

- Lack of microbial diversity
- Loss of ‘keystone’ species
- Overgrowth of opportunistic pathogens
- Poor diet/lifestyle
- Drug interactions

“Dysbiosis”

Looking inside the black box is the key to understanding disease

DISEASE

To understand disease, we need to understand health

- What are the microbes that make up a ‘healthy’ gut microflora?
- What jobs do these microbes do for us?
- What happens if they are missing?
- Can we replace missing gut microbes?
  - If so, how?

The good, the bad and the ugly

- **The Good**
- Lactic Acid Bacteria (LAB)
  - E.g. *Bifidobacterium* and *Lactobacillus* spp.
- Butyrate-producing bacteria
  - E.g. *Faecalibacterium prausnitzii, Roseburia* spp.

- **The Bad**
- Opportunistic pathogens
  - E.g. *E.coli, Pseudomonas aeruginosa, Clostridium difficile, Bacteroides fragilis*
- Sulfate-reducing bacteria
  - E.g. *Desulfovibrio* spp.

The Ugly: it really is not that clear-cut!!

Everyone is different

- Gut microbial ecosystems are highly variable in composition and abundance profiles

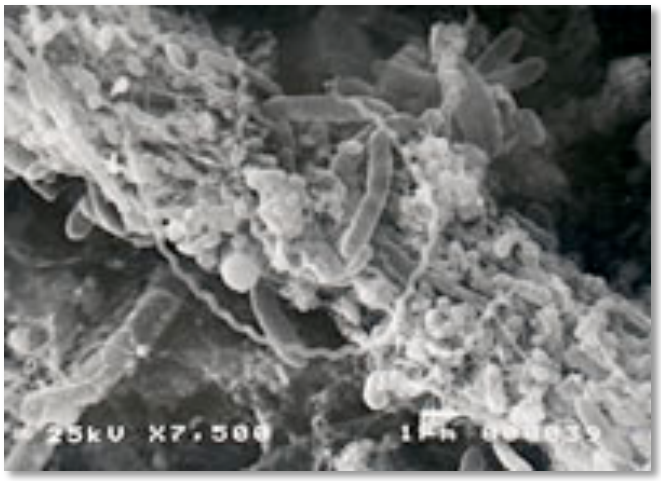
- However…
- Ecosystem function is preserved across individuals
  - It's not about what species are there, but about what the species are doing
- To understand health, we need to study gut *ecosystems*
  - We have only accessed the biology of ~30-50% of the gut microbiota
  - Remainder are 'unculturable'

Why don’t we know more than we do?

- Most of the microbes in the gut are strict anaerobes
- Require specific conditions (and equipment) to culture them
- Even then, many species refuse to grow in the lab

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



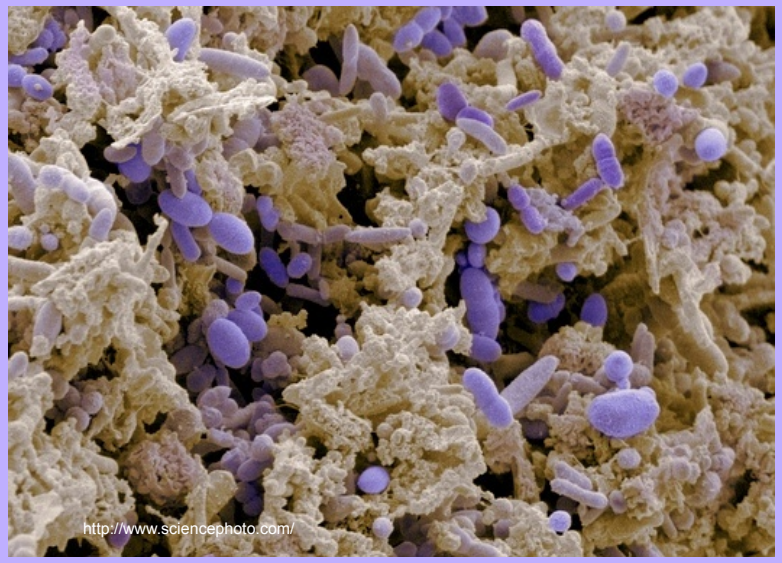
Microbes in a microbiology lab…

•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

Just like teenagers: Microbes behave differently when on their own

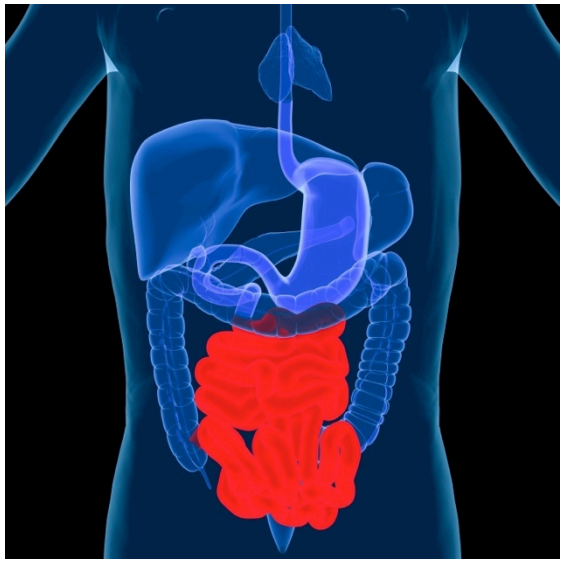
…They are usually happier when with their friends!

The human gut microbiota is a complex microbial ecosystem



Function and behaviour of this ecosystem is *best studied as a whole*

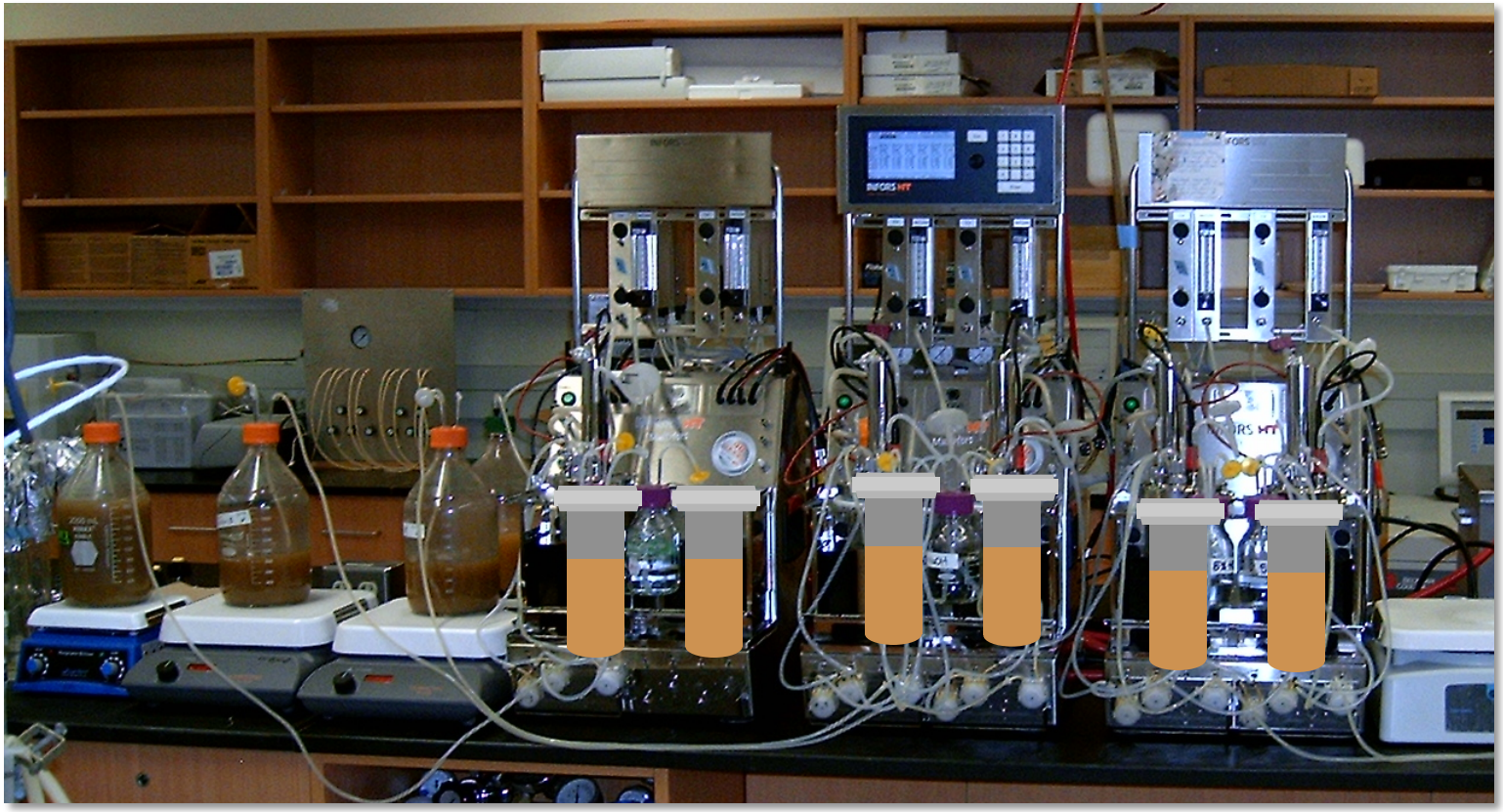
Growing microbes in communities



The human gut is a type of 'chemostat'

Rate of medium in = rate of medium out

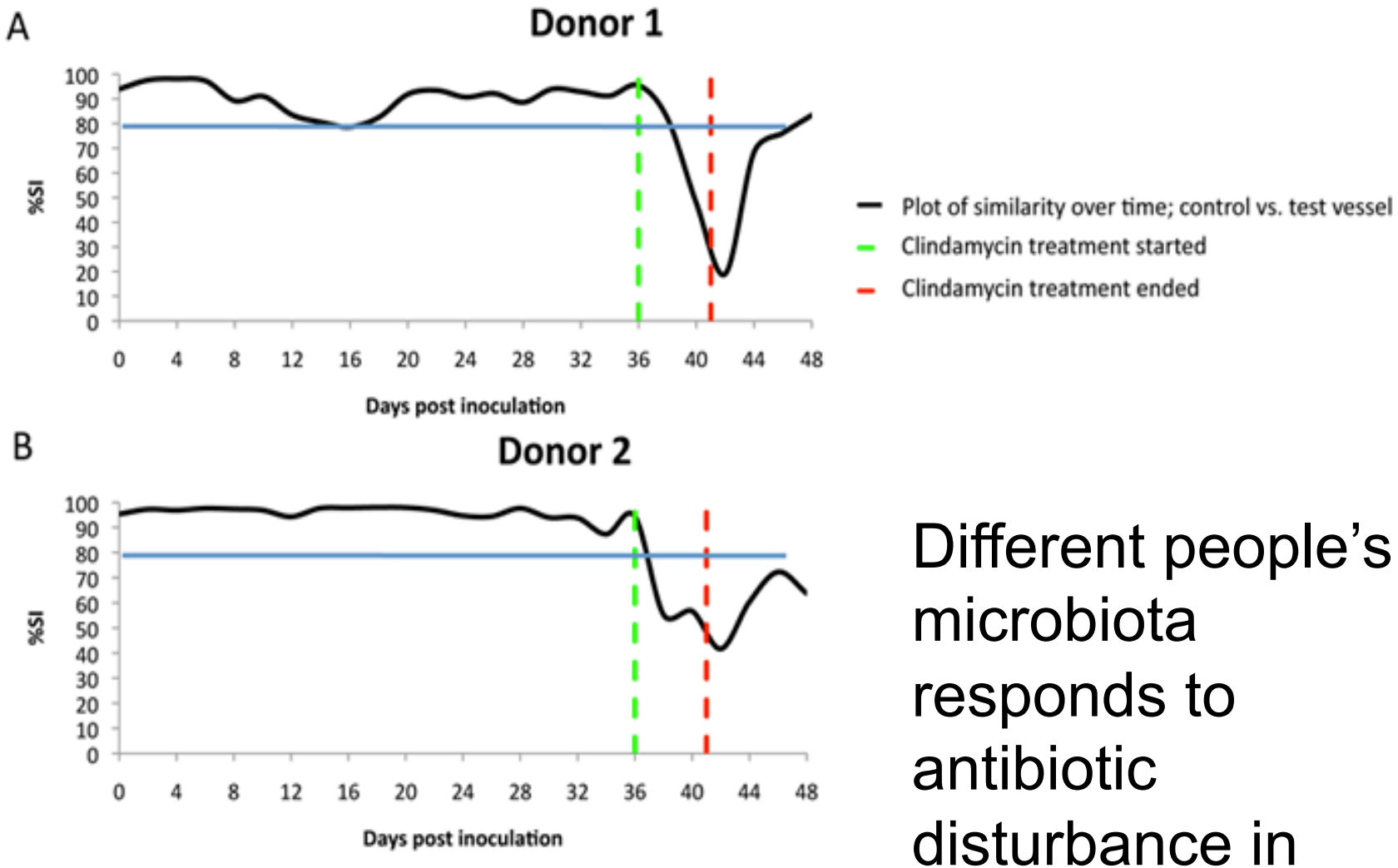
Our “Roboguts”



- Seeded with fresh feces and set to model the distal gut ecosystem
- Host-free system
- Can be used to support growth of fastidious gut anaerobes

“Liquid gold”

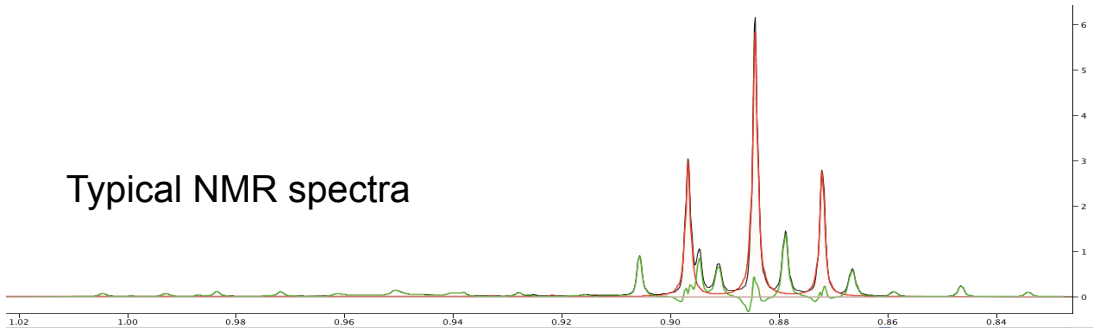

5000 mL
KIMAX
KIMBLE
EA-V
NO. 14395
APPROXIMATE VOLUMES
4000
3500
3000
2500
2000
1500
1000



Different people's microbiota responds to antibiotic disturbance in different ways

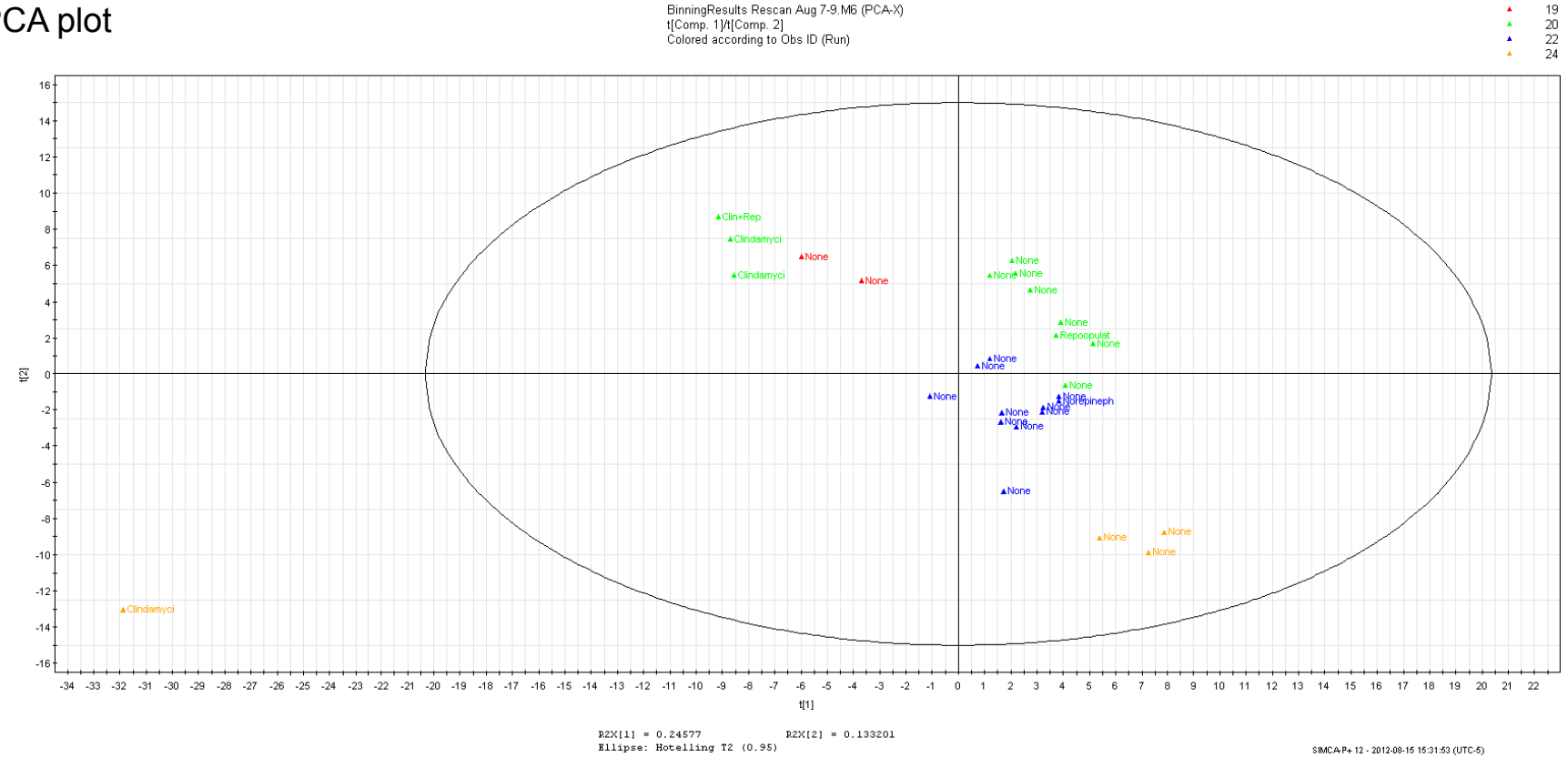
^{1}H NMR on ‘liquid gold’ samples

Typical NMR spectra



Marc Aucoin,
U Waterloo

PCA plot



Liquid gold derived from different donors produces metabolic profiles unique to the the respective hosts

So, if human health depends on microbiota health…

…how can we modulate the gut microbiota to improve health?

Why not just use existing probiotics?

- **Pros**
- ‘Generally regarded as safe’
- Many naturally ferment foodstuffs
- May have beneficial effects as they transition through the intestine
- Currently very popular

- **Cons**
- Not policed well
  - Many do not live up to their claims
- Can be very expensive
- No ‘one-size fits all’ probiotic
  - But often marketed this way
- Do not colonize; no lasting effects

The layperson's view of probiotics…

The microbial ecologist's view of probiotics

Normal gut microbiota

vs.

Probiotic

Microbial Ecosystem Therapeutics, MET

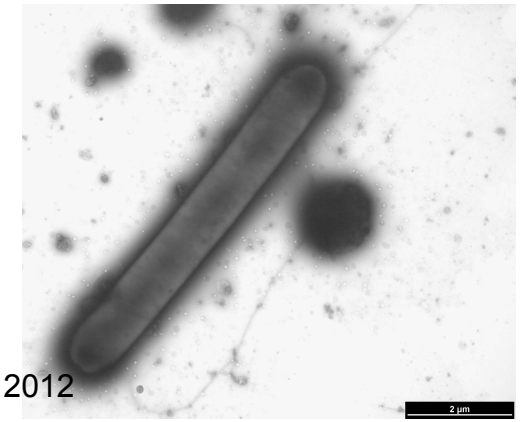
- Dysfunctional ecosystems have been associated with many diseases
  - Cause or effect not yet fully understood
- Can we cure disease by replacing a damaged microbiota?

- One disease we know is caused by gut ecosystem disturbance is *Clostridium difficile* infection, CDI

Ecosystem damage and CDI

http://www.google.com

Clostridium difficile:
Strictly anaerobic, spore-forming, Gram positive gut anaerobe



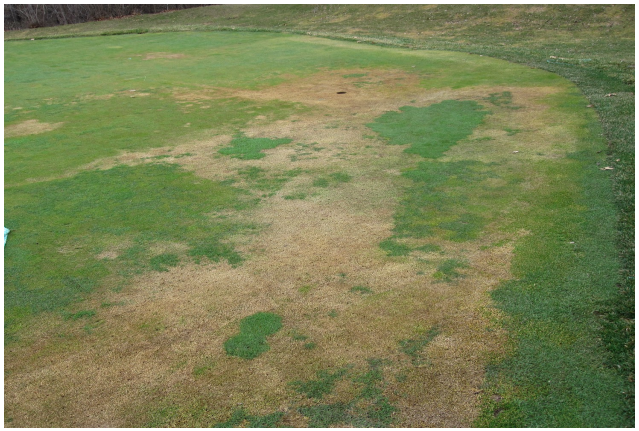
C.Carlucci, A-V lab, 2012

- Lack of diversity in the gut ecosystem allows overgrowth of *Clostridium difficile* in the niche
  - Toxin production ⇧, colitis ensues

The healthy lawn analogy

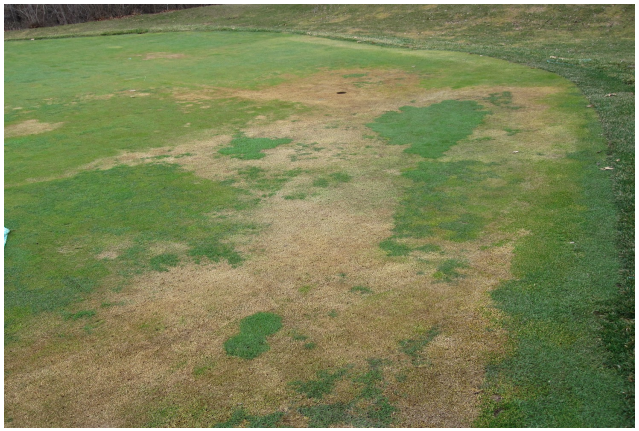
A healthy gut microbiota is like a healthy lawn:
Lush growth, no room for weeds

The healthy lawn analogy



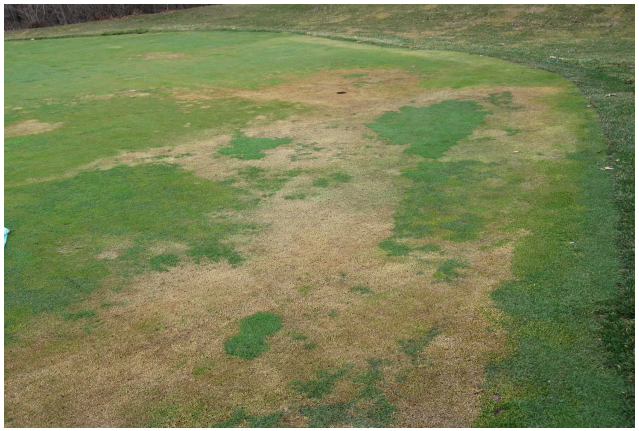
When the lawn is stressed, e.g during drought, damage ensues

The healthy lawn analogy



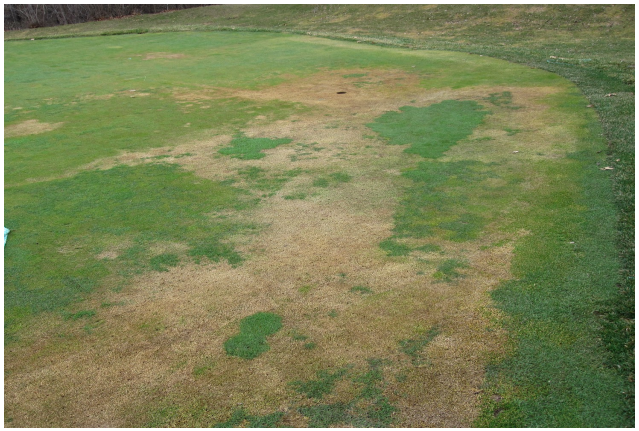
If you're unlucky, weeds can move in before the lawn recovers from the stress

The healthy lawn analogy



Applying more damage to the lawn is one way to get rid of the weeds

The healthy lawn analogy



Another approach is to replace the damaged turf with new, healthy growth

Fecal transplant/fecal bacteriotherapy (aka “re-turfing”)

CBC *This Hour has 22 Minutes,* Oct 2012

- Donor selected
- Usually close family member
- Screened for range of diseases that are potentially passed on through stool
- If ‘pass’, donation time coordinated with patient drug taper

- Fresh homogenate instilled into patient within 6 hrs of preparation
  - Rectal enema
  - Colonoscopy
  - Nasoduodenal tube
  - "Poop pills"

- Results in cure of the patient in >90% of cases
- Rapid resolution of CDI
- Only rare recurrence of disease

Pros and cons of fecal transplants

- Pros:
  - They work! ~90% of patients are cured of CDI
    - Van Nood *et al*., NEJM 2013
  - They're comparatively cheap

- Cons:
  - Somewhat primitive
  - Undefined; will vary donor to donor
    - How do you know who's healthy?
  - Despite screening, still much potential for spread of pathogens
  - They're gross – lots of psychological stigma

Can we use cultured microbes to make 'fake poop'?

- Collaboration with Dr. Elaine Petrof, Queen's University
- Plan: to develop the fecal transplant concept further by using pure bacteria – 'probiotics'
  - But not your average probiotic: "RePOOPulate"!
- By doing so should mitigate fears about:
  - Safety
  - Reproducibility
  - Delivery
  - Shelf-life
- "Microbial Ecosystem Therapeutics" (MET)

- Not really a new idea
  - But in the past, barrier to this was perceived unculturability of gut bacteria

Our healthy donor

- Healthy female in her early 40s
- average BMI
- Very healthy lifestyle
- very few or no antibiotic exposures in childhood
- 1 reported exposure to antibiotics in the last 10 years
- Cultured >70 strains from poop sample using Robogut…
  - formulated RePOOPulate (33 strains)

“RePOOPulate”

- *Acidaminococcus intestinalis*
- *Bacteroides ovatus*
- *Bifidobacterium adolescentis* (x2)
- *Bifidobacterium longum* (x2)
- *Collinsella aerofasciens*
- *Dorea longicatena* (x2)
- *Escherichia coli*
- *Eubacterium eligens*
- *Eubacterium limosum*
- *Eubacterium rectale* (x4)
- *Eubacterium ventriosum*
- *Faecalibacterium prausnitzii*
- *Lactobacillus casei*
- *Lactobacillus paracasei*
- *Parabacteroides distasonis*
- *Raoultella* sp.
- *Roseburia faecalis*
- *Roseburia intestinalis*
- *Ruminococcus torques* (x2)
- *Streptococcus mitis*
- Likely novel species (x5)
- Likely novel genus & species (x1)

(Closest species by full-length 16S alignment)
Formulation tested for ecosystem stability in our Robogut

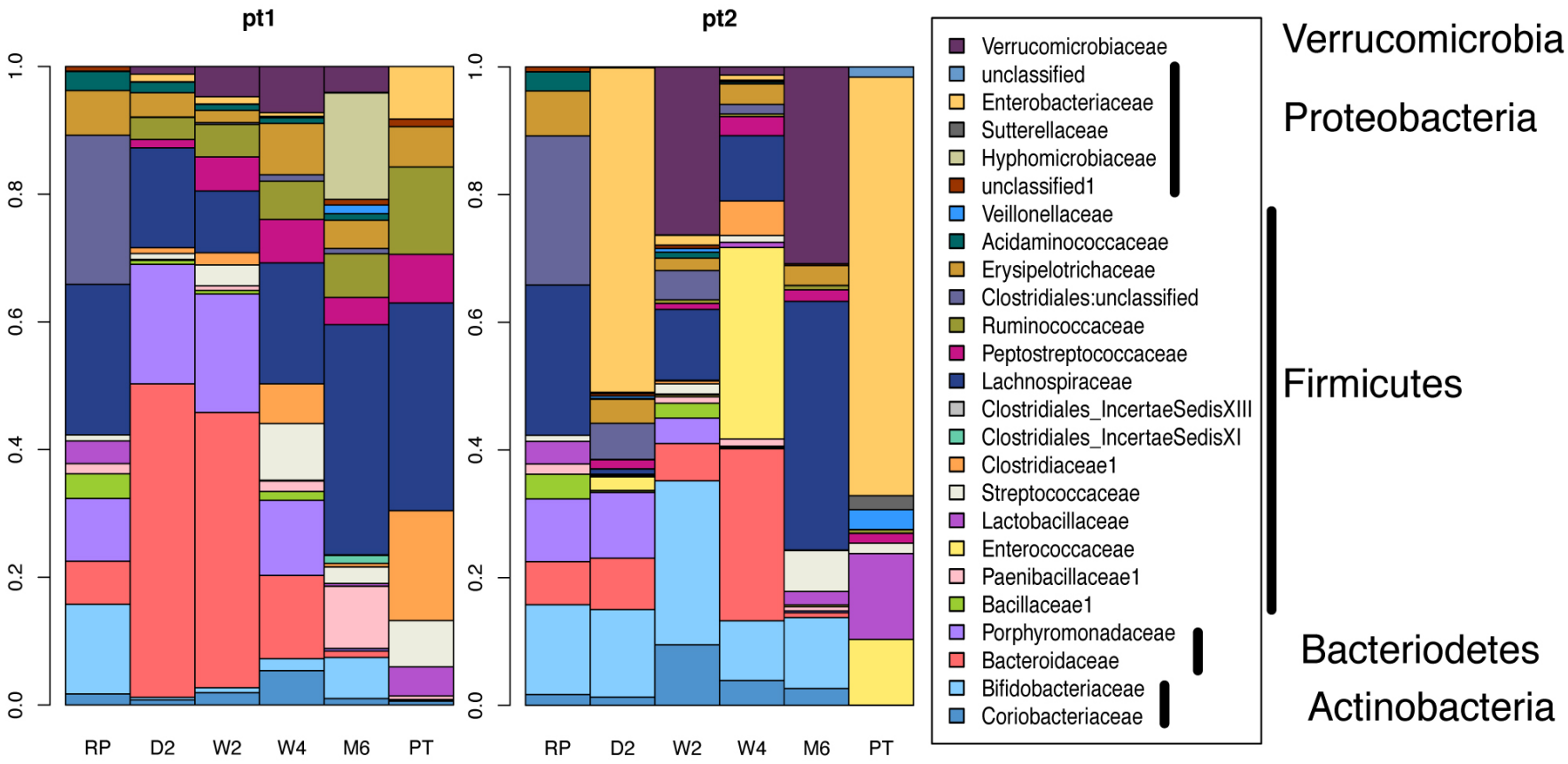
“RePOOPulate”

- *Acidaminococcus intestinalis*
- *Bacteroides ovatus*
- *Bifidobacterium adolescentis* (x2)
- *Bifidobacterium longum* (x2)
- *Collinsella aerofasciens*
- *Dorea longicatena* (x2)
- *Escherichia coli*
- *Eubacterium eligens*
- *Eubacterium limosum*
- *Eubacterium rectale* (x4)
- *Eubacterium ventriosum*
- *Faecalibacterium prausnitzii*
- *Lactobacillus casei*
- *Lactobacillus paracasei*
- *Parabacteroides distasonis*
- *Raoultella* sp.
- *Roseburia faecalis*
- *Roseburia intestinalis*
- *Ruminococcus torques* (x2)
- *Streptococcus mitis*
- Likely novel species (x5)
- Likely novel genus & species (x1)

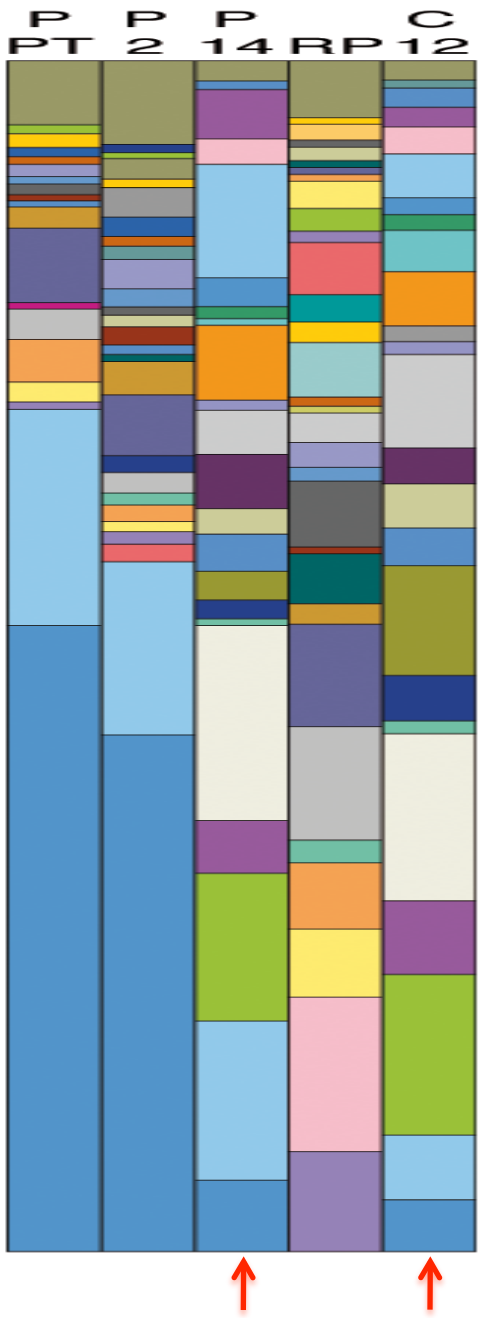
‘Lachnospiraceae’ family species

RePOOPulate proof-of-principle trial

- 2 elderly ladies with severe, recurrent *C.diff* infections were treated (April and June 2011)
- RePOOPulate made fresh at Guelph, driven to KGH, and administered via colonoscopy
  - 1 dose, 100mLs
- Both patients recovered within 2 days and have remained *C.diff*-free ever since (despite numerous subsequent antibiotic exposures)



Petrof *et al. Microbiome*, 2013



For patient 1, chemostat 16S rRNA gene profile after 12 days (steady state) closely matched patient profile after 14 days

The chemostat represents a good surrogate for the *in vivo* environment

This therapeutic ecosystem *colonized* our patients

SCIENTIFIC AMERICAN™

Fake Feces To Treat Deadly Disease: Scientists Find They Can Just Make Sh*t Up

By Christie Wilcox | January 10, 2013 | 4

I foresee a time when…

- Gut microbial ecosystem functional screening will be a critical component of all comprehensive medical check-ups
- It will be possible to enhance ecosystem functionality to maintain health by manipulating the microbiota and supporting these ecosystems with a tailored diet
- Broad spectrum antibiotics will not be used without measures to protect the microbiota
- "Symbiontology" will become a new medical specialty
  - A merger of Gastroenterology, infectious disease, microbial ecology and nutrition science (and many other specialties!)

Acknowledgements

EA-V lab members
Christian Ambrose
Ian Brown
Erin Bolte
Christian Carlucci
Kyla Cochrane
Michelle Daigneault
Kaitlyn Oliphant
Kathleen Schroeter
Mike Toh

Queen's University
Elaine Petrof
Adriana Breen
Curtis Noordhof
Julie McDonald
KGH clinical staff

U Western Ontario
Greg Gloor
Jean Macklaim

U Waterloo
Marc Aucoin
Sandi Yen

PSI FOUNDATION

Crohn's and Colitis Foundation of Canada
Fondation canadienne des maladies inflammatoires de l'intestin

SEAMO
Southeastern Ontario Academic Medical Organization

NSERC CRSNG

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

Canada Foundation for Innovation
Fondation canadienne pour l'innovation

Ontario Ministry of Agriculture, Food and Rural Affairs

Ontario Ministry of Research and Innovation

Questions?

I found the problem, Mr. Smith. Instead of probiotics, you have been taking amateur biotics.