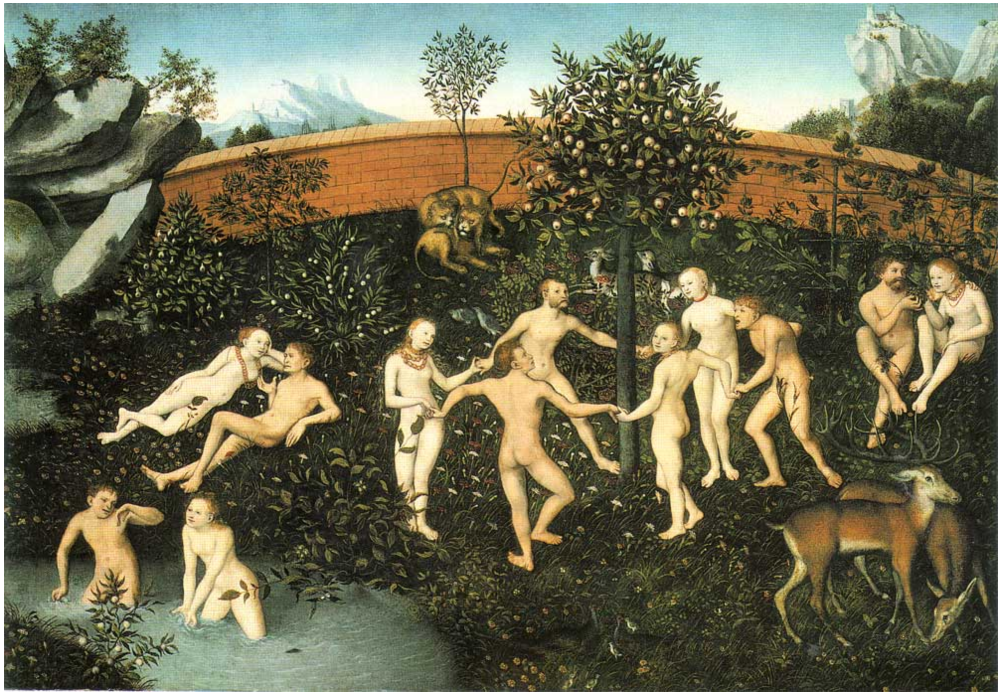
The Future of Microbiome Research??

Mad Science?

Utopia?



The Golden Age (Lucas Cranach the Elder)

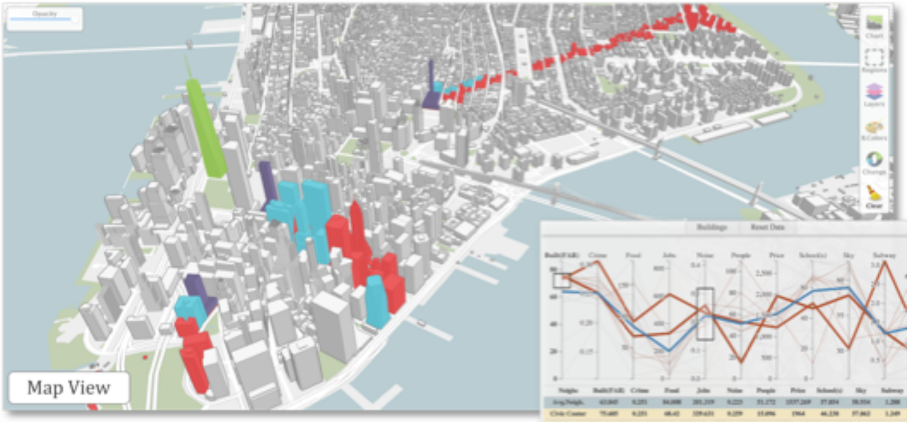
Lecture outline

1. How are **microbiome studies** informing us **now**?

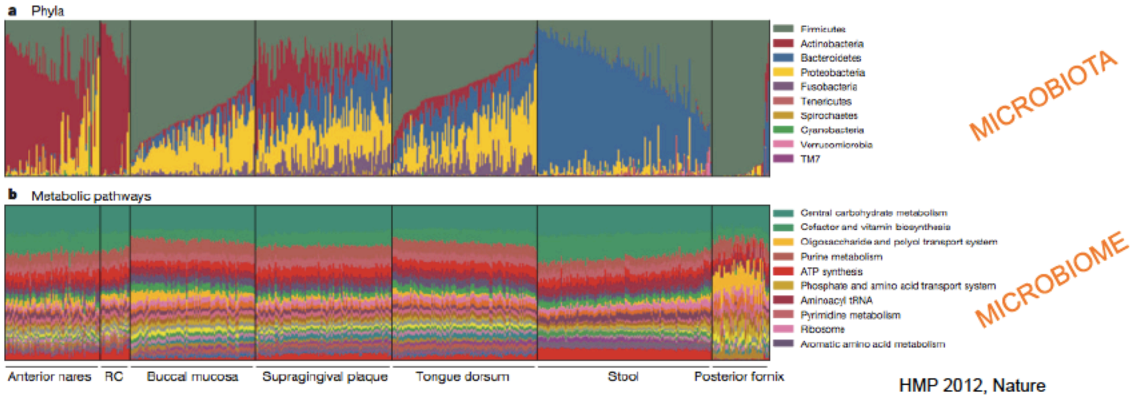
2. It's a **Brave New World**: the microbiome and new therapeutics (FMT)

3. The **Urban Microbiome**: characterizing, tracking, and mapping microbes across New York City

4. It's a **Cool New Techno-World**: microbial genomics on a USB-drive



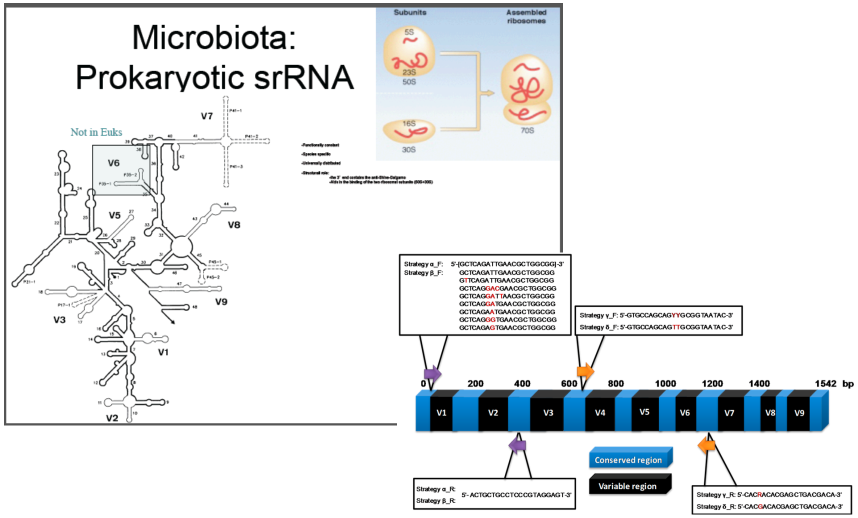
Terms and definitions



HMP 2012, Nature

Microbiota
The *microbes* inhabiting a particular environment

Microbiome
The collective *genes* of all the microorganisms inhabiting a particular environment



Strategy α_F: 5'-[GCTCAGATTGAACGCTGGCGG]-3'
Strategy β_F: GCTCAGATTGAACGCTGGCGG
GTTCAGATTGAACGCTGGCGG
GCTCAGGACGAACGCTGGCGG
GCTCAGGATTAACGCTGGCGG
GCTCAGGATGAACGCTGGCGG
GCTCAGAATGAACGCTGGCGG
GCTCAGGGTGAACGCTGGCGG
GCTCAGAGTGAACGCTGGCGG

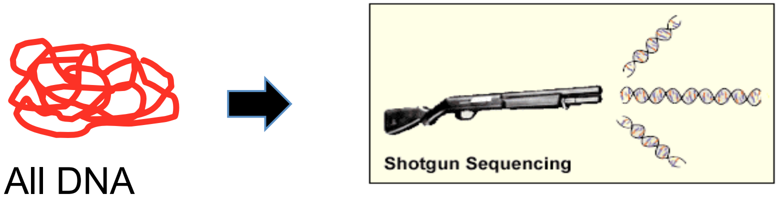
Strategy γ_F: 5'-GTGCCAGCAGYYGCGGTAATAC-3'
Strategy δ_F: 5'-GTGCCAGCAGTTGCGGTAATAC-3'

Strategy α_R:
Strategy β_R: 5'- ACTGCTGCCTCCCGTAGGAGT-3'

Strategy γ_R: 5'-CACRACACGAGCTGACGACA-3'
Strategy δ_R: 5'-CACGACACGAGCTGACGACA-3'

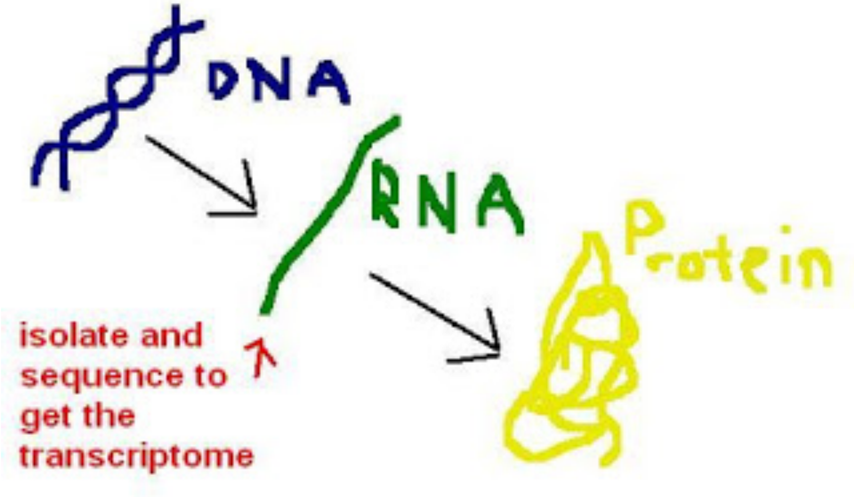
Amplicon sequencing
Single common gene amplified and sequenced, *e.g.*, 16S or 18S rRNA

Metagenomic sequencing
Shotgun-sequencing of ALL DNA in a sample & reconstruction of complete genomes



Terms and definitions

Meta-transcriptomics
How *active* the genes are in all the microorganisms inhabiting a particular environment

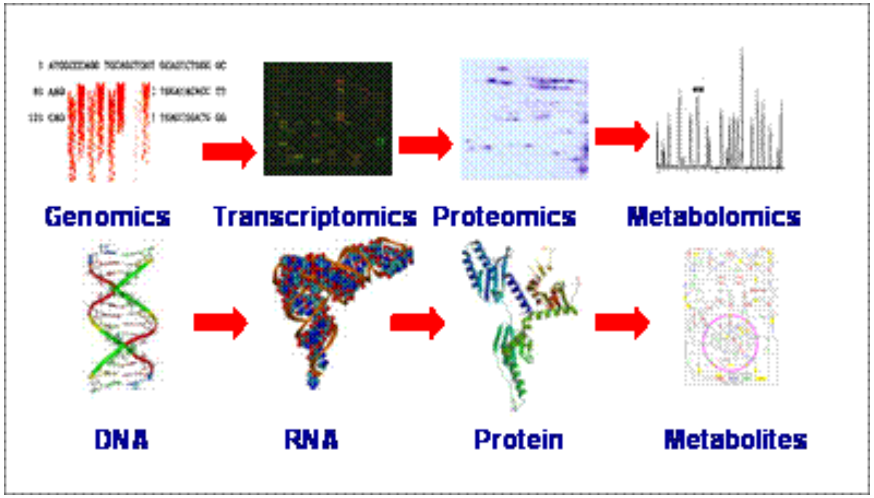


Meta-proteomics

What proteins are found in all the microorganisms inhabiting a particular environment and how they interact with each other

Metabolomics

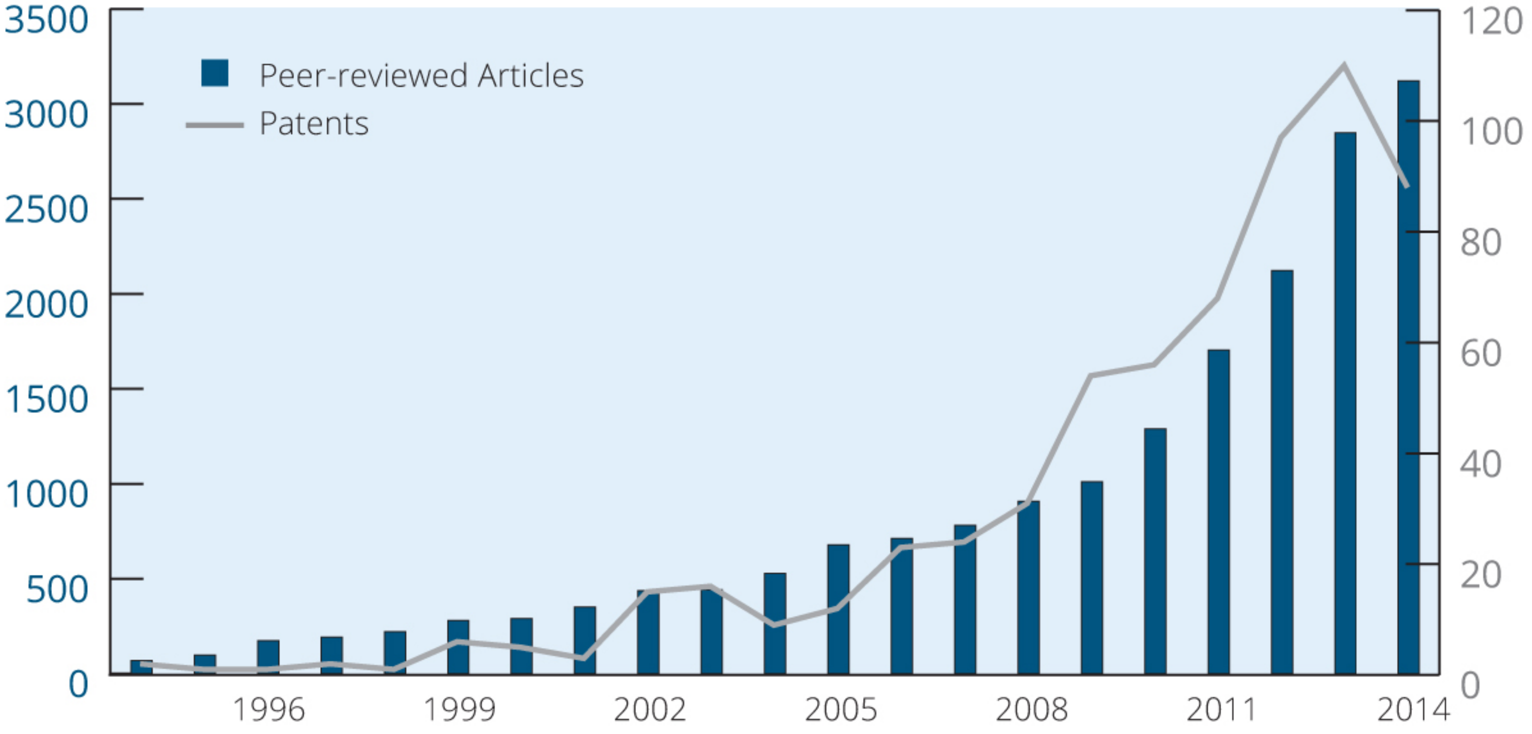
What metabolites are produced by in all the microorganisms inhabiting a particular environment



1. The Current Status of Microbiome Research

- A huge and rapid increase in the volume of publications that link health to the microbiome

- Microbiome research has also fueled a large number of patents



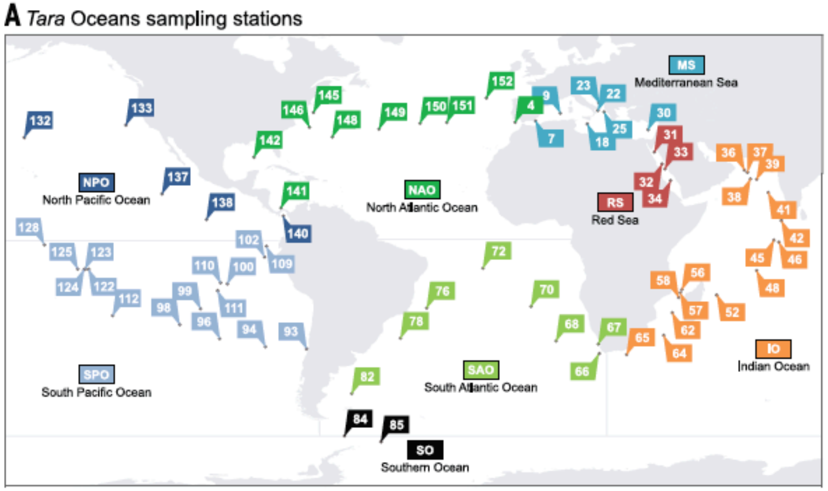
http://www.4inno.com/inno_insight/microbiome-a-scientific-revolution-in-the-making/

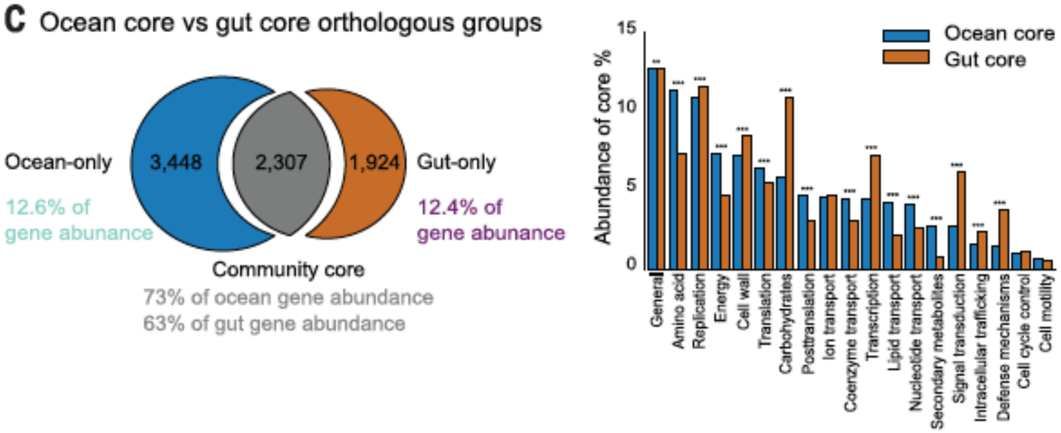
Wide variety of studies looking at microbiomes in humans, their pets and pests, livestock, wild-life and the environment

Science AAAS

Structure and function of the global ocean microbiome

Sunagawa et al., May 2015





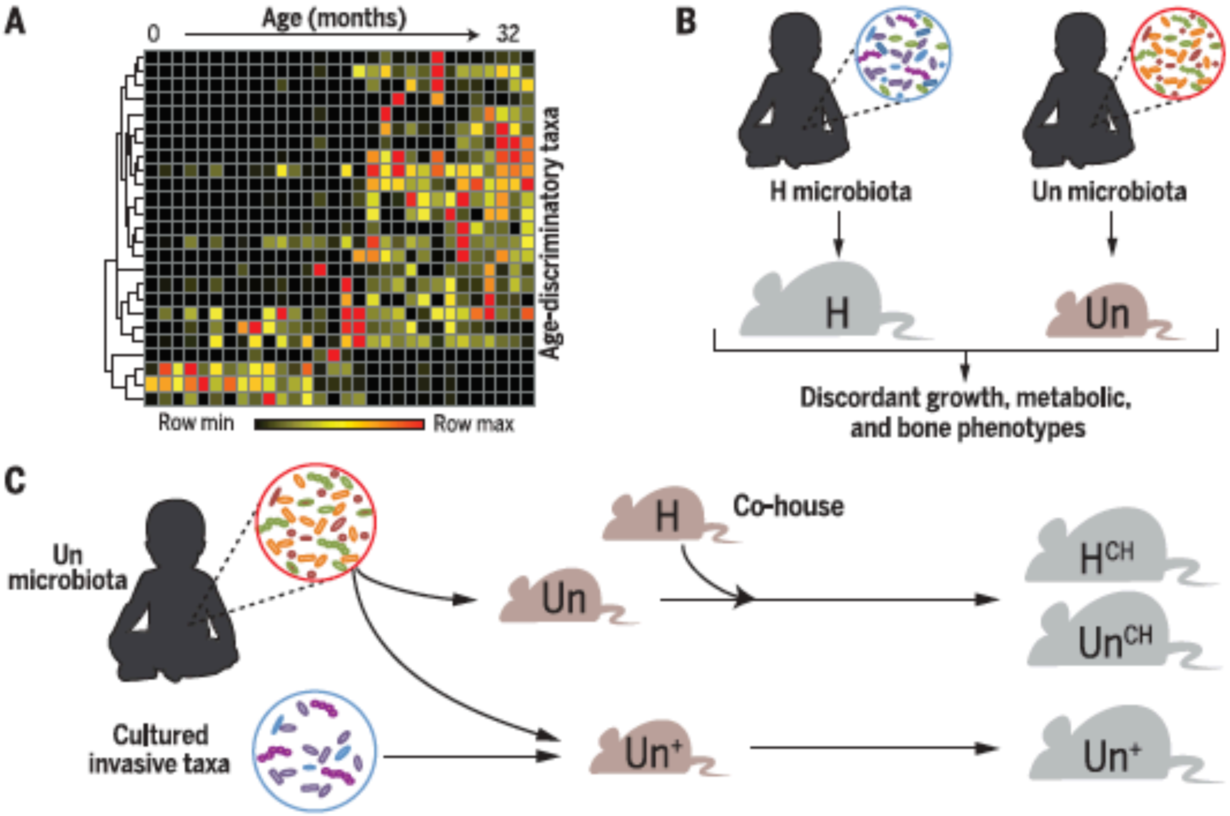
"We identify ocean microbial core functionality and reveal that >73% of its abundance is shared with the human gut microbiome despite the physicochemical differences between these two ecosystems."

Science AAAS

MICROBIOME

Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children

Laura V. Blanton, Mark R. Charbonneau, Tarek Salih, Michael J. Barratt, Siddarth Venkatesh, Olga Ilkaveya, Sathish Subramanian, Mark J. Manary, Indi Trehan, Josh M. Jorgensen, Yue-mei Fan, Bernard Henrissat, Semen A. Leyn, Dmitry A. Rodionov, Andrei L. Osterman, Kenneth M. Maleta, Christopher B. Newgard, Per Ashorn, Kathryn G. Dewey, Jeffrey I. Gordon*



Avershina *et al. Microbiome* (2015) 3:65
DOI 10.1186/s40168-015-0125-2

Microbiome

RESEARCH Open Access

Potential association of vacuum cleaning frequency with an altered gut microbiota in pregnant women and their 2-year-old children

Ekaterina Avershina[1], Anuradha Ravi[1], Ola Storrø[2], Torbjørn Øien[2], Roar Johnsen[2] and Knut Rudi[1]

Checinska *et al. Microbiome* (2015) 3:50
DOI 10.1186/s40168-015-0116-3

Microbiome

RESEARCH Open Access

Microbiomes of the dust particles collected from the International Space Station and Spacecraft Assembly Facilities

ka[1], Alexander J. Probst[2], Parag Vaishampayan[1], James R. White[3], Deepika Kumar[4],
, George E. Fox[4], Henrik R. Nilsson[5], Duane L. Pierson[6], Jay Perry[7] and Kasthuri Venkateswaran[1*]

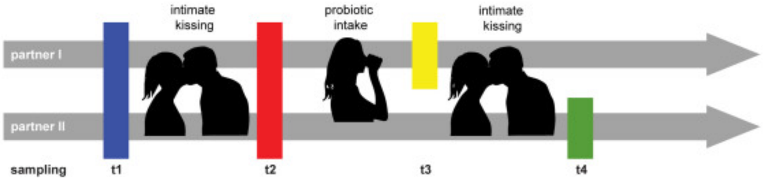
Shin *et al. Microbiome* (2015) 3:59
DOI 10.1186/s40168-015-0126-1

Microbiome

RESEARCH Open Access

The first microbial environment of infants born by C-section: the operating room microbes

Hakdong Shin[1], Zhiheng Pei[1,2], Keith A. Martinez II[1], Juana I. Rivera-Vinas[3], Keimari Mendez[3], Humberto Cavallin[4] and Maria G. Dominguez-Bello[1*]



Kort *et al. Microbiome* 2014, **2**:41
http://www.microbiomejournal.com/content/2/1/41

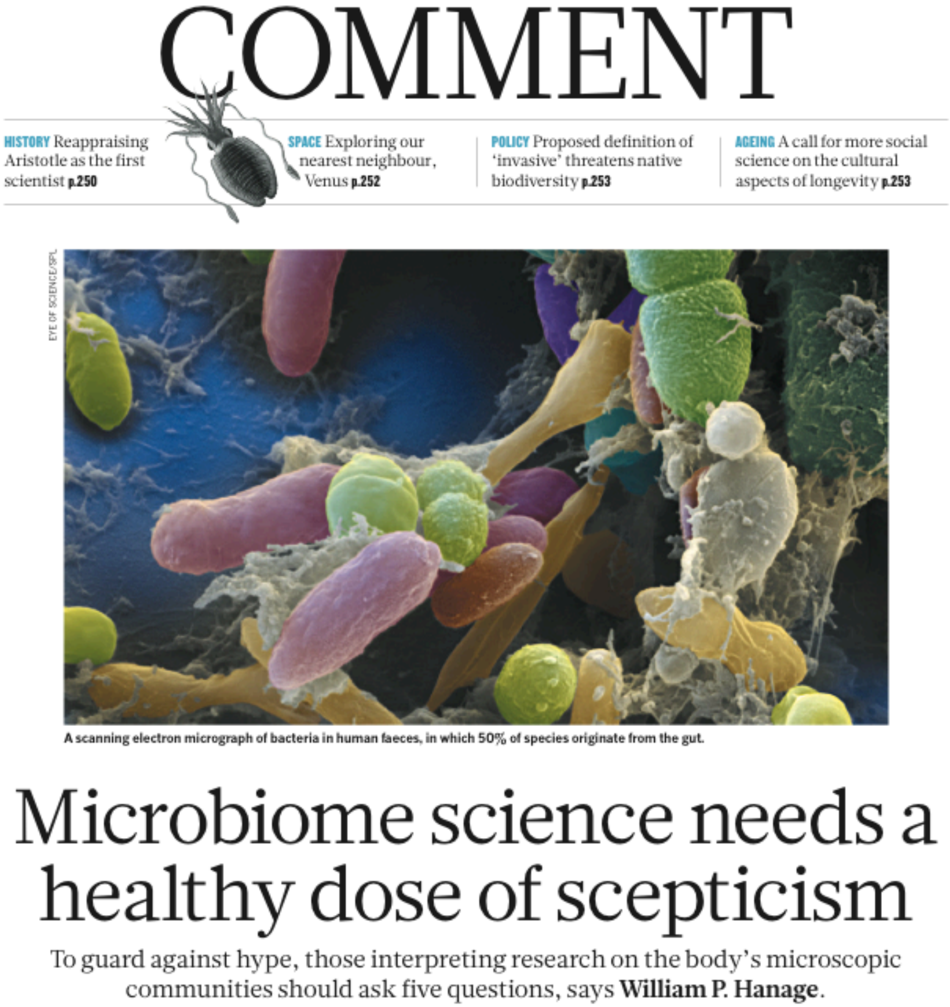
Microbiome

RESEARCH Open Access

Shaping the oral microbiota through intimate kissing

Remco Kort[1,2,3*], Martien Caspers[1], Astrid van de Graaf[2], Wim van Egmond[2], Bart Keijser[1] and Guus Roeselers[1]

Microbiome science: fad fueled by journalism or robust scientific discipline?



COMMENT

HISTORY Reappraising Aristotle as the first scientist **p.250**

SPACE Exploring our nearest neighbour, Venus **p.252**

POLICY Proposed definition of 'invasive' threatens native biodiversity **p.253**

AGEING A call for more social science on the cultural aspects of longevity **p.253**

EYE OF SCIENCE/SPL

A scanning electron micrograph of bacteria in human faeces, in which 50% of species originate from the gut.

Microbiome science needs a healthy dose of scepticism

To guard against hype, those interpreting research on the body's microscopic communities should ask five questions, says **William P. Hanage**.

Crucial questions

Five questions that anyone conducting or evaluating this research should ask to keep from getting carried away by hype:

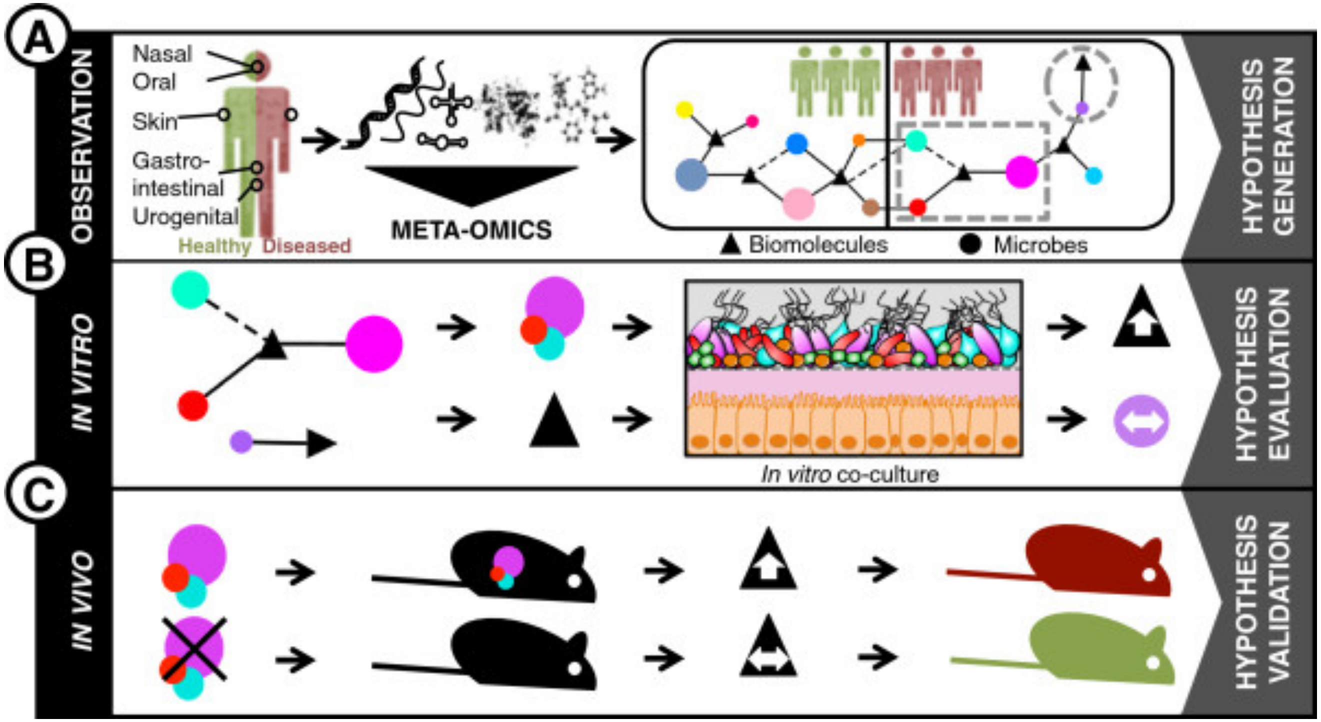
1. **Can experiments detect differences that matter?**
2. **Does the study show causation or just correlation?**
3. **What is the mechanism?**
4. **How much do experiments reflect reality?**
5. **Could anything else explain the results?**

From association to causality

- Microbiome studies produce data that are essential for defining <u>baseline healthy microbiota</u> and the identification of differences that may be associated with human disease

- BUT to <u>causally</u> link identified differences in the human microbiota with distinct human diseases, experiments are essential

- *In vitro* and *in vivo* experimental models are required: allow systematic manipulation of variables and validation of microbiome results



Fritz *et al.*, Microbiome, 2013

MICROBIOME

A unified initiative to harness Earth's microbiomes

Transition from description to causality and engineering

By **A. P. Alivisatos,* M. J. Blaser, E. L. Brodie, M. Chun, J. L. Dangl, T. J. Donohue, P. C. Dorrestein, J. A. Gilbert, J. L. Green, J. K. Jansson, R. Knight, M. E. Maxon, M. J. McFall-Ngai, J. F. Miller,† K. S. Pollard, E. G. Ruby, S. A. Taha, Unified Microbiome Initiative Consortium**

POLICY

Despite their centrality to life on Earth, we know little about how microbes (*1*) interact with each other, their hosts, or their environment. Although DNA sequencing technologies have enabled a new view of the ubiquity and diversity of microorganisms, this has mainly yielded snapshots that shed limited light on microbial functions or community dynamics. Given that nearly every habitat and organism hosts a diverse constellation of microorganisms—its "microbiome"—such knowledge could transform our understanding of the world and launch innovations in agriculture, energy, health, the environment, and more (see the photo). We propose an interdisciplinary Unified Microbiome Initiative (UMI) to discover and advance tools to understand and harness the capabilities of Earth's microbial ecosystems. The impacts of oceans and soil microbes on atmospheric CO_2 are critical for understanding climate change (*2*). By manipulating interactions at the root-soil-microbe interface, we may reduce agricultural pesticide, fertilizer, and water use enrich marginal land and rehabilitate degraded soils. Microbes can degrade plant cell walls (for biofuels), and synthesize myriad small molecules for new bioproducts, including antibiotics (*3*). Restoring normal human microbial ecosystems can save lives [e.g., fecal microbiome transplantation for *Clostridium difficile* infections (*4*)]. Rational management of microbial communities in and around us has implications for asthma, diabetes, obesity, infectious diseases, psychiatric illnesses, and other afflictions (*5*, *6*). The human microbiome is a target and a source for new drugs (*7*) and an essential tool for precision medicine (*8*).

The National Science Foundation's Microbial Observatories, the U.S. Department of Energy's Genomic Sciences program, the National Institutes of Health's Human Microbiome Project, and other efforts in the United States and abroad have served as critical first steps in revealing the diversity of microbes and their communities. However, we lack many tools required to advance beyond descriptive approaches to studies that enable a mechanistic, predictive, and actionable understanding of global microbiome processes. Developing these tools requires new collaborations between physical, life, and biomedical sciences; engineering; and other disciplines.

AREAS OF EMPHASIS. A central purpose of the UMI is to develop cross-cutting platform technologies to accelerate basic discovery and translation to applications. We highlight key needs and opportunities.

Decrypting microbial genes and chemistries. Approaches for characterizing microbiomes increasingly rely on whole-community metagenomic sequencing, yet roughly half of the genes identified in these studies encode products of unknown function, and existing functional annotations are often incomplete or inaccurate (*9*). Technologies for resolving roles of uncharacterized genes with high throughput and high accuracy are needed. These approaches must integrate improved computational methods for in silico prediction of protein and RNA functions, rapid mutagenesis of model organisms or native strains under natural conditions, multiomics and high-resolution phenotyping platforms to test functional predictions in vitro and in situ, and improved capture of information in the literature.

> *"we envision…evidence-based, model-informed microbiome management…"*

Deciphering chemistries of microbiomes is essential. In untargeted metabolomics studies using mass spectrometry, less than 2% of data can be matched to known chemical compounds, and only a fraction of those map to recognized biochemical pathways (*10*). Advances have been made in predicting structures from mass spectra, but improvements are needed in both in silico and physical

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*See the supplementary materials for authors' affiliations.
†Corresponding author. E-mail: jfmiller@ucla.edu

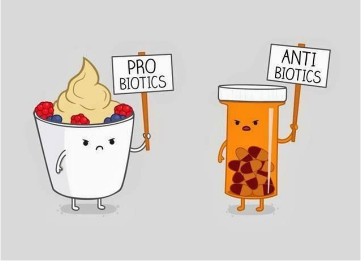
30 OCTOBER 2015 • VOL 350 ISSUE 6260 507

Published by AAAS

Unified Microbiome Initiative Consortium (UMIC)

- Plan to to organize research efforts across **disciplinary** and **geopolitical boundaries**
- Develop cross-cutting **platform technologies** to accelerate basic discovery and translation to applications.
- Encourage **data sharing, data access** and foster environment of collaboration between scientists and clinicians

2. The Microbiome and Therapeutics



- A new breed of company pursuing commercialization of microbiome-modulating therapies & diagnostics is emerging – including start-ups & venture capitalists

- **Food companies** most prolific patent assignees, e.g. Nestec (subsidiary of Nestlé) and Nutricia (subsidiary of Danone)

- **Large pharmaceutical companies** missing because the field remains in its infancy and very few proprietary agents exist that can be put into clinical development

- Patenting activity of food companies focused on **nondigestible fibers** and **bacterial strains** -- stimulate the growth of bacteria in the digestive tract in ways that are presumably beneficial to human health - **PREBIOTICS**

- **PROBIOTICS:** presumed to provide health benefits when consumed e.g. *Lactobacillus* in yoghurt, bifidobacteria in probiotic supplements – but not yet associated with health and disease states by sequencing-based microbe censuses of human cohorts

Who is Doing What?

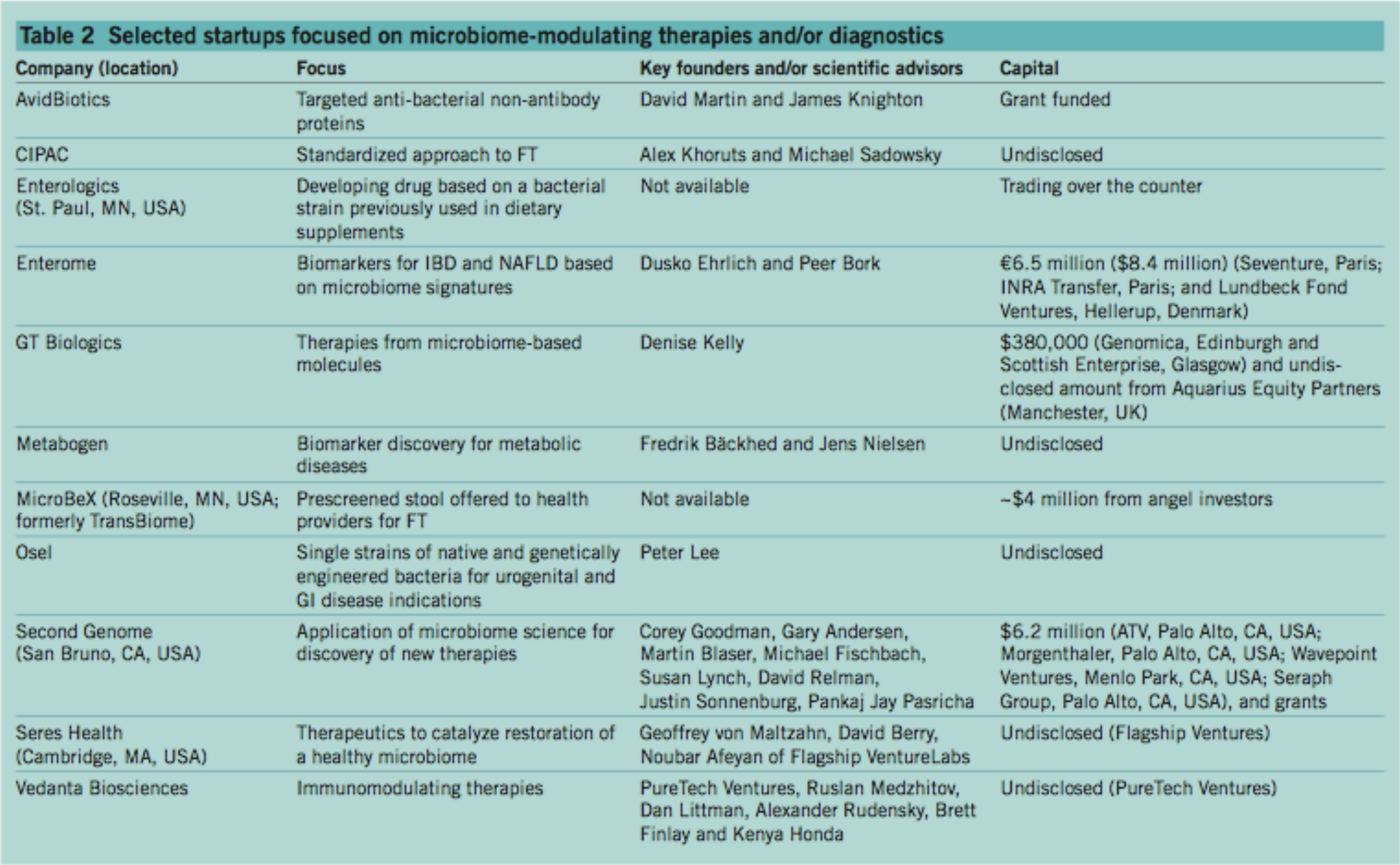
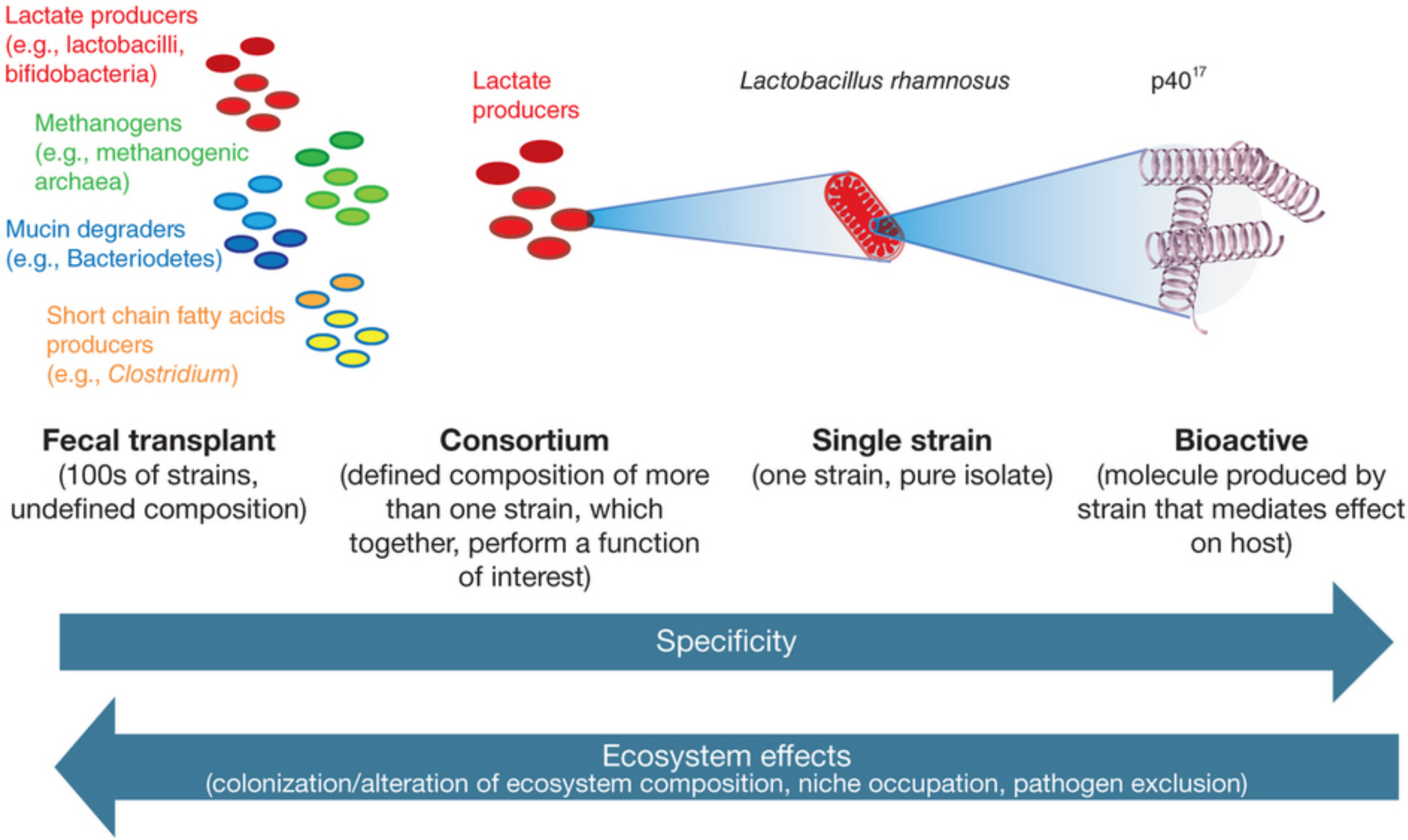


Table 2 Selected startups focused on microbiome-modulating therapies and/or diagnostics

Company (location)	Focus	Key founders and/or scientific advisors	Capital
AvidBiotics	Targeted anti-bacterial non-antibody proteins	David Martin and James Knighton	Grant funded
CIPAC	Standardized approach to FT	Alex Khoruts and Michael Sadowsky	Undisclosed
Enterologics (St. Paul, MN, USA)	Developing drug based on a bacterial strain previously used in dietary supplements	Not available	Trading over the counter
Enterome	Biomarkers for IBD and NAFLD based on microbiome signatures	Dusko Ehrlich and Peer Bork	€6.5 million ($8.4 million) (Seventure, Paris; INRA Transfer, Paris; and Lundbeck Fond Ventures, Hellerup, Denmark)
GT Biologics	Therapies from microbiome-based molecules	Denise Kelly	$380,000 (Genomica, Edinburgh and Scottish Enterprise, Glasgow) and undisclosed amount from Aquarius Equity Partners (Manchester, UK)
Metabogen	Biomarker discovery for metabolic diseases	Fredrik Bäckhed and Jens Nielsen	Undisclosed
MicroBeX (Roseville, MN, USA; formerly TransBiome)	Prescreened stool offered to health providers for FT	Not available	~$4 million from angel investors
Osel	Single strains of native and genetically engineered bacteria for urogenital and GI disease indications	Peter Lee	Undisclosed
Second Genome (San Bruno, CA, USA)	Application of microbiome science for discovery of new therapies	Corey Goodman, Gary Andersen, Martin Blaser, Michael Fischbach, Susan Lynch, David Relman, Justin Sonnenburg, Pankaj Jay Pasricha	$6.2 million (ATV, Palo Alto, CA, USA; Morgenthaler, Palo Alto, CA, USA; Wavepoint Ventures, Menlo Park, CA, USA; Seraph Group, Palo Alto, CA, USA), and grants
Seres Health (Cambridge, MA, USA)	Therapeutics to catalyze restoration of a healthy microbiome	Geoffrey von Maltzahn, David Berry, Noubar Afeyan of Flagship VentureLabs	Undisclosed (Flagship Ventures)
Vedanta Biosciences	Immunomodulating therapies	PureTech Ventures, Ruslan Medzhitov, Dan Littman, Alexander Rudensky, Brett Finlay and Kenya Honda	Undisclosed (PureTech Ventures)

B. Olle, Nature Biotechnology, 2013

Types of microbiome-derived therapeutics being pursued by biotech companies



Lactate producers (e.g., lactobacilli, bifidobacteria)

Methanogens (e.g., methanogenic archaea)

Mucin degraders (e.g., Bacteriodetes)

Short chain fatty acids producers (e.g., *Clostridium*)

Lactate producers

Lactobacillus rhamnosus

p40[17]

Fecal transplant
(100s of strains, undefined composition)

Consortium
(defined composition of more than one strain, which together, perform a function of interest)

Single strain
(one strain, pure isolate)

Bioactive
(molecule produced by strain that mediates effect on host)

Specificity

Ecosystem effects
(colonization/alteration of ecosystem composition, niche occupation, pathogen exclusion)

B. Olle, Nature Biotechnology, 2013

The most dramatic microbiome therapeutic: Fecal Microbiota Transplant (FMT)

- Infusion of fecal material (and its microbes) from a healthy donor into an individual with a specific disease for the purpose of providing relief of symptoms or cure of that disease

- First described in 4th century China

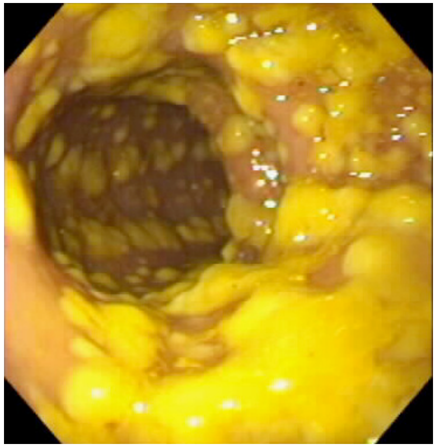
- 17th century veterinary medicine – "**transfaunation**"

- 1958: Eiseman for pseudomembranous colitis from *Micrococcus pyogenes*

- Establish a favorable shift of the intestinal microbiome

- Therapeutic armamentarium: **Antibiotics → Bacteria**

- **Used to treat recurrent *C. difficile*, Irritable Bowel Syndrome, diabetes, depression & anxiety**



Borody, et al. J Clin Gastroenterol, 2004
Eiseman. Surgery, 1958
Zhang, et al. Am J Gastroenterol, 2012

….and some of the headlines….

The New York Times

HEALTH

When Pills Fail, This, er, Option Provides a Cure
By DENISE GRADY
JAN 16, 2013

A New Kind of Transplant Bank
By PETER ANDREY SMITH
FEB 17, 2014

THE NEW YORKER

The Excrement Experiment
Treating disease with fecal transplants.
By EMILY EAKIN
DEC 1, 2014

Los Angeles Times

Fecal transplants: A therapy whose time has come
By THE TIMES EDITORIAL BOARD
MAR 5, 2014

Study: Frozen poop pills may make fecal transplants simpler and safer
By KAREN KAPLAN
OCT 11, 2014

Did fecal transplant make woman obese?
By MONTE MORIN
FEB. 4, 2015

THE HUFFINGTON POST

Artificial Poop, RePOOPulate, May Lead To Synthetic Fecal Transplants
By CHRISTIE WILCOX
JAN. 11, 2013

The Seattle Times

Wonder cure for gut: FDA allows fecal transplants
By CAROL OSTROM
OCT 26, 2013

Dr. Olga Aroniadis

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FMT Methodology

FMT Steps

(1) Donor identified

(2) Donor and recipient screened

(3) Stool prepared

(4) Fecal material infused from donor into the patient

(5) Patient monitored for symptomatic improvement and adverse events

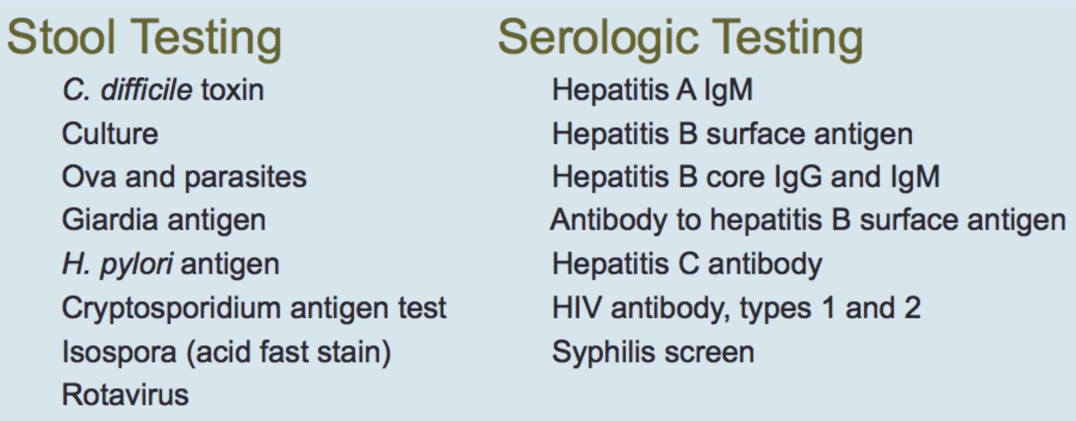
FMT Methodology

FMT Methodology

Donor Screening

History

- Antibiotic use
- Incarceration, tattoos or body piercings, high-risk sexual behaviors
- GI diseases: chronic diarrhea, constipation, IBD, IBS, colorectal polyps or cancer
- Non-GI diseases: autoimmune disorders, immunocompromised, morbid obesity, metabolic syndrome, atopy, chronic fatigue syndrome or any chronic medical condition
- Severe allergies/anaphylaxis in the recipient
- And more…



Stool Testing	Serologic Testing
C. difficile toxin	Hepatitis A IgM
Culture	Hepatitis B surface antigen
Ova and parasites	Hepatitis B core IgG and IgM
Giardia antigen	Antibody to hepatitis B surface antigen
H. pylori antigen	Hepatitis C antibody
Cryptosporidium antigen test	HIV antibody, types 1 and 2
Isospora (acid fast stain)	Syphilis screen
Rotavirus	

FMT Methodology

Past		Present
Patient-identified donor	→	Standard donor (stool biobank – OpenBiome)
Fresh Stool	→	Frozen Stool
Whole Stool	→	Specific Bacteria

Convenient
Reduced cost
Greater precision
Standard/rigorous screening protocols
Safer

Capsules

The New York Times

Fecal Transplants Made (Somewhat) More Palatable
By Andre Smith, November 9, 2015

November 18, 2015
Second dose of 25
capsules on each of
3 consecutive days.

Clostridium difficile

Pathogenesis

- Colonic infection, spore-forming organism, spores produce toxins A and B
- Fecal-oral route of transmission
- Risk factors: antibiotic use, acid-suppression, recent hospitalization, nursing home residence

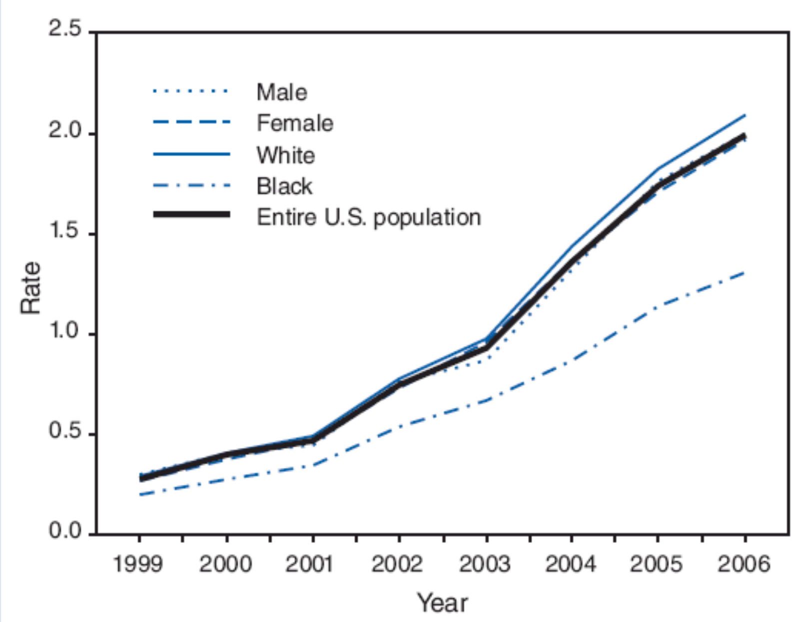
Asymptomatic to severe disease

- Diarrhea and abdominal pain
- Pseudomembranous colitis
- Toxic megacolon: colectomy, death

Treated with antibiotics

Recurrence

- First: 10-15% of patients
- Second: 40% of patients
- Third: 60% of patients
- Treatment with antibiotics unsuccessful



Morbidity and Mortality Weekly Report. CDC, 2009

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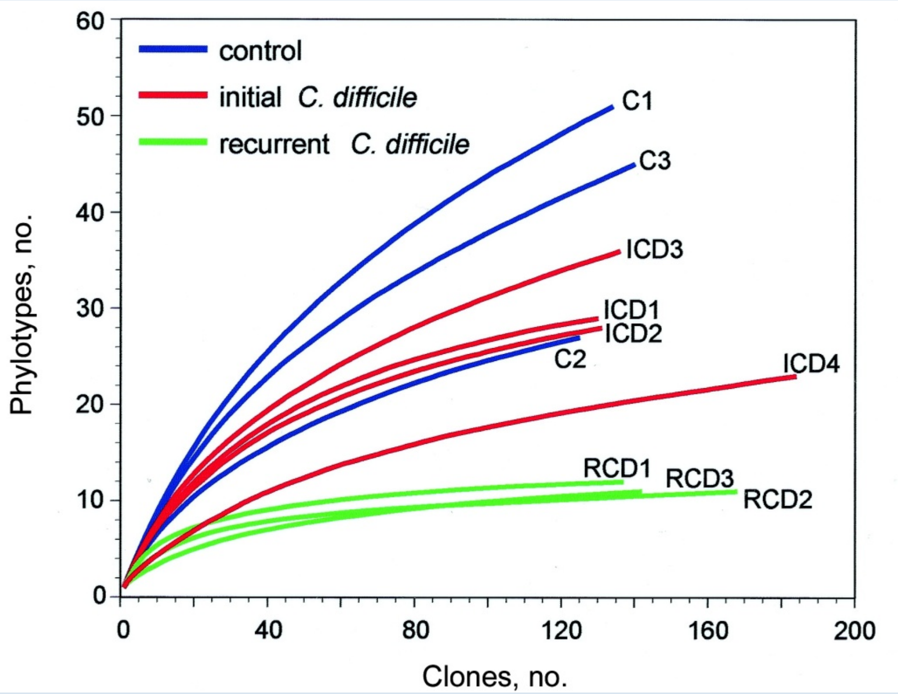
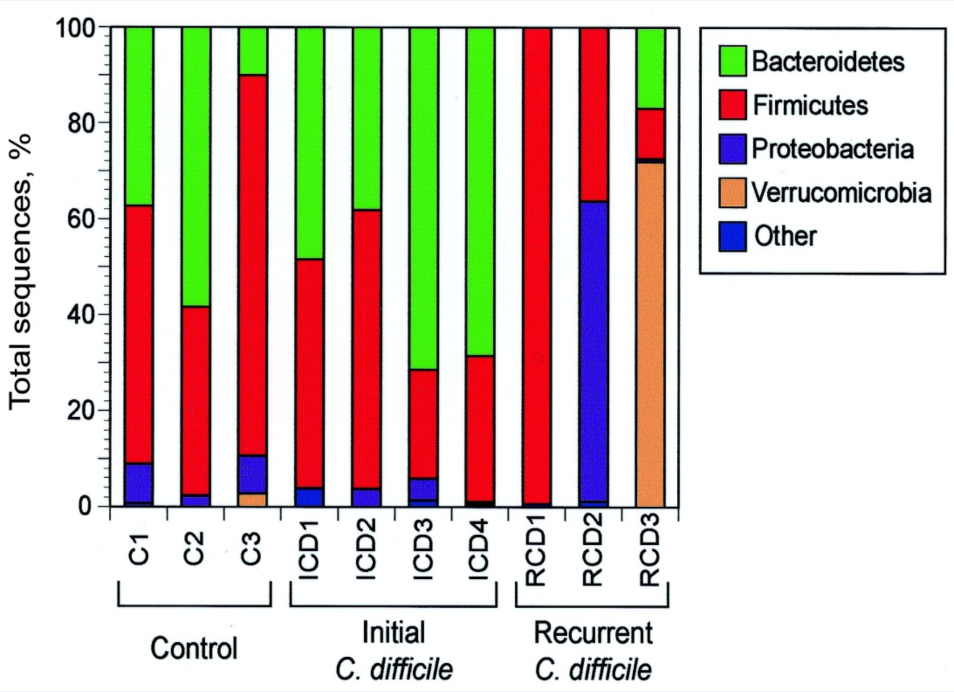
C. difficile and the colonic microbiome



Patients with recurrent *C. difficile* have decreased microbiome richness



Bacteroidetes and *Firmicutes* are reduced in patients with recurrent *C. difficile*

Chang, et al. J Infect Dis, 2008

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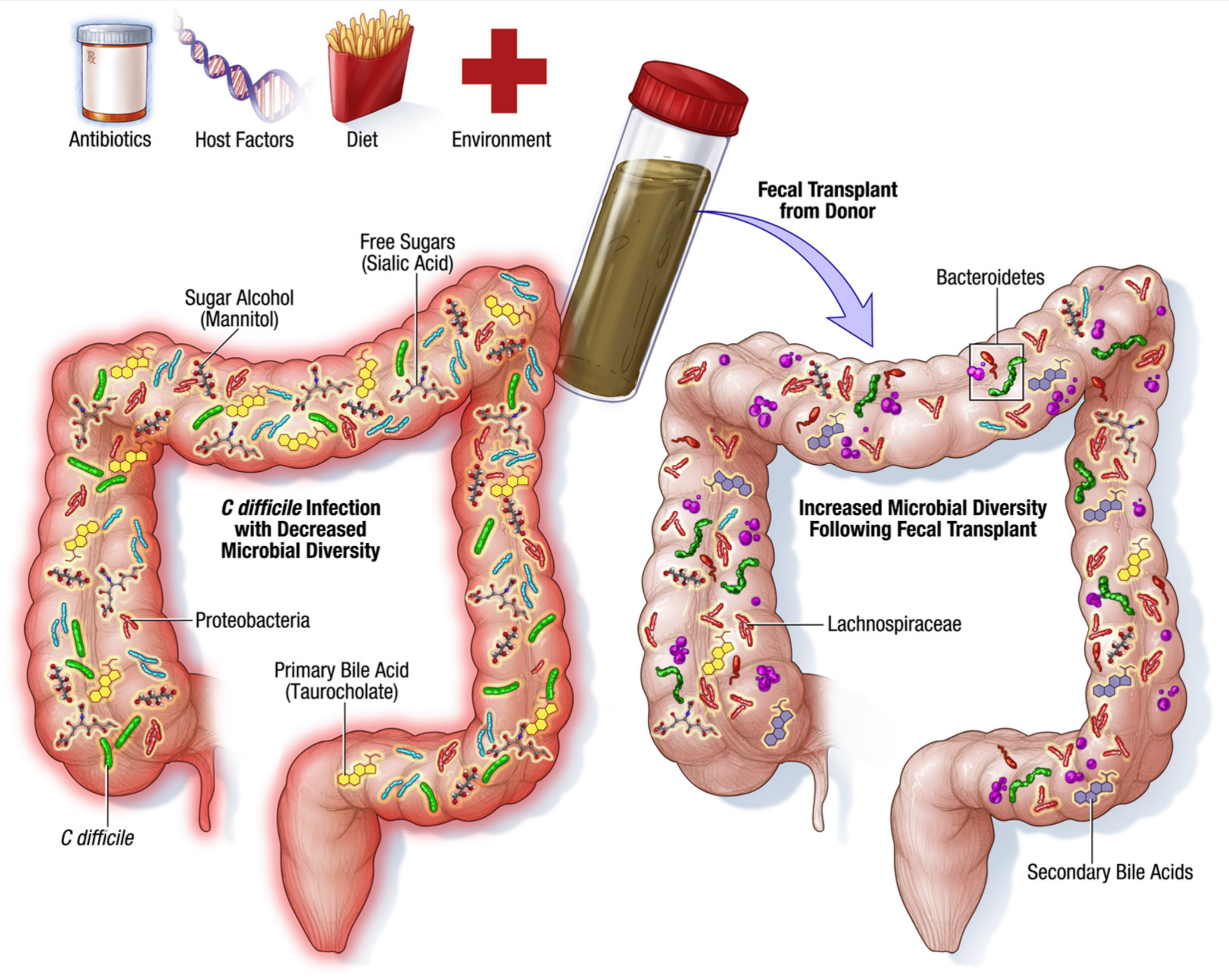
Mechanism of cure



Change in microbial community structure:

- Restoration of microbial diversity
- Increase in phyla
  Bacteroidetes
  Firmicutes
- Increase in bile acids

Kelly, et al. Gastroenterology, 2015

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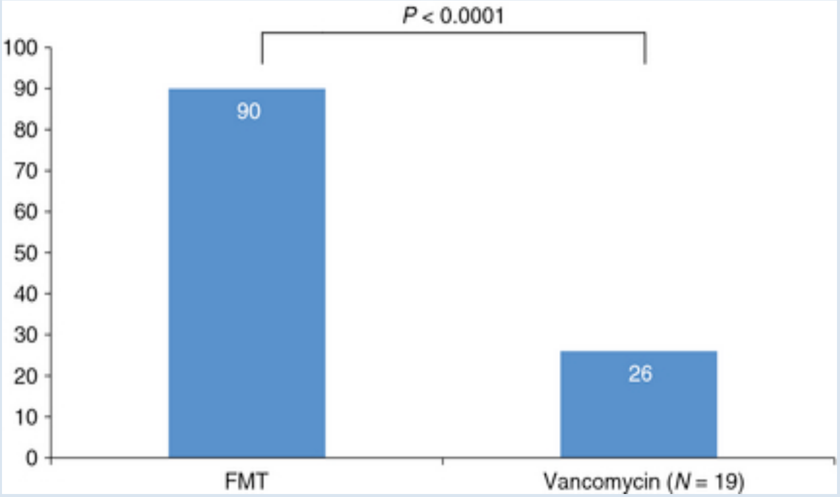
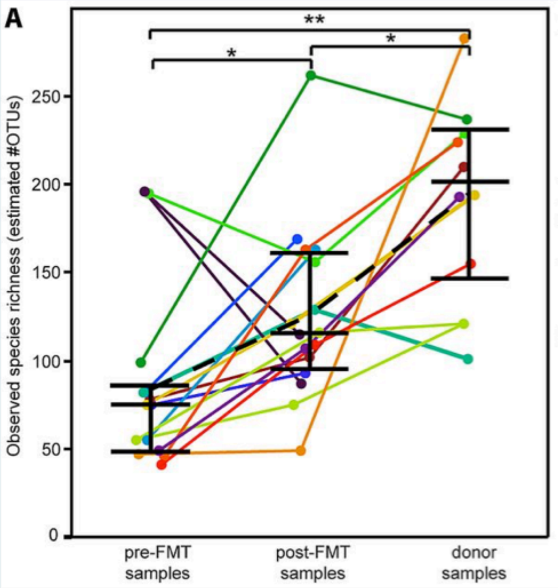
FMT treatment of Recurrent *C. difficile*

5 clinical trials:

- 20 treated with colonoscopic FMT
- 19 treated with vancomycin
- Cure: resolution of diarrhea at 10 wks
- FMT: 90% cure; vancomycin: 26% cure



Cammarota, et al. Aliment Pharmacol Ther, 2015



Seekatz, et al. mBio, 2014

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FDA Regulations

July 2013 – FDA announces stool is a drug/biologic

- Investigational New Drug (IND) application required
- FMT unavailable to the community physician

September 2013 – FDA liberalizes the restriction on FMT for *C. difficile* infection while maintaining discretionary regulation

- FMT available for CDI without an IND
- IND required for all other indications

Summary and Ethical Concerns

- FMT is **highly successful for *C. difficile* treatment** – but further data needed for other diseases
- **Safety** of paramount importance & adverse events need to be rigorously monitored
- Avoid a premature rush to use microbiome data and FMT in the clinical setting – **more clinical trials required**
- Currently physicians cannot make diagnoses or give clear guidance based on an **individual's microbiome profile**
- **Ethical issues** surround type of consent appropriate for fecal storage in biorepositories in order to preserve confidentiality

3. The urban microbiome of New York City

- 8 mill people, ~300 sq miles, major international gateway to US
- Global hub for finance, media, art, science, entertainment
- City of immigrants & their children (36% foreign born in many countries)
- Most extensive mass transit system in US
- Extensive sewer system: 1.5 billion gallons of waste water/day through 7,400 miles of sewer conduits
- A precious resource: requires monitoring to sustain and secure against acts of bioterrorism, environmental or epidemic threats


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Mapping the New York City Microbiome

We propose to use advances in DNA sequencing technology with Big Data solutions and statistical analysis to identify, map and ultimately track beneficial and infectious microbial communities in NYC, and to integrate and visualize these data with existing NYC urban datasets.

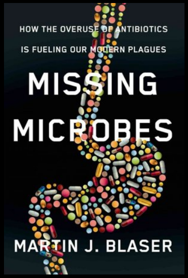
Can we track species of parasitic protist and determine their zoonotic potential?

Carlton

Can we measure the NYC 'virome'?

Ghedin

Can we monitor the spread of antibiotic resistance?



HOW THE OVERUSE OF ANTIBIOTICS IS FUELING OUR MODERN PLAGUES

MISSING MICROBES

MARTIN J. BLASER

Blaser

Can we survey bacteria, especially spore-forming members of the Bacillaceae & Clostridia?

Eichenberger

What statistical & visualization methods are needed for these Big Data sets?

Silva

Bonneau

CENTER FOR GENOMICS AND SYSTEMS BIOLOGY NEW YORK UNIVERSITY

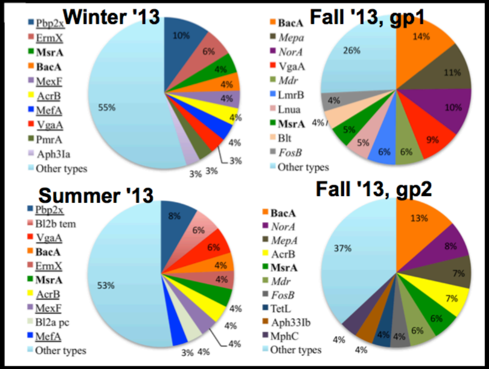
NYU School of Medicine
NYU LANGONE MEDICAL CENTER

NYU
GIPH

CUSP
CENTER FOR URBAN SCIENCE + PROGRESS

"Dirty Money Project"

microbes on paper currency circulating in NYC

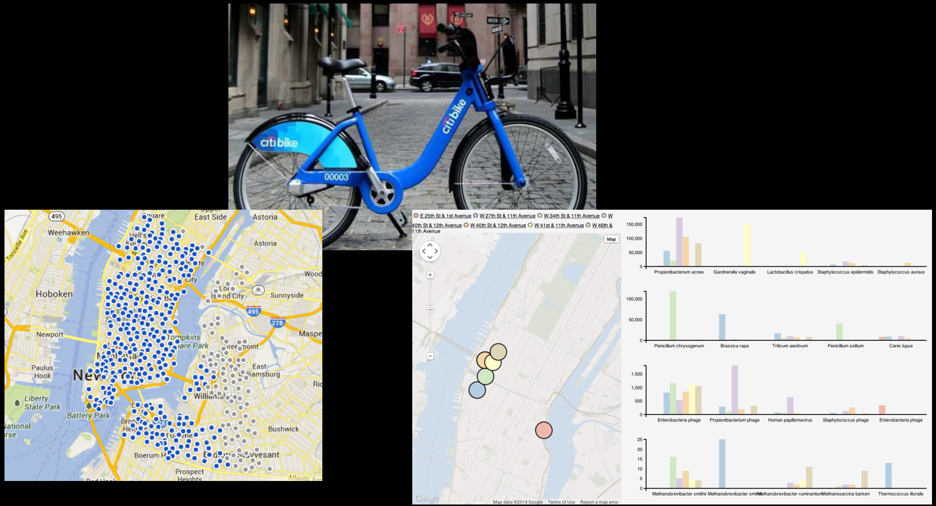


NYC Subway Project

all 468 subway stations

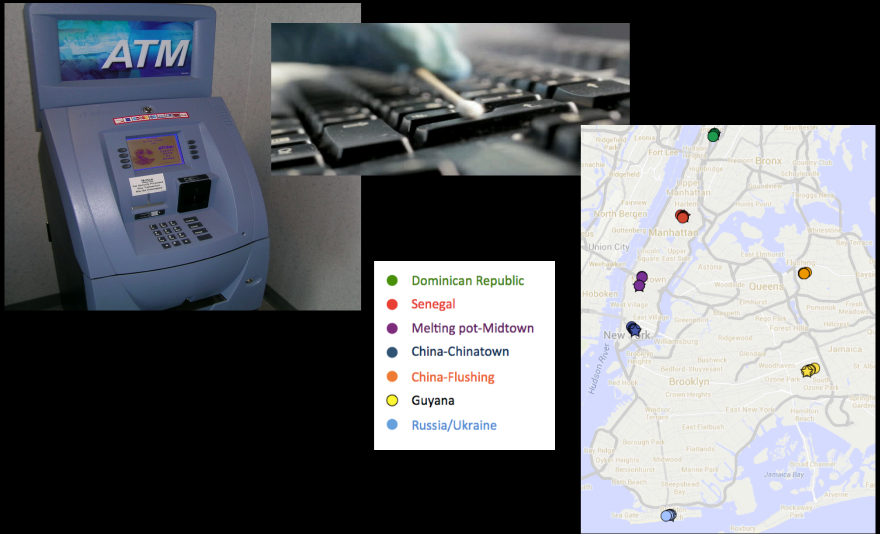
Micro"bike"ome Project:

microbes on public bike schemes in NYC & London



ATM Project

microbes on ATM buttons in ethnic regions of NYC



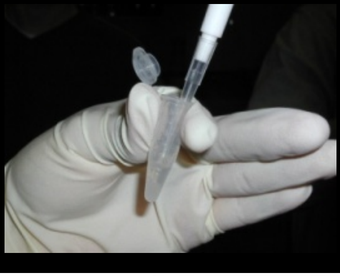
- ● Dominican Republic
- ● Senegal
- ● Melting pot-Midtown
- ● China-Chinatown
- ● China-Flushing
- ○ Guyana
- ● Russia/Ukraine

What microbes are present on circulating $1 bills in NYC?

Two time points:
Feb 2013 (Winter)
July 2013 (Summer)

20 $1 bills

Swabbed front and back



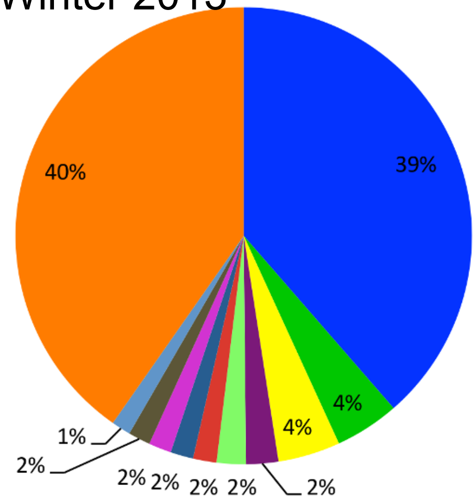
DNA extraction
Mobio PowerSoil kit

Shotgun metagenomic
library construction

2X100 Illumina HiSeq run

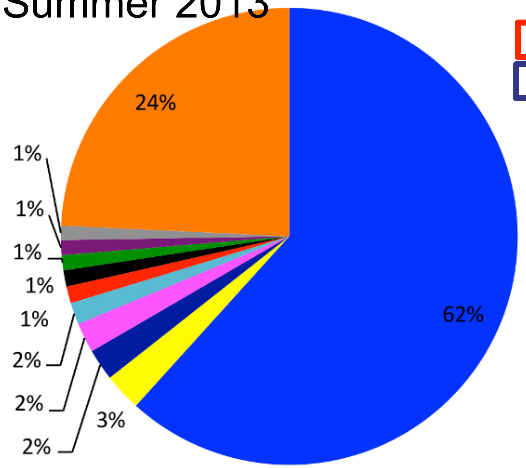
A diverse array of abundant bacterial species

Winter 2013



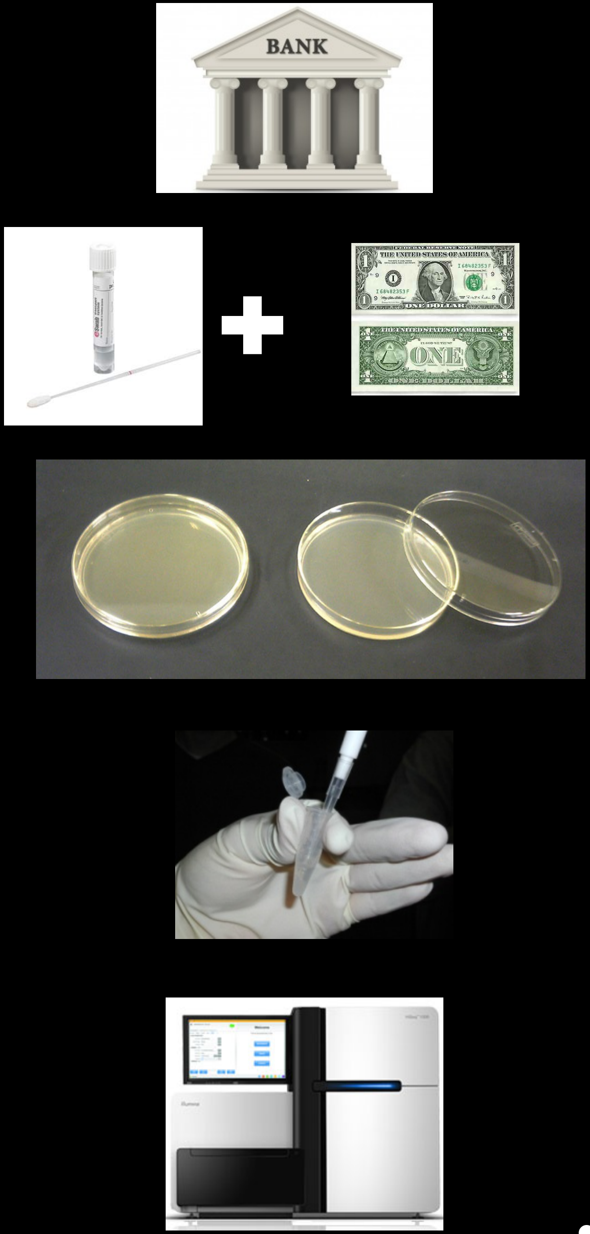
	Relative abundance	Association with human flora
🟦 *Propionibacterium acnes**	38.60	Linked to skin acne
🟩 *Micrococcus luteus**	4.55	Commensal skin flora, common soil microbe
🟨 *Staphylococcus epidermidis**	4.41	Commensal skin flora
🟪 *Streptococcus pneumoniae**	2.28	Linked to community acquired pneumonia
🟩 *Streptococcus oralis*	2.06	Commensal oral flora
🟥 *Gardnerella vaginalis*	1.66	Linked to disruption of normal vagina flora
🟦 *Rothia mucilaginosa*	1.61	Commensal oral and respiratory tract flora
🟪 *Streptococcus mitis*	1.60	Commensal oral flora
🟫 *Streptococcus parasanguinis*	1.57	Commensal oral flora
🟦 *Streptococcus salivarius*	1.36	Commensal oral and respiratory tract flora
🟧 Other species	40.28	N/A

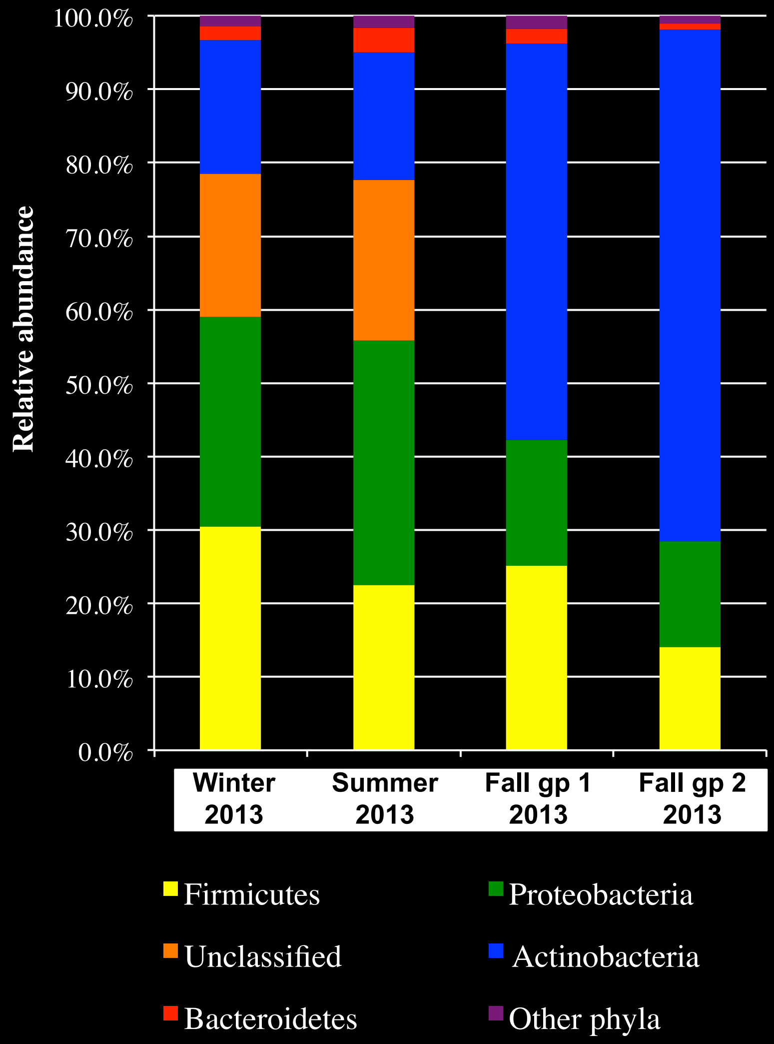
Summer 2013



	Relative abundance	Association with human flora
🟦 *Propionibacterium acnes**	61.71	Linked to skin acne
🟨 *Staphylococcus epidermidis**	2.67	Commensal skin flora
🟦 *Xanthomonas campestris*	2.24	Plant pathogen
🟪 *Helicobacter pylori*	2.12	Linked to gut ulcers
🟦 *Micrococcus luteus**	1.55	Commensal skin flora, common soil microbe
🟥 *Pseudomonas fluorescens*	1.19	Antibiotic and dairy production, common soil microbe
⬛ *Rothia dentocariosa*	1.15	Commensal oral and respiratory tract flora
🟩 *Lactococcus lactis*	1.07	Important dairy production microbe
🟪 *Streptococcus pneumoniae**	1.06	Linked to community acquired pneumonia
⬜ *Corynebacterium kroppenstedtii*	1.01	Associated with skin flora
🟧 Other species	24.25	N/A

Are the microbes found on $1 bills viable?



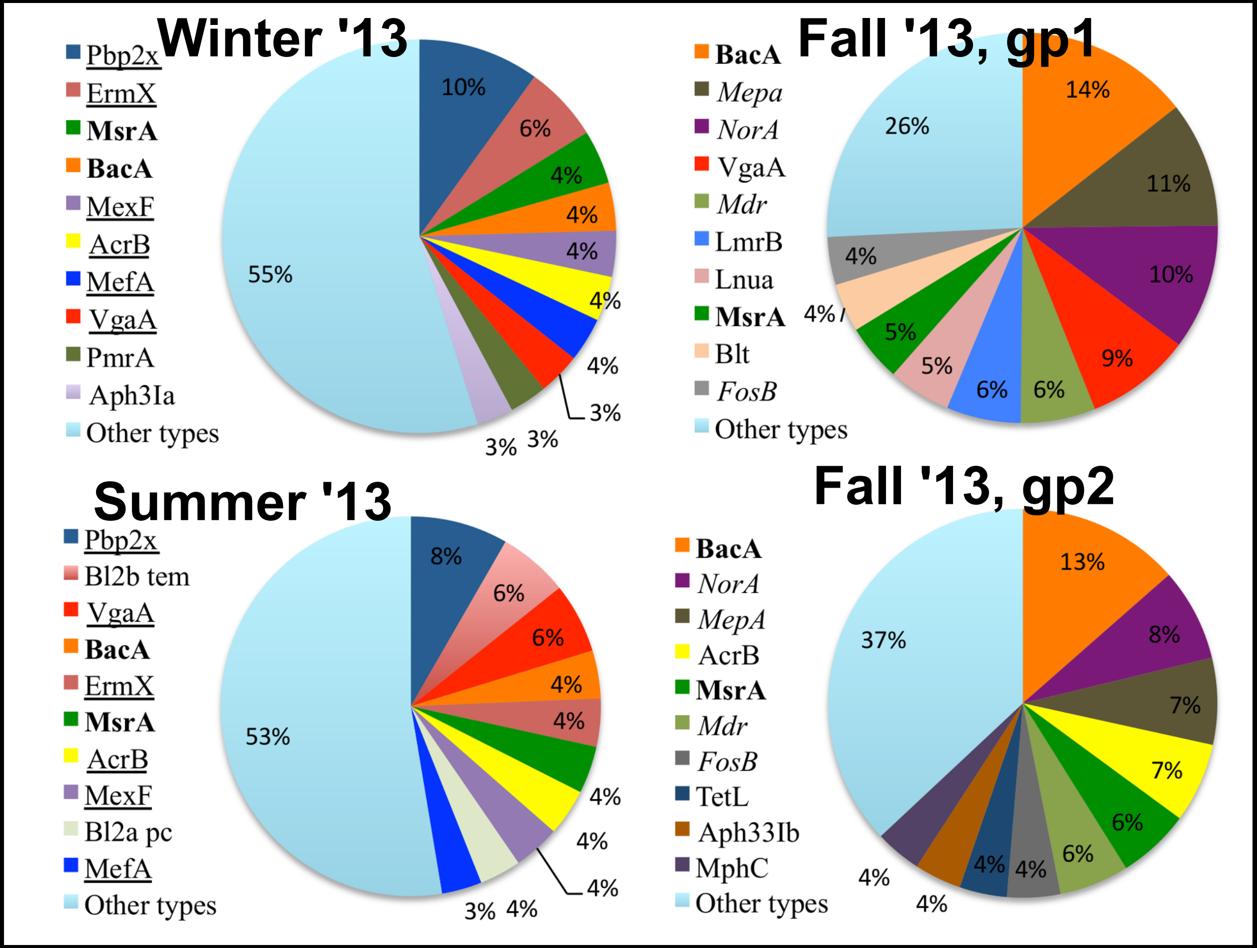


- Similar phyla as previously identified

Several clinically important bacteria and protists detected

Species	Clinical relavence	Winter 2013	Summer 2013	Fall gp 1 2013	Fall gp 2 2013
Bacillus anthracis	Can cause anthrax	0.01	0	3.35	0.1
Bacillus cereus	Can cause food-borne illness	0.04	0.02	12.3	0.45
Clostridium difficile	Causes antibiotic-associated dairrhea	0.02	0.01	0	0
Corynebacterium diptheriae	Causes diptheria	0.15	0.01	0	0
Escheria coli	Can cause serious food poisoning	0.11	0.19	0	0
Gardnerella vaginalis	Can cause bacterial vaginosis	1.66	0.32	0	0
Helicobacter pylori	Associated with gut ulcers	0.01	2.12	0.45	0.33
Salmonella enterica	Causes salmonella	0.06	0.03	0	0.02
★ *Staphylococcus aureus*	A variety of skin infections, MRSA antibiotic resistance	0.42	0.24	1.7	0.3
Staphylococcus saprophyticus	Linked to urinary tract infections	0.43	0.21	19.3	15.7
Trichomonas vaginalis	Causes trichomoniasis	0	0.02	NA	NA
Candida albicans	Causes candidiasis (oral thrush)	0.04	0	NA	NA
Aspergillus flavus	Can cause asthma and other pulmonary infections	0.02	0	NA	NA

Can money transmit antibiotic resistance genes?



- Most abundant in winter/summer groups was **Pbp2x** (penicillin resistance)
- Most abundant in both cultured groups was **BacA** (bacitracin resistance)

One hypothesis to be tested: does paper currency found in hospital cafeterias contain a higher ratio of AR genes?

Dirty money project summary

- Environmental metagenomic sequencing is an effective way to detect microbial genetic material
- - But transfer, storage and analysis of datasets of this size represent significant computational challenges

- Viable bacteria are present on money
  - Probably more than identified here or in previous studies

- Money could serve as a mode of transmission for antibiotic resistance genes
  - More studies needed

- Money is an interface for human-microbe interaction: use as a "biomarker" to monitor public health?

News of this study went viral – even before publication

THE WALL STREET JOURNAL.
WSJ

Why You Shouldn't Put Your Money Where Your Mouth Is

By Robert Lee Hotz

Talk about dirty money: Scientists are discovering a surprising number of microbes living on cash.

In the first comprehensive study of the DNA on dollar bills, researchers at **New York University**'s Dirty Money Project found that currency is a medium of exchange for hundreds of different kinds of bacteria as bank notes pass from hand to hand.

By analyzing genetic material on $1 bills, the NYU researchers identified 3,000 types of bacteria in all—many times more than in previous studies that examined samples under a microscope. Even so, they could identify only about 20% of the non-human DNA they found because so many microorganisms haven't yet been cataloged in genetic data banks.

Easily the most abundant species they found is one that causes acne. Others were linked to gastric ulcers, pneumonia, food poisoning and staph infections, the scientists said. Some carried genes responsible for antibiotic resistance.

"It was quite amazing to us," said Jane Carlton, director of genome sequencing at **NYU's Center for Genomics and Systems Biology** where the university-funded work was performed. "We actually found that microbes grow on money."

Their unpublished research offers a glimpse into the international problem of dirty money. From rupees to euros, paper money is one of the most frequently passed items in the world. Hygienists have long worried that it could become a source of contagion.

"A body-temperature wallet is a petri dish," said Philippe Etienne, managing director of Innovia Security Pty Ltd., which makes special bank-note paper for 23 countries.

Central banks and state treasurys usually worry more about counterfeiting and durability than microbiology, several currency experts said. With nearly 150 billion new bank notes circulated every year around the world, governments spend nearly $10 billion annually to provide people with notes that are fit to hold.

A U.S. one-dollar bill, printed on a cotton-linen blend, lasts little more than 21 months. In all, the U.S. Federal Reserve System is spending $826.7 million on new money this year to make 7.8 billion bank notes with a total face value of $297.1 billion.

To make cash more durable, countries from Canada to the Kingdom of Bhutan are printing bank notes on sheets of flexible plastic polymer film, with implications for the microbiology of money.

In a study of the public-health effect of new currency materials, researchers at Australia's University of Ballarat recently tested bills taken in change from supermarkets, coffee shops and cafeterias in 10 countries.

While levels of bacteria varied widely from place to place, they usually found fewer on polymer bills than on cotton-based ones, according to their 2010 study in the journal Foodborne Pathogens and Disease.

"The thing about a polymer note is that it is not absorbent," said Mr. Etienne. "There is a cleanliness benefit, which is a health issue."

Other researchers tried growing bacteria on seven different currencies. They found that some germs survived longer on the plastic bank notes, according to tests published last year in Antimicrobial Resistance and Infection Control.

A human touch compounds the problem. Bacteria can feed on the waxy residue of skin and oils that builds up on bills in circulation.

"We provide the nutrients when we handle the bank notes," said Brown University physicist Nabil Lawandy, who is president of Spectra Systems Corp. in Rhode Island, which designs currency-security features for 19 central banks.

Researchers have also explored the fibrous surface of paper money. Using traditional cell-culture techniques, research groups in India, the Netherlands and the U.S. have isolated about 93 species of bacteria clinging to paper bills. In 2012, microbiologists at Queen Mary University of London found that about 6% of English bank notes tested had levels of e.coli bacteria comparable to a toilet seat.

Generally, the NYU researchers could identify so many more species than previous efforts because high-speed gene sequencing and computerized database analysis allowed them to recognize life forms by their DNA, rather than by isolating the cells in culture and studying them under a microscope.

In their experiment, the NYU researchers analyzed the DNA found on 80 $1 bills that they collected last year from an unnamed bank in Manhattan.

All told, the dollar bills yielded about 1.2 billion DNA segments. It took 320 gigabytes of digital storage to hold all the genetic data—roughly the amount needed to hold an entire library of traditional medical texts. "We were casting the broadest possible net," said NYU senior research scientist Steven Sullivan.

The DNA was as diverse as New York. About half of it was human. The researchers found bacteria, viruses, fungi and plant pathogens. They saw extremely minute traces of anthrax and diphtheria. They identified DNA from horses and dogs—even a snippet or two of white rhino DNA.

"We had a lot of the spectrum of life represented on money," said NYU genome researcher Julia Maritz, who did much of the DNA analysis.

Andrew Lamberson for The Wall Street Journal

NYU researchers found that currency is a medium of exchange for hundreds of different kinds of bacteria as bills pass from hand to hand.

Dirty Dollars

NYU researchers identified 3,000 types of bacteria on a set of one-dollar bills collected in New York.

Total DNA found: 1.2 billion segments

Percentage human: 27%-48%

Bacterial DNA: 54 million segments

Sampler of bacteria identified:

Acinetobacter species: antibiotic-resistant infections
Staphyloccus aureus: skin infections
Bacillus cereus: food-borne illness
Eschera coli: food poisoning
Heliobacter pylori: gastric ulcers
Coroynebactrium diptheriae: diphtheria

Sources: New York University, US Federal Reserve System.

TIME

NEWSFEED SCIENCE

Science Confirms Dollar Bills Are Covered in a Bajillion Gross Germs

8:30 AM ET

Dolla dolla bills, y'all

Science is pretty cool, and it gets even cooler when it's confirming things we already know through intuition or anecdote. This time, science confirms what my mom (and

public health

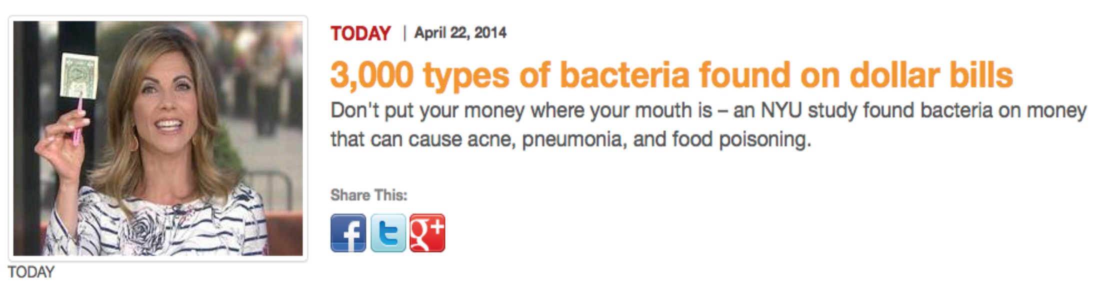
Dirty Money: A Microbial Jungle Thrives In Your Wallet

by MICHAELEEN DOUCLEFF

April 23, 2014 7:40 AM ET

Even some euro bank notes may need a good scrubbing. Like dollar bills, these notes are made from cotton and they harbor an array of bacteria.

Thomas Leuthard/The Preiser Project/Flickr



TODAY | April 22, 2014

3,000 types of bacteria found on dollar bills

Don't put your money where your mouth is – an NYU study found bacteria on money that can cause acne, pneumonia, and food poisoning.

Share This:

How do we manage the publicity that comes with characterizing the good and bad microbes in NYC?

- It's our duty as good scientific citizens not to scare-monger or jump on the publicity band-wagon

- We should also work in collaboration and maintain good relations with NYC government offices, NGOs & policy-makers

- These aims can be in conflict with journalists who just want a good story!

- But ultimately it is our collective responsibility as scientists to aim for robust data sets, conservative data interpretation & aiming towards making NYC - & urban cities everywhere - a better place

4. It's a Cool New Techno-World

TIGR c. 2002

Next generation sequencers

illumina

PACIFIC BIOSCIENCES

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Image Capture

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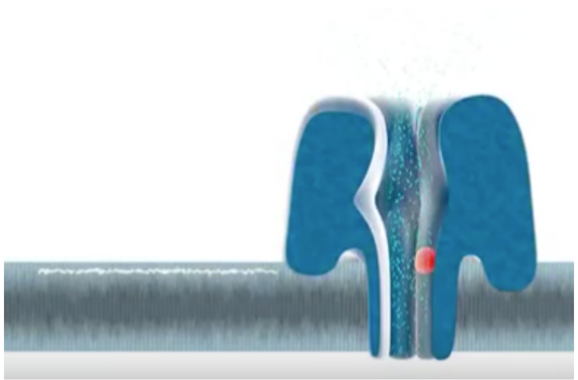
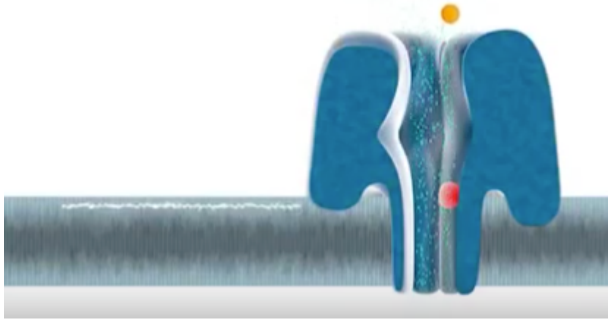
Data Processing

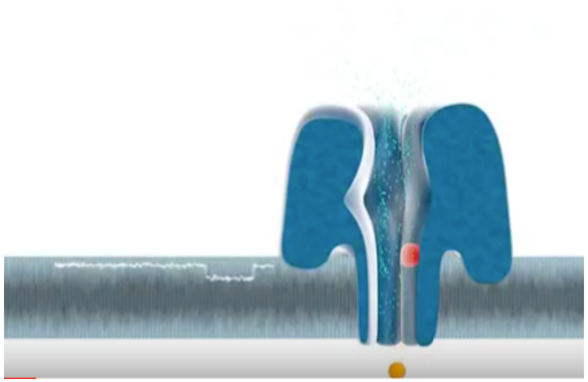
Genome Sequencer FLX Instrument

life technologies

Oxford Nanopore MinION portable sequencer








MinION
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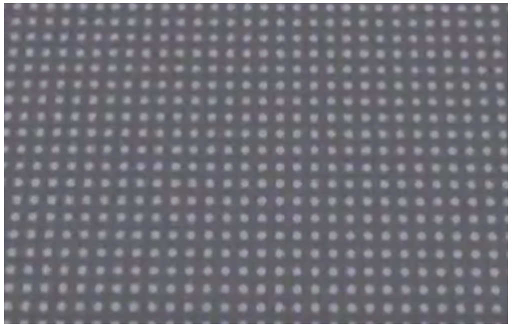

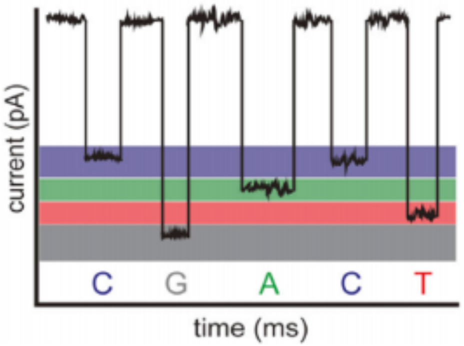
current (pA)
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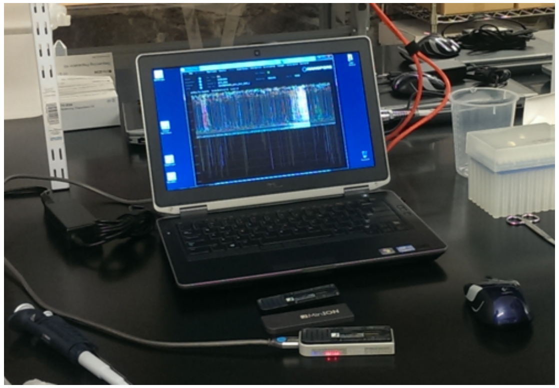
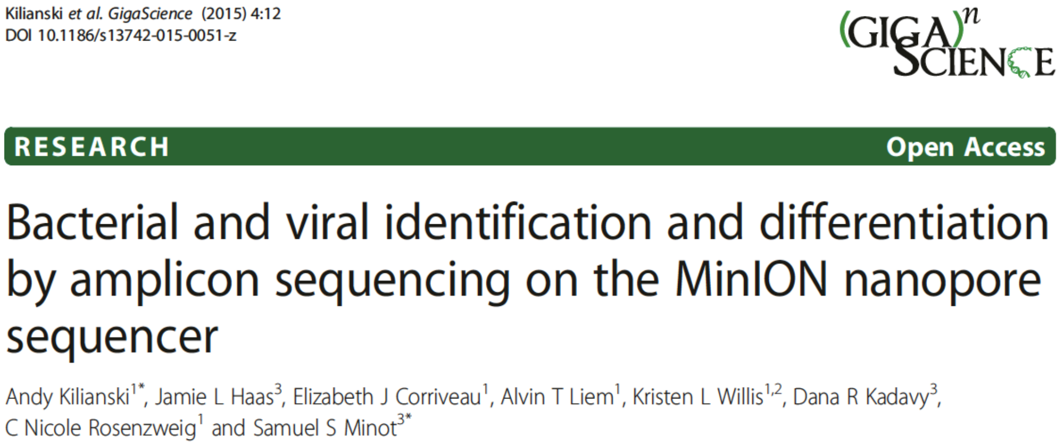
time (ms)



MinION is already being used to sequence clinical samples in the field, as well as in clinics



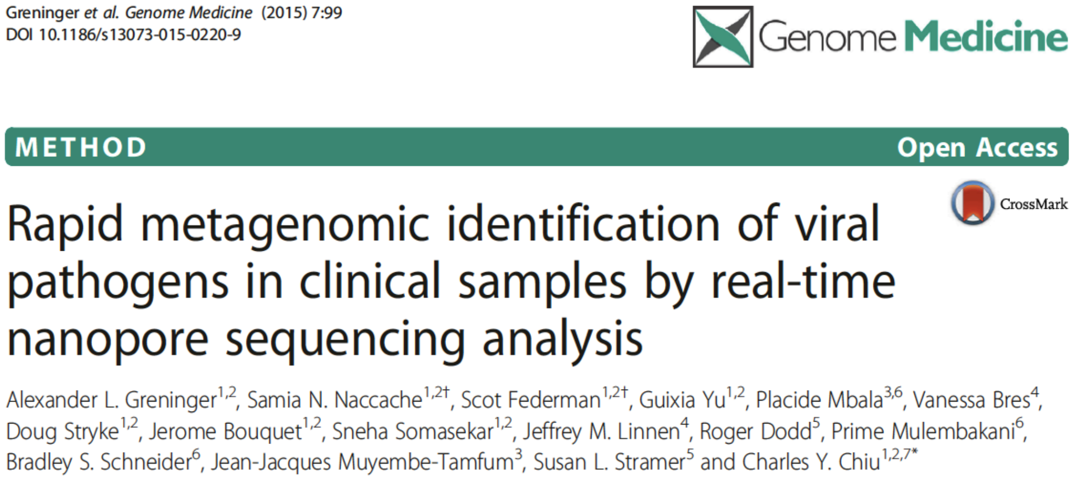
Kilianski *et al. GigaScience* (2015) 4:12
DOI 10.1186/s13742-015-0051-z

(GIGA)^n SCIENCE

RESEARCH Open Access

Bacterial and viral identification and differentiation by amplicon sequencing on the MinION nanopore sequencer

Andy Kilianski[1*], Jamie L Haas[3], Elizabeth J Corriveau[1], Alvin T Liem[1], Kristen L Willis[1,2], Dana R Kadavy[3], C Nicole Rosenzweig[1] and Samuel S Minot[3*]



Greninger *et al. Genome Medicine* (2015) 7:99
DOI 10.1186/s13073-015-0220-9

Genome **Medicine**

METHOD Open Access

CrossMark

Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis

Alexander L. Greninger[1,2], Samia N. Naccache[1,2†], Scot Federman[1,2†], Guixia Yu[1,2], Placide Mbala[3,6], Vanessa Bres[4], Doug Stryke[1,2], Jerome Bouquet[1,2], Sneha Somasekar[1,2], Jeffrey M. Linnen[4], Roger Dodd[5], Prime Mulembakani[6], Bradley S. Schneider[6], Jean-Jacques Muyembe-Tamfum[3], Susan L. Stramer[5] and Charles Y. Chiu[1,2,7*]

Quick *et al. Genome Biology* (2015) 16:114
DOI 10.1186/s13059-015-0677-2

Genome **Biology**

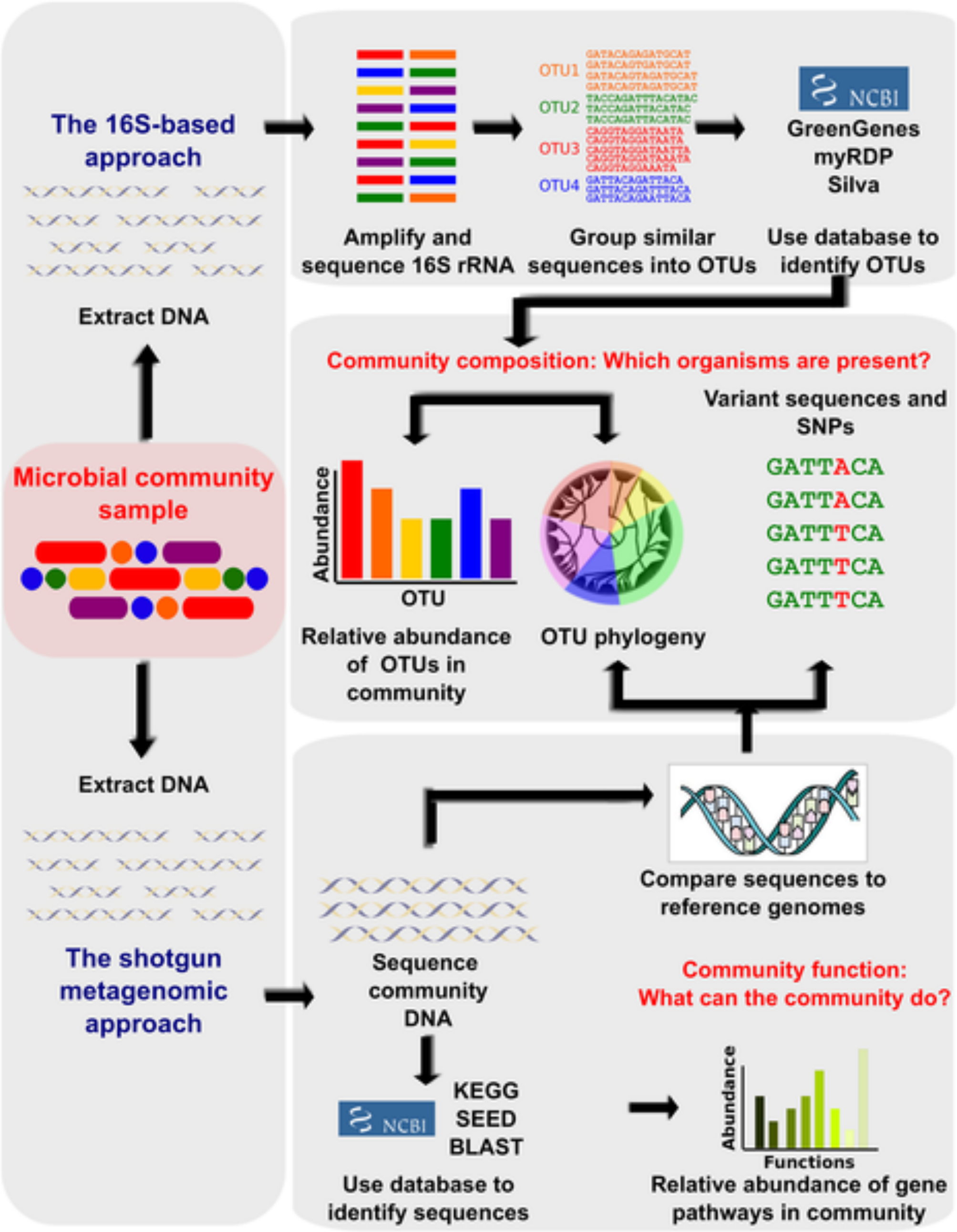
RESEARCH Open Access

CrossMark

Rapid draft sequencing and real-time nanopore sequencing in a hospital outbreak of *Salmonella*

Joshua Quick[1,2†], Philip Ashton[3†], Szymon Calus[1,2], Carole Chatt[4], Savita Gossain[5], Jeremy Hawker[4], Satheesh Nair[3], Keith Neal[4], Kathy Nye[5], Tansy Peters[3], Elizabeth De Pinna[3], Esther Robinson[6], Keith Struthers[5], Mark Webber[2], Andrew Catto[7], Timothy J. Dallman[3], Peter Hawkey[1,5*] and Nicholas J. Loman[1*]

The Future of Microbiome Research??


The 16S-based approach
Extract DNA
Amplify and sequence 16S rRNA
OTU1
OTU2
OTU3
OTU4
Group similar sequences into OTUs
NCBI
GreenGenes
myRDP
Silva
Use database to identify OTUs
Community composition: Which organisms are present?
Abundance
OTU
Relative abundance of OTUs in community
OTU phylogeny
Variant sequences and SNPs
GATTACA
GATTACA
GATTTCA
GATTTCA
GATTTCA
Microbial community sample
Extract DNA
The shotgun metagenomic approach
Sequence community DNA
Compare sequences to reference genomes
Community function: What can the community do?
NCBI
KEGG
SEED
BLAST
Use database to identify sequences
Abundance
Functions
Relative abundance of gene pathways in community

NIH HUMAN
MICROBIOME
PROJECT