Bacterial Genomics and the Human Microbiome

Dr. Bob Blumenthal, UTHSC Robert.Blumenthal@utoledo.edu

BRIM 6200/6800
Biomarker Discovery, Validation and Implementation

BIPG 6400/8400
Applications of Bioinformatics and Proteomics/Genomics

Bacterial Genomics and the Human Microbiome

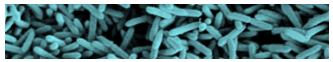
Bacterial Genomics and the Human Microbiome

1. **Background**
   - A. Distribution and ubiquity of bacteria
   - B. Still learning very basic features about bacteria
   - C. Metagenomics to escape the limits of culturability
   - D. 16S rRNA and "Next Generation Sequencing"
2. **Genome sequence as "parts list"**
   - A. Gene numbers
   - B. Identifying pathways, virulence factors, drug/vaccine targets
   - C. Synthetic chromosomes (minimal cells, new amino acids)
3. **Regulatory architecture as "wiring diagram"**
   - A. Operon, regulon, stimulon
   - B. Predicting regulation from the genomes of poorly-characterized bacteria
4. **The human microbiome and how it is characterized**
   - A. Bacterial "species", and how they change
   - B. Microbiome microbes C. Composition at different body sites
   - D. Twin studies E. α *vs.* β diversity F. Stability and resilience
5. **Medical conditions correlated with microbiome properties**
6. **Microbiome properties that can serve as biomarkers**
   - A. Specific genes/organisms B. Community structure
7. **Manipulation of the microbiome in individualized medicine**
   - A. Diet and pre/pro/syn-biotics B. Antibiotics and vaccines C. Transplants
8. **Homework assignments**

1. Background – A. Distribution and ubiquity of bacteria

Given their ecological, geochemical, agricultural and medical importance, we know surprisingly little about bacteria.

- Great majority of dissolved organic N in seawater is bacterial cell wall material (peptidoglycan).



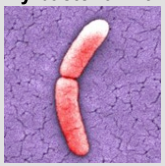
- Sediments under open ocean contain _____ bacteria/cm^3 (Soil has ~10^9/g, ~2-10,000 spp.)

- Soil microbes are historically the richest source of antibiotics used in human medicine. >99% of soil bacteria are resistant to culturing, and their antibiotics remain inaccessible by traditional means.

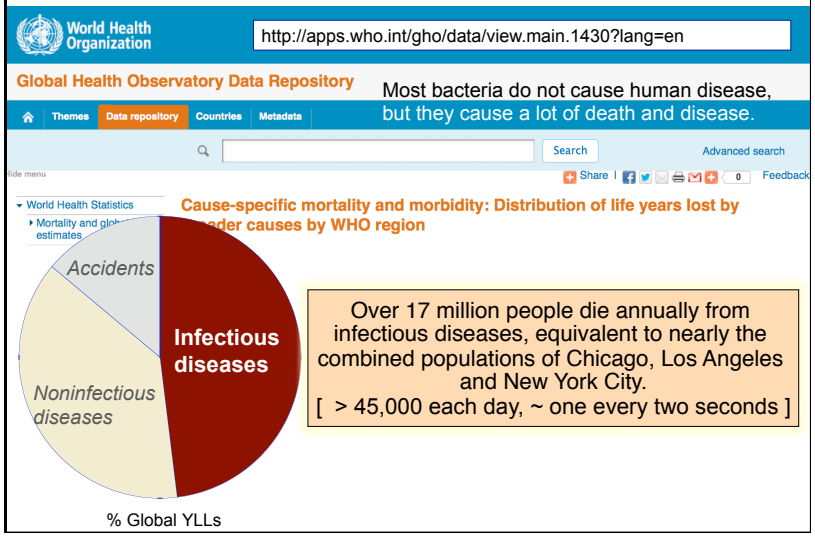
1. Background – A. Distribution and ubiquity of bacteria

- The **great** majority of infectious agents are **not** pathogenic to humans.
  - – Molecular methods indicate >400 species of bacteria in the human oral cavity. Only ~150 of those species have been cultured in laboratories and identified.
  - –Similar situation for human colon.
  - –Total estimated number of bacteria on Earth: _____

10–100 trillion (10^{13}-10^{14}) symbiotic microbial cells harbored by each person, primarily bacteria in the gut



1. Background – A. Distribution and ubiquity of bacteria



Most bacteria do not cause human disease, but they cause a lot of death and disease.

% Global YLLs

Over 17 million people die annually from infectious diseases, equivalent to nearly the combined populations of Chicago, Los Angeles and New York City.
[> 45,000 each day, ~ one every two seconds]

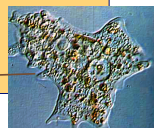
1. Background – B. Still learning very basic features about bacteria

Historical assumption:

Bacteria are small enough, and have a high enough surface-to-volume ratio, that no internal cytoplasmic organization is needed…

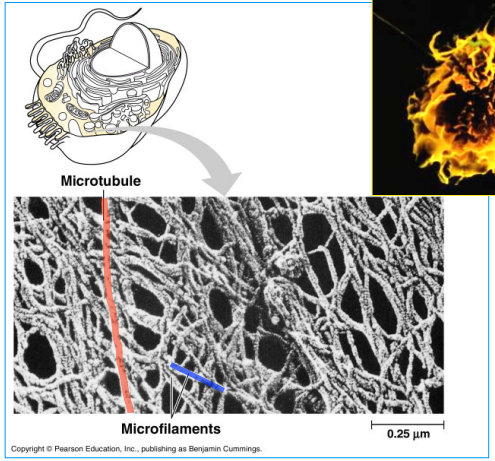
Surface area of sphere = $4\pi r^2$ Volume of sphere = $^4/_3\pi r^3$

Radius	Surface Area	Volume	SA:Vol
1 µm	12.57	4.19	3.0
2	50.29	33.52	1.5
4	201.14	268.19	0.75
10 µm	1257.14	4190.47	0.3

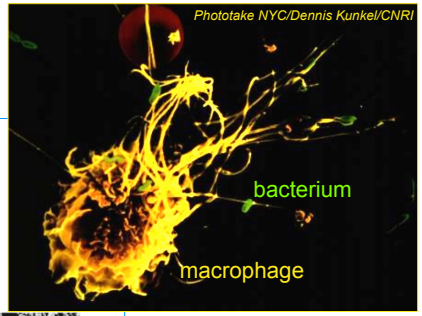


…while eukaryotic cells are large enough to require a complex cytoskeleton.

Eukaryotic cells have structurally-organized cytoplasm (cytoskeleton)



35.9.122.184/images/ 07-TourOfTheCell/



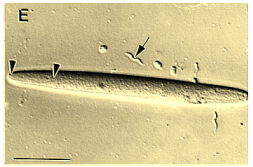
Typical bacteria are much smaller; have been believed to lack cytoskeleton.

1. Background – B. Still learning very basic features about bacteria

Much larger bacteria are now being discovered, and may require cytoskeletons

Epulopiscium fishelsoni is a gut symbiont of the brown surgeonfish in the Red Sea (light micrograph, bar = 50 µm). Note the presumed spirillum (arrow) with a length of ~18 µm.

http://jb.asm.org/cgi/content/full/180/21/5601/F1



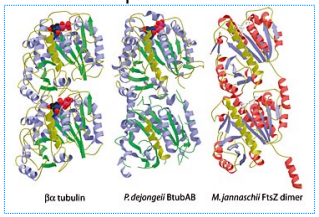
The colossus among bacteria, ~1 mm diameter, is a single-celled giant that lives in oceanic sulfur-rich sediment off Namibia and is named *Thiomargarita namibiensis.*

http://www.sciencenews.org/pages/sn_arc99/4_17_99/fob5.htm

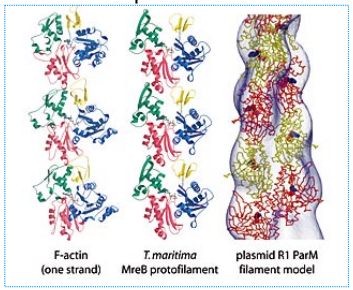
1. Background – B. Still learning very basic features about bacteria

Bacteria contain both actin and tubulin-like proteins that polymerize *in vivo* and *in vitro* into filaments similar to their counterparts in eukaryotes

Tubulin-like proteins in bacteria



Actin-like proteins in bacteria



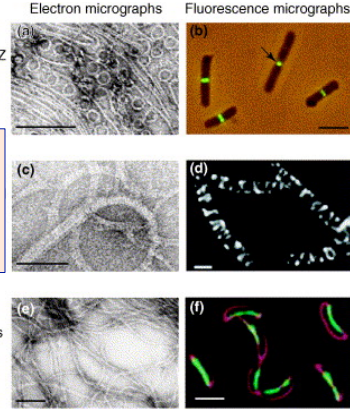
http://www2.mrc-lmb.cam.ac.uk/SS/Lowe_J/

1. Background – B. Still learning very basic features about bacteria

Increasing complexity of the bacterial cytoskeleton

Jakob Møller-Jensen and Jan Löwe

Current Opinion in Cell Biology 2005, 17:75–81



Size bars are 100nm in (a, c, e) and 2µm (2000nm) in (b, d, f).

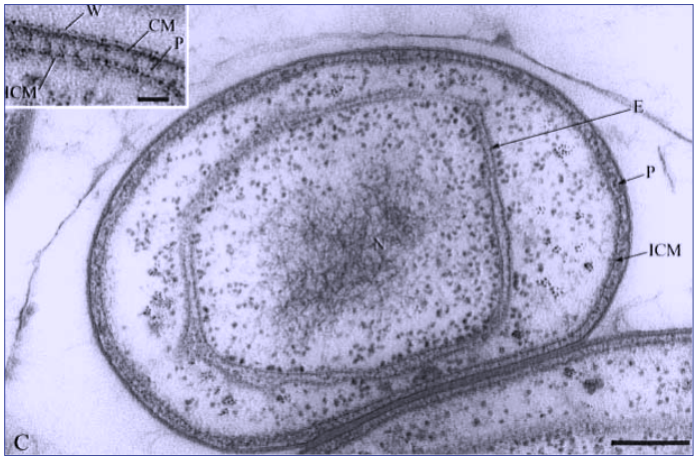
(a) Straight and curved FtsZ protofilaments. **(b)** Immunofluorescent localization of FtsZ in *E. coli*. Arrow indicates a constricting Z-ring. **(c)** MreB filaments and sheets. **(d)** Helical filaments formed by the MreB-like protein Mbl fused to GFP in *Bacillus subtilis*. **(e)** Intermediate filaments formed by crescentin. **(f)** Crescentin-GFP localisation to the inner cell curvature of *Caulobacter crescentus*. The cell membrane is stained in red.

Okay, bacteria have cyto-skeletons, but they don't have nuclei. Right?

Margaret R. Lindsay · Richard I. Webb · Marc Strous
Mike S. M. Jetten · Margaret K. Butler
Rebecca J. Forde · John A. Fuerst

Arch Microbiol (2001) 175 : 413–429

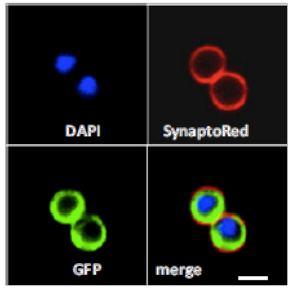
Cell compartmentalisation in planctomycetes: novel types of structural organisation for the bacterial cell

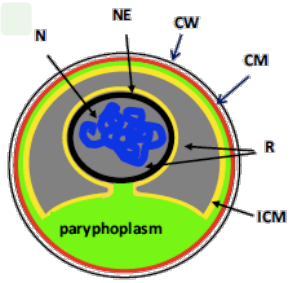


Endocytosis-like protein uptake in the bacterium *Gemmata obscuriglobus*

PNAS | July 20, 2010 | vol. 107 | no. 29 | 12883–12888

Thierry G. A. Lonhienne[a,b,1], Evgeny Sagulenko[a,1], Richard I. Webb[c], Kuo-Chang Lee[a], Josef Franke[d], Damien P. Devos[e], Amanda Nouwens[a], Bernard J. Carroll[a,b], and John A. Fuerst[a,2]

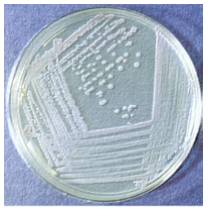




SOME bacteria are large, nucleated, and/or endocytic, have introns, *etc.*

G. obscuriglobus cells were incubated with GFP and then stained with DAPI and SynaptoRed. A GFP-containing region is seen in the cytoplasm bounded by the cytoplasmic membrane as defined by the SynaptoRed staining and is separated from the nuclear body (DAPI staining). **N**, nucleoid; **NE**, nuclear envelope; **ICM**, intracytoplasmic membrane; **R**, riboplasm; **CM**, cytoplasmic membrane; **CW**, cell wall.

1. Background – C. Metagenomics to escape the limits of culturability



Growth parameters commonly dealt with:

pH salinity temperature pO_2

simple nutrients (too many?) light osmolarity

Difficult to accommodate:

obligate partnerships

complex nutrients *etc.*

The great majority of bacteria (>>95%) have never been grown in the laboratory.

1. Background – C. Metagenomics to escape the limits of culturability

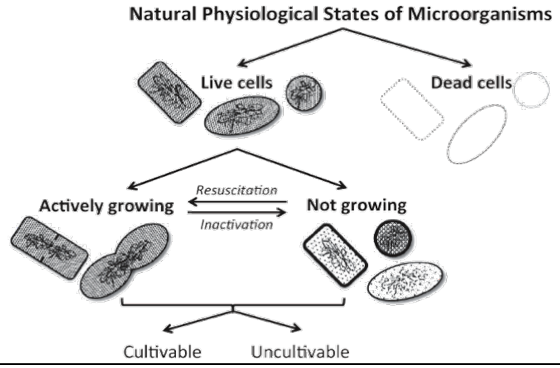
Microbes Environ. Vol. 27, No. 4, 356–366, 2012
https://www.jstage.jst.go.jp/browse/jsme2 doi:10.1264/jsme2.ME12092

MICROBES AND ENVIRONMENTS

Minireview

Are Uncultivated Bacteria Really Uncultivable?

INDUN DEWI PUSPITA[1], YOICHI KAMAGATA[1,2], MICHIKO TANAKA[1], KOZO ASANO[1], and CINDY H. NAKATSU[1,3]*



Need to determine conditions for resuscitation AND growth

1. Background – C. Metagenomics to escape the limits of culturability

MINIREVIEW FEMS Microbiol Lett **309** (2010) 1–7

Strategies for culture of 'unculturable' bacteria

Sonia R. Vartoukian, Richard M. Palmer & William G. Wade

The finding that certain bacterial species have never been identified by culture may be a simple matter of coincidence: an organism that has a low prevalence or is particularly slow-growing may have been overlooked in cultural analyses. Additionally, many genetically distinct phylotypes are phenotypically indistinguishable and are lumped together if conventional biochemical methods for identification are used. Conversely, **some bacteria are genuinely resistant to culture in isolation on conventional media**.

An alternative approach for the culture of as-yet-uncultivated organisms is to simulate their natural environment *in vitro*. Kaeberlein *et al.* (2002) constructed a **diffusion chamber** that allowed the passage of substances from the natural environment (intertidal marine sediment) across a membrane and successfully grew bacteria from marine sediment that were previously uncultivated.

1. Background – C. Metagenomics to escape the limits of culturability

THE WALL STREET JOURNAL. ≡ U.S.

http://www.wsj.com/articles/scientists-discover-new-antibiotic-a-potential-weapon-against-a-range-of-diseases-1420654892

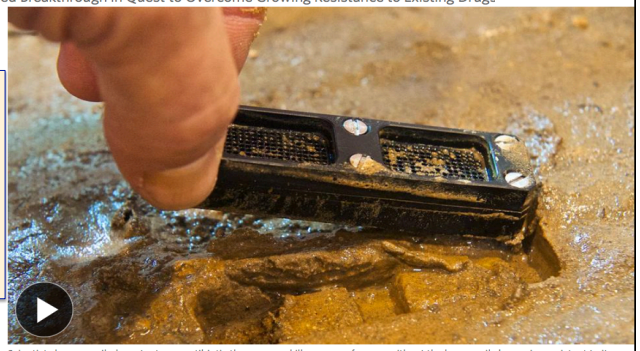
U.S. NEWS

Scientists Discover Potent Antibiotic, A Potential Weapon Against a Range of Diseases

By GAUTAM NAIK

January 8, 2015

Discovery Is a Much-Needed Breakthrough in Quest to Overcome Growing Resistance to Existing Drugs

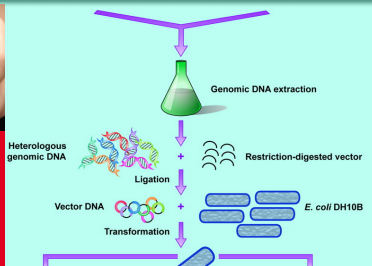


Scientists have unveiled a potent new antibiotic they say can kill an array of germs without the bugs easily becoming resistant to it. WSJ's Gautam Naik discusses the implications with Sara Murray. Photo: Slava Epstein/Northeastern University.

Even if one *could* culture 1000s of individual species, would that be the best approach?

Metagenomics

"The soil work is like trying to assemble 40,000 human genomes simultaneously."
— Craig Venter



Jo Handelsman

Microbiol Mol Biol Revs, 2004, 68: 669-685.

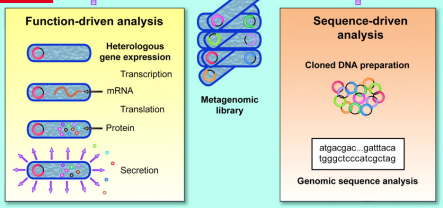
Metagenomics: Application of Genomics to Uncultured Microorganisms

V. Gewin

Nature, 2006, 439: 384-386.

Discovery in the Dirt

1g rich soil has ~10,000 species of bacteria



1. Background – C. Metagenomics to escape the limits of culturability

THE WALL STREET JOURNAL. ≡ U.S.

http://www.wsj.com/articles/big-data-and-bacteria-mapping-the-new-york-subways-dna-1423159629

HEALTH POLICY

Big Data and Bacteria: Mapping the New York Subway's DNA

Scientists in 18-Month Project Gather DNA Throughout Transit System to Identify Germs, Study Urban Microbiology

Researcher Christopher Mason from Weill Cornell Medical College collecting DNA samples at the 68th Street subway station in New York City last summer. The scientists identified hundreds of types of bacteria, most harmless, in the transit system as a way to study the microbiology of urban environments. Photo: Katie Orlinsky for The Wall Street Journal

By ROBERT LEE HOTZ

Feb. 5, 2015 1:07 p.m. ET

74 COMMENTS

Metagenomics in the news; 05-Feb-2015

[The research team found bacteria associated with everything from human diseases to mozzarella cheese.

Future: track outbreaks]

OPEN ACCESS Freely available online

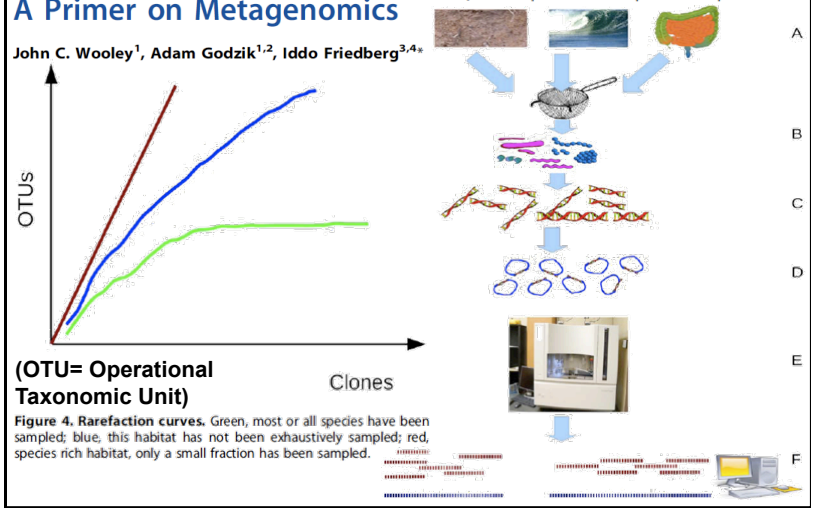
PLoS COMPUTATIONAL BIOLOGY

Review

February 2010 | Volume 6 | Issue 2 | e1000667

A Primer on Metagenomics

John C. Wooley[1], Adam Godzik[1,2], Iddo Friedberg[3,4]*

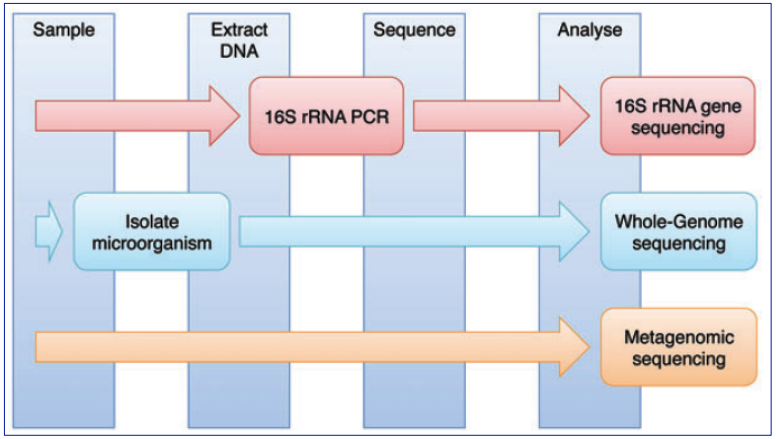


(OTU= Operational Taxonomic Unit)

Figure 4. Rarefaction curves. Green, most or all species have been sampled; blue, this habitat has not been exhaustively sampled; red, species rich habitat, only a small fraction has been sampled.

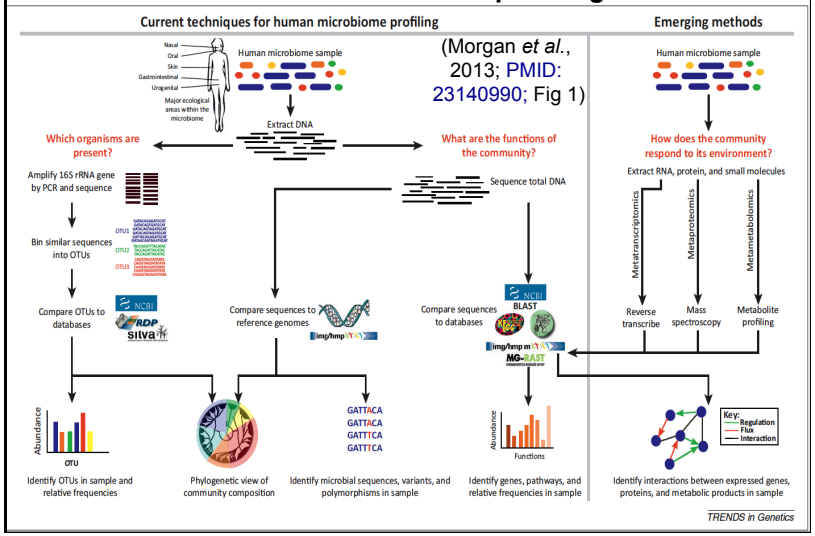
1. Background – D. 16S rRNA and "Next Generation" Sequencing

(Cox *et al.*, 2013; PMID: 23943792; Fig. 1)



1. Background – D. 16S rRNA and "Next Generation" Sequencing

(Morgan *et al.*, 2013; PMID: 23140990; Fig 1)



Analytical Tools and Databases for Metagenomics in the Next-Generation Sequencing Era

Genomics Inform 2013;11(3):102-113

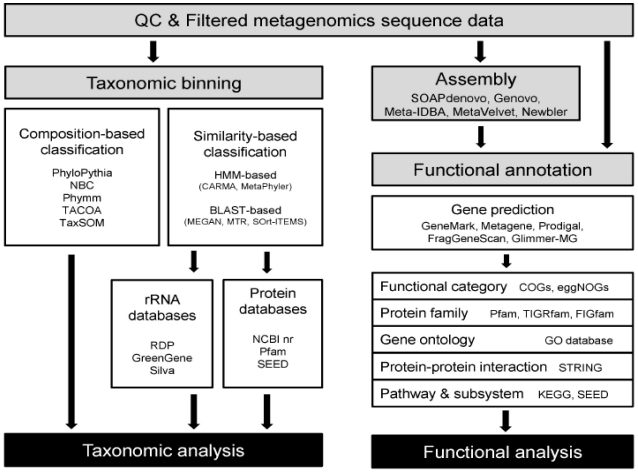
Mincheol Kim[1], Ki-Hyun Lee[1], Seok-Whan Yoon[1], Bong-Soo Kim[2], Jongsik Chun[1,2], Hana Yi[3,4,5*]

- Aside from experimental design and contextual data, metagenomic data have inherent limitations that must be overcome in the future.
  - ✓ Metagenomic reads commonly show a relatively low genomic coverage compared to that of a single genome
  - ✓ The short length of sequencing reads makes only fragmented information by the incomplete assembly and annotation processes accessible.
- Initiatives are already under way for filling the gap between metagenomic reads by doing
  - ✓ co-assembly with single-cell genomics
  - ✓ joint analysis between multiple metagenomes simultaneously, assuming that the same species must exist in different samples and that the co-occurrence helps extract shared information.
- The ultimate goal of metagenomics is a comprehensive understanding of our ecosystem.

Analytical Tools and Databases for Metagenomics in the Next-Generation Sequencing Era

Genomics Inform 2013;11(3):102-113

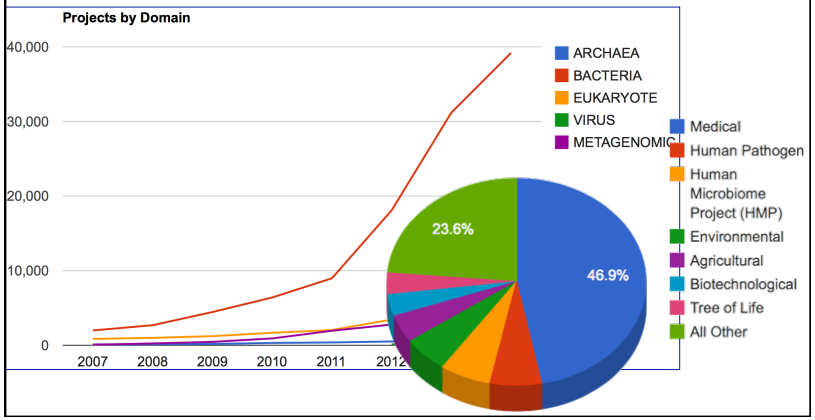
Mincheol Kim[1], Ki-Hyun Lee[1], Seok-Whan Yoon[1], Bong-Soo Kim[2], Jongsik Chun[1,2], Hana Yi[3,4,5*]



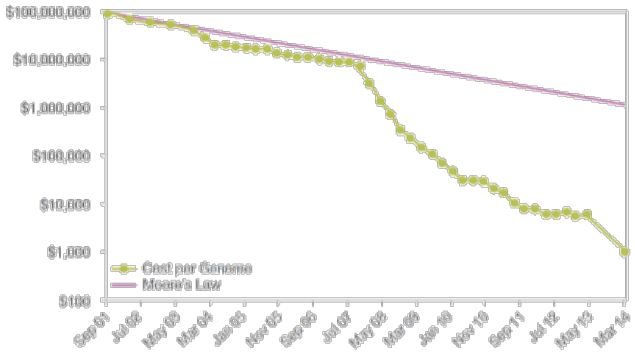
1. Background – D. 16S rRNA and "Next Generation" Sequencing

GOLD JGI JOINT GENOME INSTITUTE UNITED STATES DEPARTMENT OF ENERGY

As of Feb 2015: https://gold.jgi-psf.org/statistics



1. Background – D. 16S rRNA and "Next Generation" Sequencing



"The HiSeq X retails for $1 million a unit – and Illumina won't sell you fewer than ten. Despite the massive upfront costs, the math seems to check out."

What You Need to Know About Illumina's New Sequencers

Bio·IT World *http://www.bio-itworld.com/BioIT_Article.aspx?id=133900*

By Aaron Krol January 15, 2014

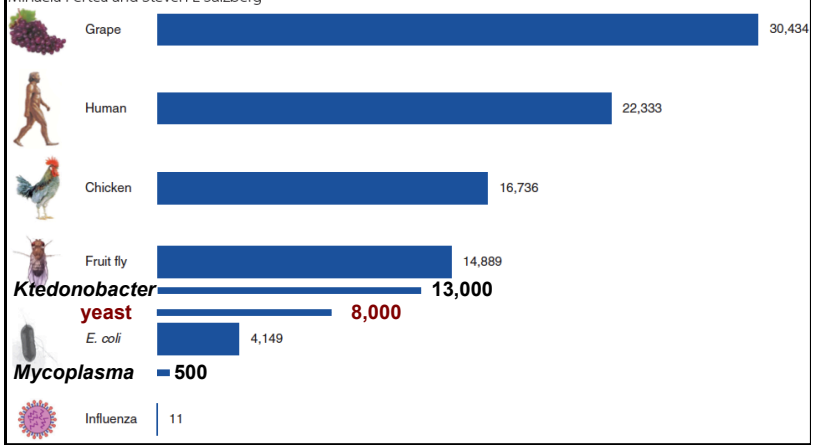
2. Genome as "Parts List" – A. Gene Numbers

REVIEW

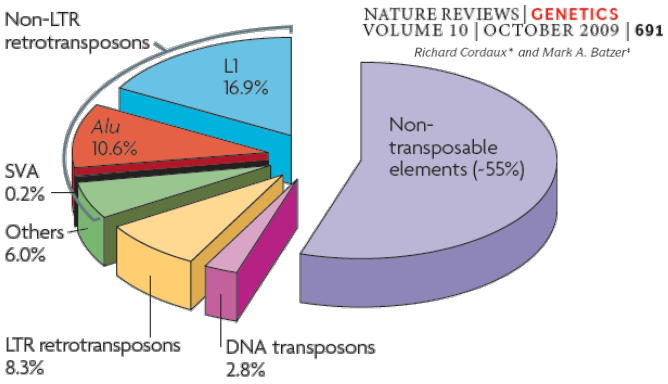
Between a chicken and a grape: estimating the number of human genes

http://genomebiology.com/2010/11/5/206

Mihaela Pertea and Steven L Salzberg*



2. Genome as "Parts List" – A. Gene Numbers



NATURE REVIEWS | GENETICS
VOLUME 10 | OCTOBER 2009 | 691

Richard Cordaux* and Mark A. Batzer‡

Approximately 45% of the human genome is currently recognized as being derived from transposable elements.
~1.5% codes for proteins.

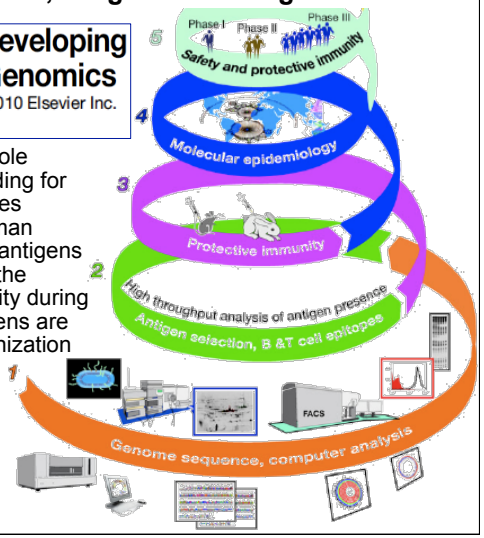
2. Genome as "Parts List" – B. Identifying Pathways, Virulence Factors, Drug/Vaccine Targets

Reverse Vaccinology: Developing Vaccines in the Era of Genomics

530 Immunity 33, October 29, 2010 ©2010 Elsevier Inc.

Alessandro Sette[1] and Rino Rappuoli[2,*]

(1) Computer analysis of the whole genome identifies the genes coding for predicted antigens, and eliminates antigens with homologies to human proteins. **(2)** Then the identified antigens are screened for expression by the pathogen and for immuno-genicity during infection. **(3)** The selected antigens are then used to test whether immunization induces a protective response. **(4)** Protective antigens are then tested for their presence and conservation across the species (molecular epidemiology). **(5)** Selected antigens are produced in large scale for clinical trials.



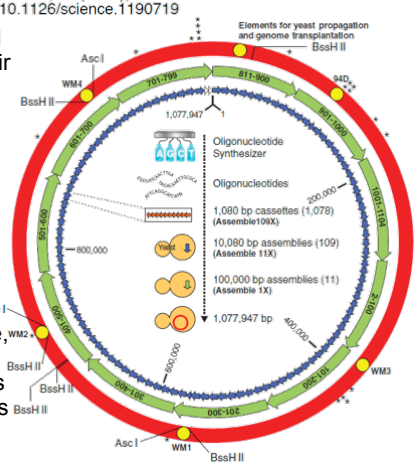
2. Genome as "Parts List" – C. Synthetic Chromosomes

Science
AAAS

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome
Daniel G. Gibson, *et al.*
Science **329**, 52 (2010);
DOI: 10.1126/science.1190719

"We report the design, synthesis, and assembly of the 1.08–mega–base pair *Mycoplasma mycoides* JCVI-syn1.0 genome:

- ✓ starting from digitized genome sequence information
- ✓ transplantation into a *M. capricolum* recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome.
- ✓ The only DNA in the cells is the designed synthetic DNA sequence, including "watermark" sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process."

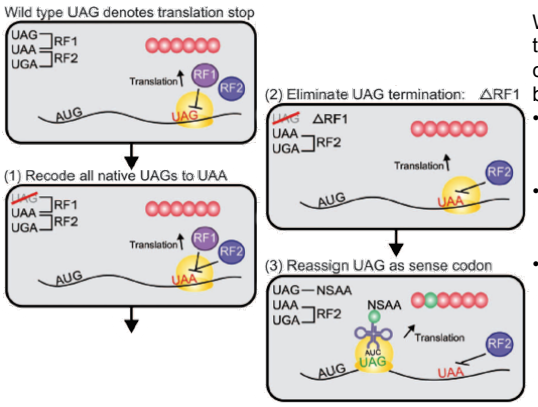


2. Genome as "Parts List" – C. Synthetic Chromosomes

Genomically Recoded Organisms Expand Biological Functions

Marc J. Lajoie,[1,2] Alexis J. Rovner,[3,4] Daniel B. Goodman,[1,5] Hans-Rudolf Aerni,[4,6] Adrian D. Haimovich,[3,4] Gleb Kuznetsov,[1] Jaron A. Mercer,[7] Harris H. Wang,[8] Peter A. Carr,[9] Joshua A. Mosberg,[1,2] Nadin Rohland,[1] Peter G. Schultz,[10] Joseph M. Jacobson,[11,12] Jesse Rinehart,[4,6] George M. Church,[1,13,*] Farren J. Isaacs[3,4,*]

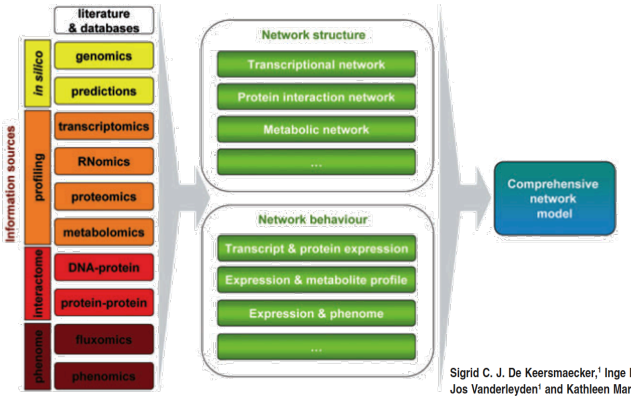
Science 342, 357 (2013)
Science
AAAS



We selected UAG as our first target for genome-wide codon reassignment because:

- UAG is the rarest codon in *E. coli* MG1655 (321 known instances)
- prior studies demonstrated the feasibility of amino acid incorporation at UAG
- a rich collection of translation machinery capable of incorporating nonstandard amino acids has been developed for UAG. [*E.g.*, phosphoserine, *p*-acetyl-phenylalanine]

3. Regulatory architecture as "wiring diagram"

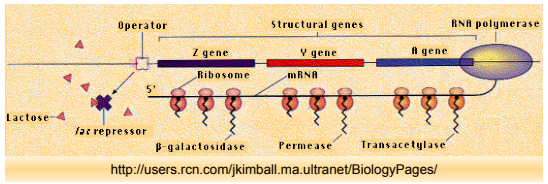


Sigrid C. J. De Keersmaecker,[1] Inge M. V. Thijs,[1] Jos Vanderleyden[1] and Kathleen Marchal[1,2]*

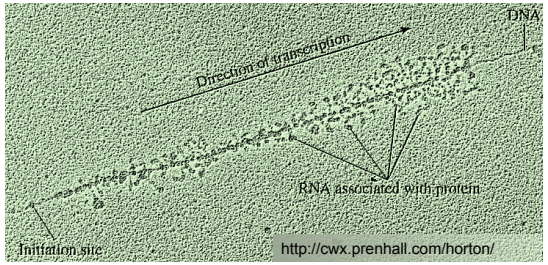
Fig. 1. Integration of omics data. Different information sources, i.e. omics data, literature and computational predictions, can be integrated to infer the structure of the transcriptional network, the protein interaction network or the metabolic network (network structure). Besides unravelling structures, omics data allow analysing the responses (mRNA, protein and metabolite profiles) triggered by each of these networks and studying their mutual relation (network behaviour). The ultimate goal in data integration will be to combine the transcriptional, protein interaction and metabolic network to construct comprehensive network models.

Integration of omics data: how well does it work for bacteria? Molecular Microbiology (2006) **62**(5), 1239–1250

3. Regulatory architecture as "wiring diagram" – A. Operon, regulon, stimulon

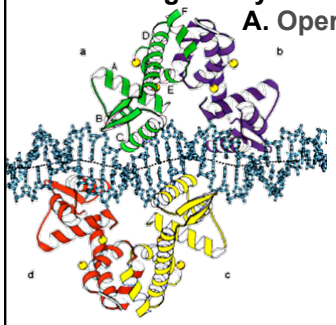


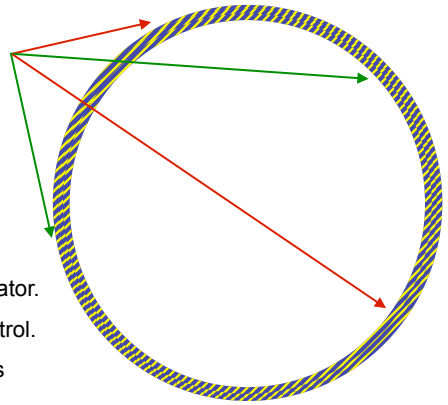
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/



http://cwx.prenhall.com/horton/

3. Regulatory architecture as "wiring diagram" – A. Operon, regulon, stimulon





Regulon – a group of operons controlled by the same regulator.

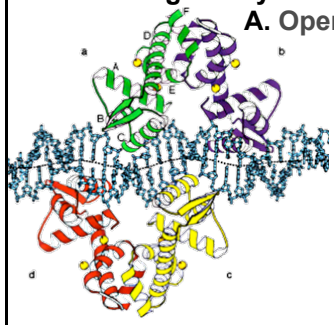
Can have a mix of + and – control.

Can have overlapping regulons (multiple regulators).

DtxR^{C102D} • Ni^{++} • *tox*
(White *et al.*, 1998, Nature 394: 502-6)

E.g., *E. coli* ArgR regulon includes 18 genes.

3. Regulatory architecture as "wiring diagram" – A. Operon, regulon, stimulon



Even for best-studied organism on the planet (*E. coli*), we know relatively little:

- There are about ~320 transcription regulatory proteins; only ~ half have been characterized.
- Some of the uncharacterized 1/2 of regulators could be "global" (subset of key regulators that each control 100s of genes)

- This is a problem, because people are trying to use our RELATIVELY extensive knowledge of *E. coli* to predict the regulatory architecture of organisms about which we know next to nothing.

3. Regulatory architecture as "wiring diagram" – A. Operon, regulon, stimulon

Stimulus

(heat, O_2, nutrients, population density, surface attachment, UV, pH, *etc.*)

?

"Half of the known and predicted transcriptional regulators in *E. coli* have predicted domains for the binding of small metabolites, whereas 10% have a CheY-like response regulator receiver domain that are phosphorylated by kinases in two-component signal transduction systems."

?

Marinez-Antonio & Collado-Vides, 2003, Curr Opinion Microbiol 6: 482-489.

Regulator Regulator Regulator Regulator Regulator Regulator Regulator

3. Regulatory architecture as "wiring diagram" – B. Predicting regulation from the genomes of poorly-characterized bacteria

Problem:

- Huge number of poorly-studied bacteria
- Laboratory analysis of which genes are expressed under which conditions (where the bacteria can be grown) is accurate, but very labor intensive.
- Would like to predict gene regulation via bioinformatics
- This is (currently) exceptionally difficult.
- Example: predicting promoters

Genome-Wide Identification of Transcription Start Sites, Promoters and Transcription Factor Binding Sites in *E. coli*

PLoS ONE | October 2009 | Volume 4 | Issue 10 | e7526

Alfredo Mendoza-Vargas[1], Leticia Olvera[1], Maricela Olvera[1], Ricardo Grande[4], Leticia Vega-Alvarado[2], Blanca Taboada[2], Verónica Jimenez-Jacinto[4], Heladia Salgado[3], Katy Juárez[1], Bruno Contreras-Moreira[3¤], Araceli M. Huerta[3], Julio Collado-Vides[3], Enrique Morett[1]*

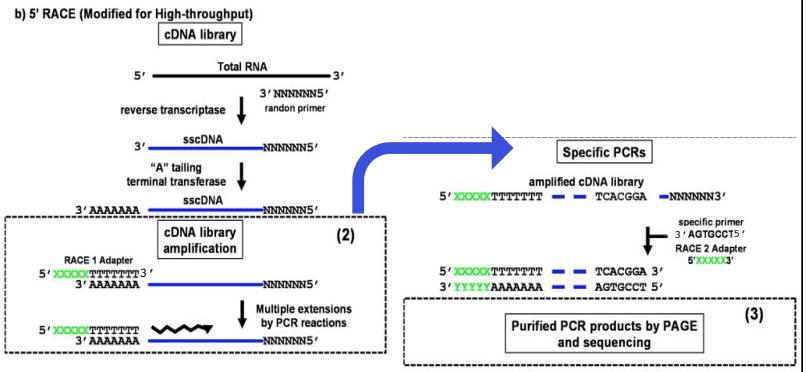
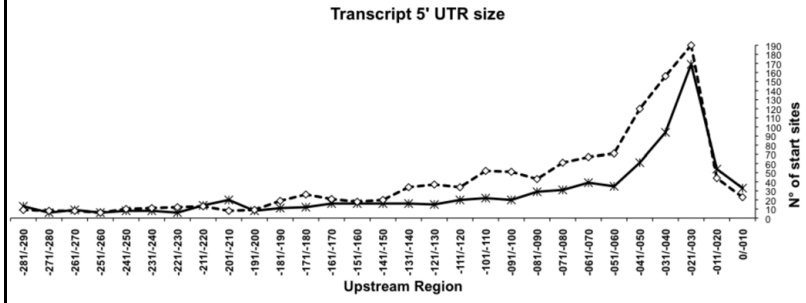


Figure 1. Directed Mapping of Transcription Start Sites

Genome-Wide Identification of Transcription Start Sites, Promoters and Transcription Factor Binding Sites in *E. coli*

PLoS ONE | October 2009 | Volume 4 | Issue 10 | e7526

Alfredo Mendoza-Vargas[1], Leticia Olvera[1], Maricela Olvera[1], Ricardo Grande[4], Leticia Vega-Alvarado[2], Blanca Taboada[2], Verónica Jimenez-Jacinto[4], Heladia Salgado[3], Katy Juárez[1], Bruno Contreras-Moreira[3¤], Araceli M. Huerta[3], Julio Collado-Vides[3], Enrique Morett[1]*



Hard to identify promoters from sequence alone.
Reason #1: Variable distance from ATG initiation codon

Genome-Wide Identification of Transcription Start Sites, Promoters and Transcription Factor Binding Sites in *E. coli*

PLoS ONE | October 2009 | Volume 4 | Issue 10 | e7526

Alfredo Mendoza-Vargas[1], Leticia Olvera[1], Maricela Olvera[1], Ricardo Grande[4], Leticia Vega-Alvarado[2], Blanca Taboada[2], Verónica Jimenez-Jacinto[4], Heladia Salgado[3], Katy Juárez[1], Bruno Contreras-Moreira[3¤], Araceli M. Huerta[3], Julio Collado-Vides[3], Enrique Morett[1]*

- RNAP can associate with any of the seven σ factors present in *E. coli*
  - ✓ Each σ factor recognizes different consensus promoter motifs
  - ✓ The majority of genes expressed during exponential growth involve the "housekeeping" σ70 factor.
  - ✓ The other six "alternative" σ factors have specific roles in stress survival and adaptation to environmental conditions.
- Unambiguous determination of which form of RNAP holoenzyme is transcribing a gene is not straightforward.

Hard to identify promoters from sequence alone.
Reason #2: Multiple sigma factors

Genome-Wide Identification of Transcription Start Sites, Promoters and Transcription Factor Binding Sites in *E. coli*

PLoS ONE | October 2009 | Volume 4 | Issue 10 | e7526

Alfredo Mendoza-Vargas[1], Leticia Olvera[1], Maricela Olvera[1], Ricardo Grande[4], Leticia Vega-Alvarado[2], Blanca Taboada[2], Verónica Jimenez-Jacinto[4], Heladia Salgado[3], Katy Juárez[1], Bruno Contreras-Moreira[3¤], Araceli M. Huerta[3], Julio Collado-Vides[3], Enrique Morett[1]*

- The canonical model for the σ^{70}-DNA promoter sequence:
  - ✓ –35 hexamer, consensus sequence = TTGACA
  - ✓ Spacer of 15 to 21 nucleotides
  - ✓ –10 hexamer, consensus sequence = TATAAT
    - ❖ –10 element is essential; stabilizes initial RNAP-σ binding to promoter
- Other elements can modify the canonical model of the σ^{70} promoters:
  - ✓ extended –10 region (TG), that is located 1 nucleotide upstream of the –10 element
    - ❖ Promoters with extended –10 normally have poor –35 element
  - ✓ UP element, usually positioned 4 nucleotides upstream of the –35 promoter region.

Hard to identify promoters from sequence alone.
Reason #3: Promoters have only short "consensus" sequences

Genome-Wide Identification of Transcription Start Sites, Promoters and Transcription Factor Binding Sites in *E. coli*

PLoS ONE | October 2009 | Volume 4 | Issue 10 | e7526

Alfredo Mendoza-Vargas[1], Leticia Olvera[1], Maricela Olvera[1], Ricardo Grande[4], Leticia Vega-Alvarado[2], Blanca Taboada[2], Verónica Jimenez-Jacinto[4], Heladia Salgado[3], Katy Juárez[1], Bruno Contreras-Moreira[3¤], Araceli M. Huerta[3], Julio Collado-Vides[3], Enrique Morett[1]*

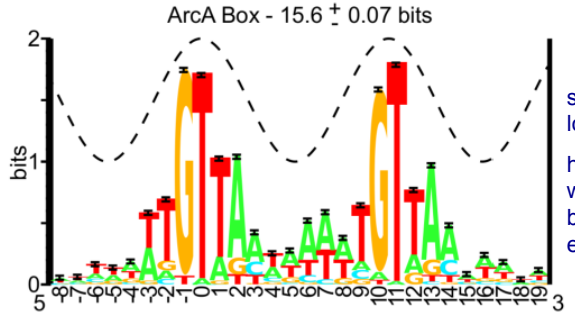
- Different combinations of promoter elements vary the basal strength
- On average, σ^{70}-dependent promoters preserve just 8/12 canonical nucleotides of the –35 and –10 hexamers
- ~10% of promoters match the consensus in only about half the nucleotides and yet still serve as sites for σ binding.
- Activated promoters often poorly match consensus (otherwise they would not need to be activated).
- [Also, in bacteria having genomes with low %GC, TATAAT –10 consensus occurs very frequently on a random basis.]

Hard to identify promoters from sequence alone.
Reason #4: Promoters do not always fit "consensus"

The Bacterial Response Regulator ArcA Uses a Diverse Binding Site Architecture to Regulate Carbon Oxidation Globally

PLOS Genetics | October 2013 | Volume 9 | Issue 10 | e1003839

Dan M. Park[1¤a], Md. Sohail Akhtar[2¤b], Aseem Z. Ansari[2], Robert Landick[2,3,4], Patricia J. Kiley[1,4]*



sequence logos:

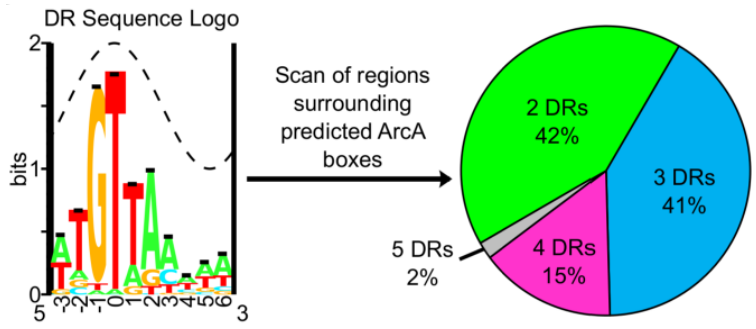
http://weblogo.berkeley.edu/

Also hard to identify regulator binding sites from sequence alone.
Reason #1: Operators do not always fit "consensus"

The Bacterial Response Regulator ArcA Uses a Diverse Binding Site Architecture to Regulate Carbon Oxidation Globally

PLOS Genetics | October 2013 | Volume 9 | Issue 10 | e1003839

Dan M. Park[1¤a], Md. Sohail Akhtar[2¤b], Aseem Z. Ansari[2], Robert Landick[2,3,4], Patricia J. Kiley[1,4]*

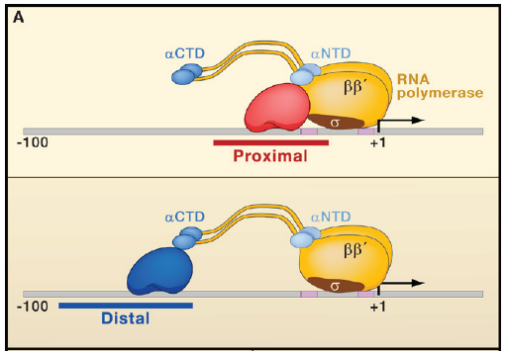


Also hard to identify regulator binding sites from sequence alone.
Reason #2: If there are multiple cooperative operators, none of them needs to be particularly strong.

Evolution of Transcriptional Regulatory Circuits in Bacteria

Cell *138*, July 24, 2009 ©2009 Elsevier Inc. 233

J. Christian Perez[1,2] and Eduardo A. Groisman[1,*]



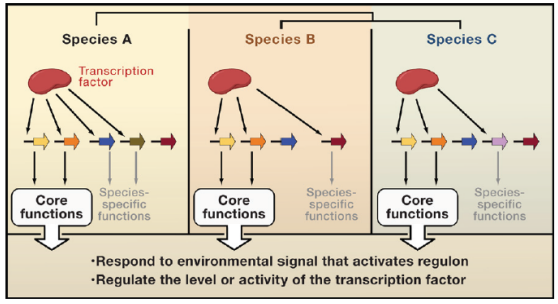
Also hard to identify regulator binding sites from sequence alone.
Reason #3: Operator-promoter spacing is variable.

Evolution of Transcriptional Regulatory Circuits in Bacteria

Cell *138*, July 24, 2009 ©2009 Elsevier Inc. 233

J. Christian Perez[1,2] and Eduardo A. Groisman[1,*]

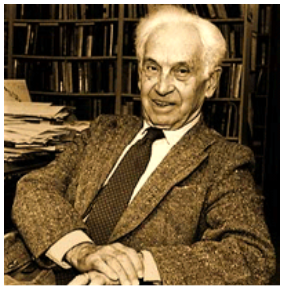
…organisms of the most different sorts are constructed from the very same battery of genes. The diversity of life forms results from small changes in the regulatory systems that govern expression of these genes.
– François Jacob,
Of Flies, Mice, and Men



Also hard to identify regulator binding sites from sequence alone.
Reason #4: Role of conserved regulators is variable between species.

4. The human microbiome and how it is characterized – A. Bacterial "species" and how they change

"Species are groups of interbreeding natural populations that are reproductively isolated from other such groups."



Ernst Mayr (1905 - 2005)

Bacteria reproduce asexually (and most have single chromosomes).

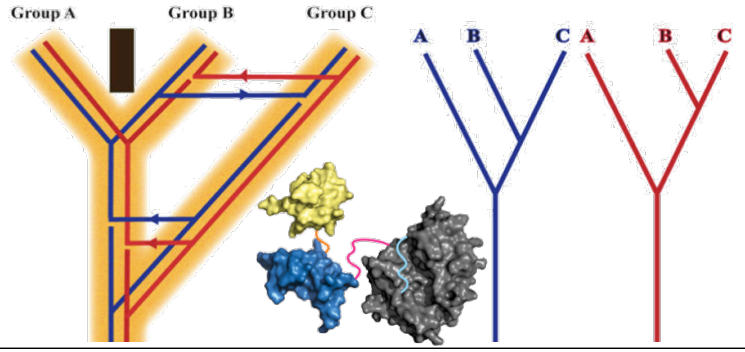
4. The human microbiome and how it is characterized – A. Bacterial "species" and how they change

Natural taxonomy in light of horizontal gene transfer

Biol Philos (2010) 25:589–602

Cheryl P. Andam · David Williams · J. Peter Gogarten

Evolution of organismal lineages | Molecular phylogenies



4. The human microbiome and how it is characterized – A. Bacterial "species" and how they change

- Clusters of similar sequences = "operational taxonomic units" (OTUs)
- 97% rRNA sequence identity → same species
- 93% rRNA sequence identity → same genus
- Warning: up to 5% of 16S rRNA sequences in GenBank may be erroneous (Ashelford *et al*., 2005; PMID 16332745)

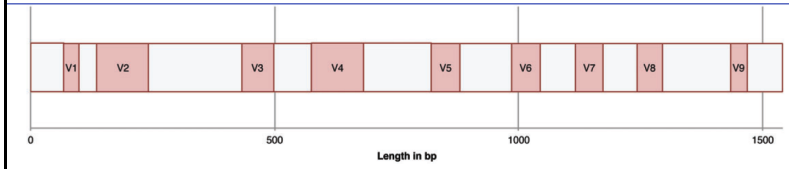
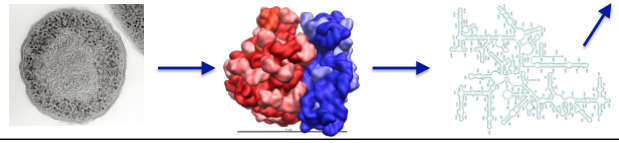


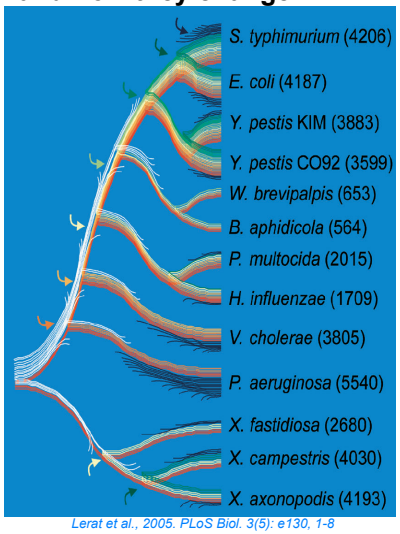
Figure 2. Approximately 1.5 kb 16S rRNA gene of *E.coli* showing the nine variable regions that make it an ideal target as a phylogenetic marker gene.



4. The human microbiome and how it is characterized – A. Bacterial "species" and how they change

Genome Evolution in γ-Proteo-bacteria

Only a small proportion of genes have been retained since the common ancestor of γ-proteo-bacteria (red). If ancestral and contemporary genome sizes are similar, most genes from this ancestral genome (white) have been replaced by nonhomologous genes (yellow to **green**), usually via LGT from organisms outside of this clade. The abundance of genes unique to a species (blue) indicates that these bacteria (with the exception of the endosymbionts) constantly acquire new genes, most of which do not persist long-term within lineages. (Numbers of non-IS or -phage ORFs are shown in parentheses).



Lerat et al., 2005. PLoS Biol. 3(5): e130, 1-8

Evolution of Pan-Genomes of *Escherichia coli*, *Shigella* spp., and *Salmonella enterica*

Journal of Bacteriology p. 2786–2792
June 2013 Volume 195 Number 12

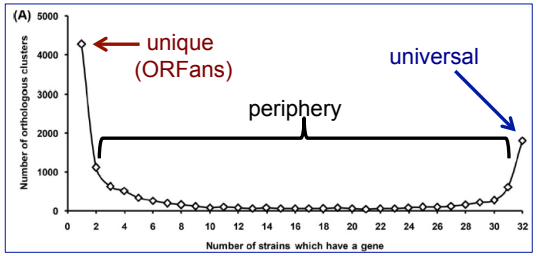
Evgeny N. Gordienko,[a] Marat D. Kazanov,[b] Mikhail S. Gelfand[b,c]

- The **pan-genome** is the total complement of genes from all sequenced strains of the same species, genus, or a larger group.
- The pan-genome consists of three parts:
  - ✓ the **universal** genome with genes common for all strains
  - ✓ the **unique** genome with strain-specific genes (known as ORFans)
  - ✓ the **periphery** (genes that are present in a subset of strains).
- In most studied bacterial species, the gene content of strains varies widely, and each additional sequenced strain adds new genes to the pan-genome.
  - ✓ The *E. coli* pan-genome is open (far from saturation).
- The distribution of the OGs by the number of strains in which they are present has a well-known U-shape form.
  - ✓ The periphery genes tend to be rare (present in just 2-3 strains) or almost universal (absent from only a few strains).
  - ✓ This holds true for the *E. coli*-plus-*Shigella* distribution .
  - ✓ Overrepresented functions in the unique genome tend to be plasmid related, *e.g.*, "DNA restriction-modification system" or "response to mercury ion."

Evolution of Pan-Genomes of *Escherichia coli*, *Shigella* spp., and *Salmonella enterica*

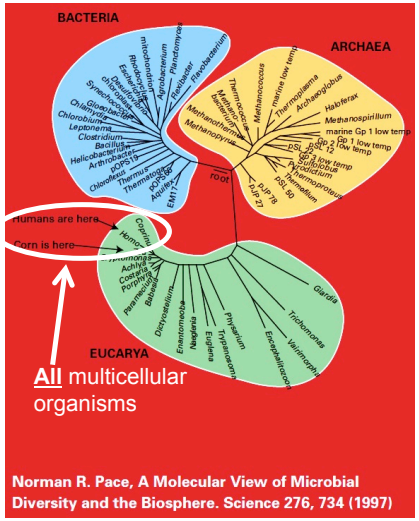
Evgeny N. Gordienko,[a] Marat D. Kazanov,[b] Mikhail S. Gelfand[b,c]

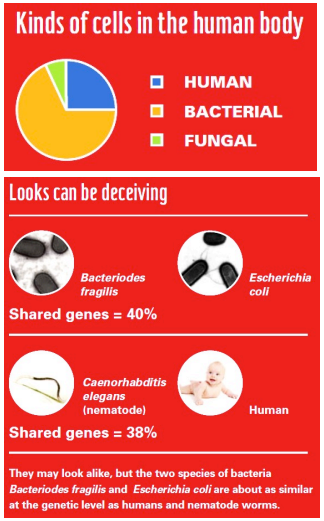
Journal of Bacteriology p. 2786–2792
June 2013 Volume 195 Number 12



- The distribution of the OGs by the number of strains in which they are present has a well-known U-shape form.
  - ✓ The periphery genes tend to be rare (present in just 2-3 strains) or almost universal (absent from only a few strains).
  - ✓ This holds true for the *E. coli*-plus-*Shigella* distribution .
  - ✓ Overrepresented functions in the unique genome tend to be plasmid related, *e.g.*, "DNA restriction-modification system" or "response to mercury ion."

4. The human microbiome and how it is characterized – B. Microbiome microbes





4. The human microbiome and how it is characterized – C. α *vs.* β diversity

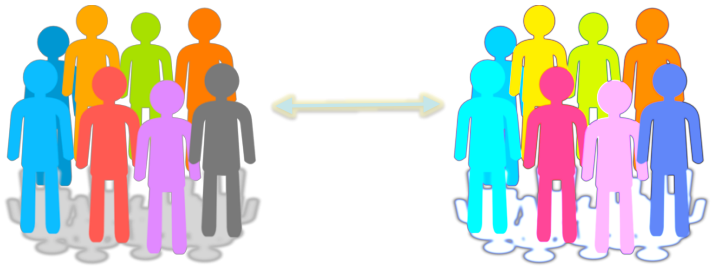
α Diversity: the organismal diversity within a sample. Quantification metrics may emphasize richness (total number of organisms in sample) and/or evenness (whether they are evenly distributed, in contrast to some more abundant than others).

Could refer to diversity within a given population, or (*e.g.*) within a given gut.

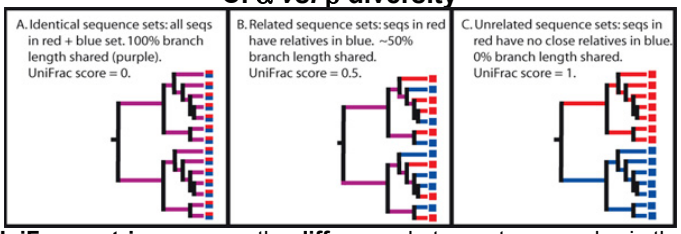
4. The human microbiome and how it is characterized – C. α *vs.* β diversity

α Diversity: the organismal diversity within a sample. Quantification metrics may emphasize richness (total number of organisms in sample) and/or evenness (whether they are evenly distributed, in contrast to some more abundant than others).

β Diversity: the organismal diversity shared between two or more communities, for example, from two or more different people sampled at the same body site. Can be measured either taxonomically (counting shared microbes) or phylogenetically (quantifying shared phylogenetic branches).



4. The human microbiome and how it is characterized – C. α *vs.* β diversity



The **UniFrac metric** measures the **difference** between two samples in the **branch length that is unique** to one sample or the other.

LEFT: every sequence in the top set has a very similar counterpart in the bottom set, so all branch length comes from nodes that have descendants in both samples (purple). This gives the minimum UniFrac distance of **0.0**.

MIDDLE: there is about as much branch length *unique* to each sample as is *shared* between samples, so the UniFrac distance is **~0.5**.

RIGHT: the division between the two samples occurs very early in the tree, so that all of the branch length is unique to one sample or the other. This results in the maximum UniFrac distance possible, **1.0**.

http://unifrac.colorado.edu/root?tool_id=unifrac_significance

4. The human microbiome and how it is characterized – D. Composition at different body sites

Structure, function and diversity of the healthy human microbiome

The Human Microbiome Project Consortium*

14 JUNE 2012 | VOL 486 | NATURE | 207

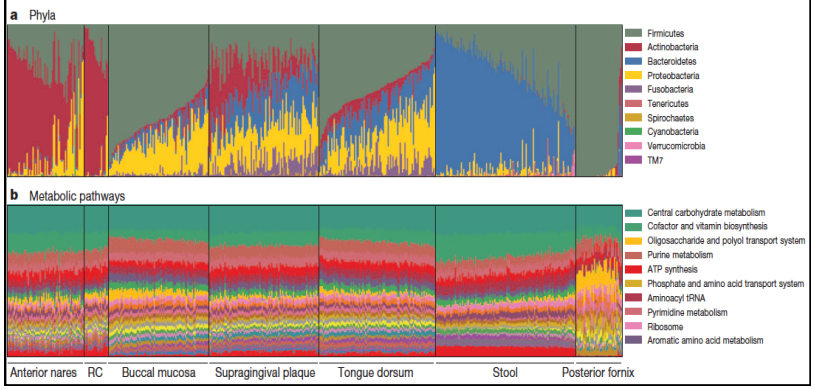
A total of 4,788 specimens from 242 screened and phenotyped adults (129 males, 113 females) were available for this study, representing the majority of the target Human Microbiome Project (HMP) cohort of 300 individuals.

4. The human microbiome and how it is characterized – D. Composition at different body sites

Structure, function and diversity of the healthy human microbiome

The Human Microbiome Project Consortium*

14 JUNE 2012 | VOL 486 | NATURE | 207



4. The human microbiome and how it is characterized – D. Composition at different body sites

Review

Trends in Genetics January 2013, Vol. 29, No. 1

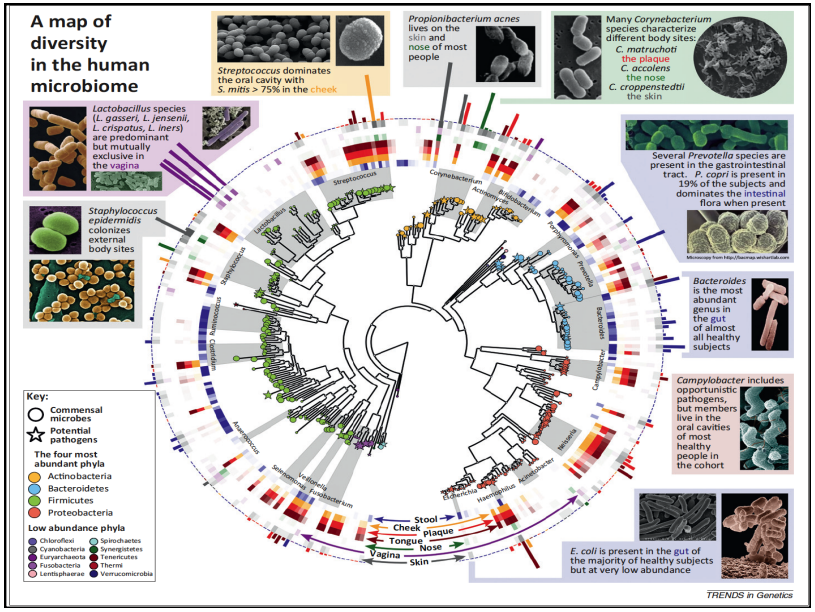
PMID: 23140990

Biodiversity and functional genomics in the human microbiome

Xochitl C. Morgan[1], Nicola Segata[1], and Curtis Huttenhower[1,2]

Habitat-specific functionality as a principle of human microbiome organization

Several human microbiome studies have reported the metagenomic distribution of **pathways within each body habitat to be much more consistent among individuals than are microbial abundances** …[S]pecific sets of site-specific gene function were maintained within each habitat regardless of the taxa present. The **stool microbiome was particularly abundant in genes related to complex carbohydrate degradation** despite highly variable Bacteroidetes:Firmicutes ratios… The **oral cavity microbiome, for example, was optimized for simple sugar metabolism** and particularly for dextran, whereas the **vaginal microbiome was optimized for glycogen and peptidoglycan degradation**. Much like individual bacterial genomes, **each habitat thus seems to have a core metagenome present in most hosts, in addition to a pan-metagenome** of more flexible auxiliary genes carried by the community of each habitat.



4. The human microbiome and how it is characterized – E. Twin studies

"The genetic and transcriptional diversity of the human gut microbiome is remarkable. Much of this diversity has not been previously identified through sequencing cultured human gut isolates; 64% of the gene clusters present in our microbiome bins had no representative in a set of 122 human gut microbial genomes, and **only 17% were shared between the two cotwins**. This diversity, even between genetically identical individuals, provides an expanded view of our multicellularity and interpersonal genetic variation."

"We deeply sampled the organismal, genetic, and transcriptional diversity in fecal samples collected from a monozygotic (MZ) twin pair…"

Organismal, genetic, and transcriptional variation in the deeply sequenced gut microbiomes of identical twins

PNAS | April 20, 2010 | vol. 107 | no. 16 | 7503–7508

Peter J. Turnbaugh[a,1], Christopher Quince[b], Jeremiah J. Faith[a], Alice C. McHardy[c], Tanya Yatsunenko[a], Faheem Niazi[d], Jason Affourtit[d], Michael Egholm[d], Bernard Henrissat[e], Rob Knight[f], and Jeffrey I. Gordon[a,2]

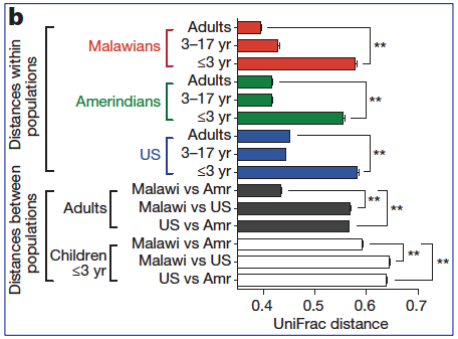
4. The human microbiome and how it is characterized – E. Twin studies

Human gut microbiome viewed across age and geography

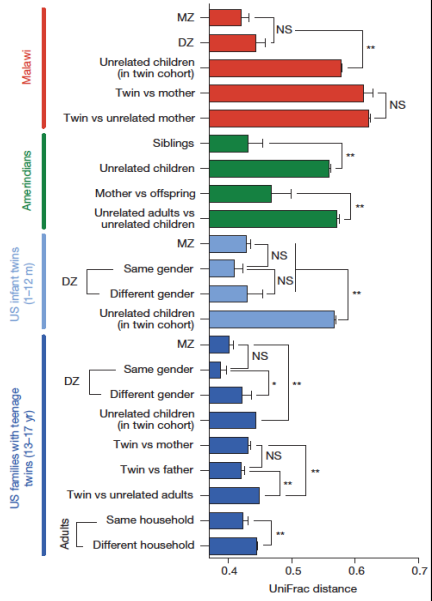
222 | NATURE | VOL 486 | 14 JUNE 2012

Tanya Yatsunenko[1], Federico E. Rey[1], Mark J. Manary[2,3], Indi Trehan[2,4], Maria Gloria Dominguez-Bello[5], Monica Contreras[6], Magda Magris[7], Glida Hidalgo[7], Robert N. Baldassano[8], Andrey P. Anokhin[9], Andrew C. Heath[9], Barbara Warner[2], Jens Reeder[10], Justin Kuczynski[10], J. Gregory Caporaso[11], Catherine A. Lozupone[10], Christian Lauber[10], Jose Carlos Clemente[10], Dan Knights[10], Rob Knight[10,12] & Jeffrey I. Gordon[1]

To examine how gut microbiomes differ among human populations, here we characterize bacterial species in fecal samples from 531 individuals, plus the gene content of 110 of them. The cohort encompassed healthy children and adults from the Amazonas of Venezuela, rural Malawi and US metropolitan areas and included mono- and dizygotic twins.



"We previously observed that adult monozygotic twins are no more similar to one another in terms of their gut bacterial community structure than are adult dizygotic twins. This result suggests that **the overall heritability of the microbiome is low**. We confirmed that the phylogenetic architecture of the fecal microbiota of monozygotic Malawian co-twins ≤3 years of age is no more similar than the microbiota of similarly aged dizygotic cotwins (n=15 monozygotic and 6 dizygotic twin pairs). We found that this is also true for monozygotic and dizygotic twin pairs aged 1–12 months (n=16 twin pairs), as well as teenaged twins (13– 17 years old; n=50 pairs) living together in the United States."



4. The human microbiome and how it is characterized – F. Stability and resilience

The human microbiome: ecosystem resilience and health

David A Relman

Nutrition Reviews® Vol. 70(Suppl. 1):S2–S9

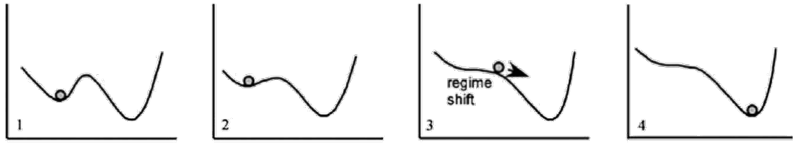


Figure 1 **"Basins of attraction," or alternative stable states or regimes, in a stability landscape.** External forces and internal change can alter the landscape (from scheme 1 to 4) such that an ecosystem (represented as the ball) becomes less resilient and attracted to a new stable state (or regime). Because the landscape topography predicts the near-future state of the ecosystem, the topography for a specific human habitat may have clinical utility in patient management. Reproduced with permission from Folke et al.[27].

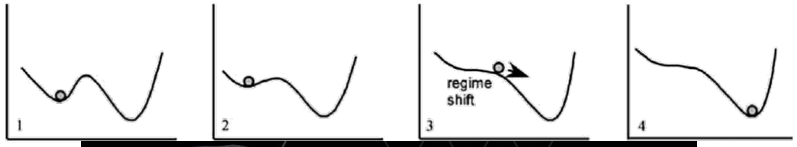
"The responses to an antibiotic are individualized and are influenced by prior experience with the same antibiotic. Besides serving to reveal critical underlying functional attributes, microbial interactions, and keystone species within the indigenous microbiota, the response to a standardized disturbance may have value in predicting future instability and disease and in restoring a preferred ecosystem regime."

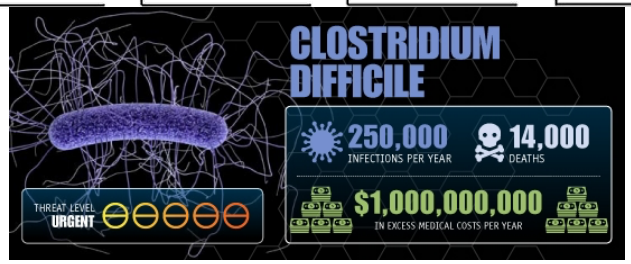
4. The human microbiome and how it is characterized – F. Stability and resilience

The human microbiome: ecosystem resilience and health

David A Relman

Nutrition Reviews® Vol. 70(Suppl. 1):S2–S9





http://www.cdc.gov/drugresistance/threat-report-2013/

5. Medical conditions correlated with microbiome properties

(Morgan *et al.*, 2013; PMID: 23140990)

Table 1. Conditions recently associated with changes in the human microbiome

Disease	Microbiome-related findings	Refs	PMID
Asthma	Large increase in airway microbial diversity; Comamonadaceae, Sphingomonadaceae, Oxalobacteraceae increases correlate with bronchial hyperresponsiveness	[69]	21194740
Atopic dermatitis	Decreased microbial diversity, increased *Staphylococcus aureus*; therapy restored commensals	[70]	22310478
Colorectal cancer	Tumors were enriched in typical gut microbes relative to surrounding healthy tissue; healthy tissue enriched in *Citrobacter, Cronobacter, Salmonella,* and *Shigella*	[71]	21647227
Kidney stones	Lack of *Oxalobacter formigenes* increases urinary oxalate levels and risk of kidney stones. Supplementation may be beneficial; results mixed	[9,72]	18322162 21460356
Periodontitis	Shift from *Actinomyces* and *Streptococcus* to *Haemophilus* and *Selenomonas*	[73]	22675498
Psoriasis	Decrease in commensal *Staphylococcus* and *Propionibacterium* and increase in Proteobacteria	[74]	22065152

5. Medical conditions correlated with microbiome properties

TABLE. Diseases and Conditions With Potential Links to the Human Microbiome

Disease or condition	Proposed mechanism	Evidence	Possible therapies available to alter microbiota
CDI	Reduced microbial diversity	Animal and human studies	FMT for treatment of recurrent CDI and questionable use of probiotics for prevention of CDI
IBS	Reduced microbial diversity and decreased Bacteroidetes	Animal and human studies	Probiotics for treatment of IBS
Inflammatory bowel disease	Reduced microbial diversity	Human studies	Probiotics (VSL #3) for treatment of pouchitis, trials of FMT ongoing
Obesity and metabolic derangements	Reversed Firmicutes to Bacteroides ratio	Animal and human studies	Trials of FMT ongoing
Allergic disorders	Reduced microbial diversity	Animal and human studies	Studies of probiotics ongoing
MDRO colonization	Reduced microbial diversity	Human studies	Studies of probiotics ongoing
Neuropsychiatric illnesses	Disruption of intestinal barrier	Animal and human studies	None

CDI = *Clostridium difficile* infection; FMT = fecal microbiota transplantation; IBS = irritable bowel syndrome; MDRO = multidrug-resistant organism.

MAYO CLINIC

CONCISE REVIEW FOR CLINICIANS

 ■ Mayo Clin Proc. 2014;89(1):107-114

A Clinician's Primer on the Role of the Microbiome in Human Health and Disease

PMID: 24388028

Sahil Khanna, MBBS, MS, and Pritish K. Tosh, MD

5. Medical conditions correlated with microbiome properties

ORAL DISEASES

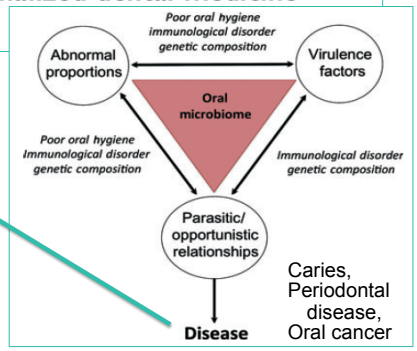
Oral Diseases (2012) 18, 109–120 doi:10.1111/j.1601-0825.2011.01851.x

www.wiley.com

INVITED MEDICAL REVIEW

The oral microbiome in health and disease and the potential impact on personalized dental medicine

MF Zarco, TJ Vess*, GS Ginsburg



Possible systemic effects:
Inflammatory mediators
Clotting activation
Bacteremia
etc.

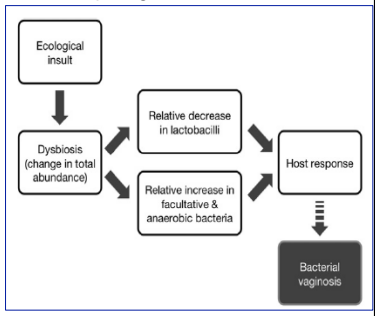
5. Medical conditions correlated with microbiome properties

Understanding vaginal microbiome complexity from an ecological perspective

Translational Research
Volume 160, Number 4 2012

ROXANA J. HICKEY, XIA ZHOU, JACOB D. PIERSON, JACQUES RAVEL, and LARRY J. FORNEY

- Bacterial vaginosis (BV) is the most common vaginal disorder of reproductive age women, resulting in millions of health care visits annually in the US alone.
- In nonpregnant women: associated with infertility, endometritis, and pelvic inflammatory disease, as well as an increased risk of acquiring HIV, *Neisseria gonorrhoeae* , and other STIs.
- During pregnancy: associated with preterm delivery, spontaneous abortion, premature rupture of membranes, preterm birth, amniotic fluid infections, postpartum endometritis, and endometritis following Caesarian section.
- Fallacies permeate thinking about the diagnosis and treatment of BV. One is that BV is an infectious disease. Though true for a number of pathogens, it may be inadequate to explain other diseases caused by **mixtures** of organisms."



5. Medical conditions correlated with microbiome properties

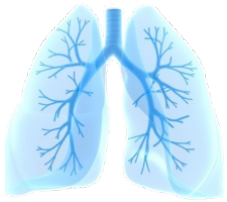
CHEST Translating Basic Research Into Clinical Practice

2013; 144(2):632–637

The Airway Microbiome and Disease

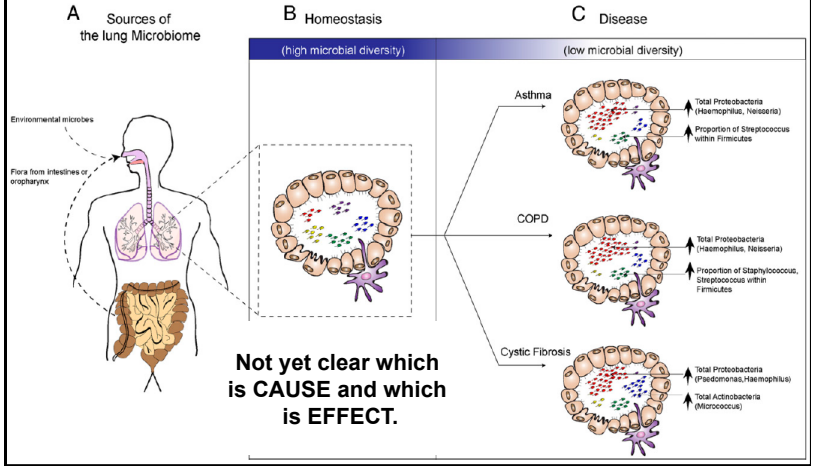
Benjamin J. Marsland, PhD; Koshika Yadava, MSc; and Laurent P. Nicod, MD

- [O]ne reason underlying the expectation that the airways were sterile was that the bacteria present there could not be cultured.
- It is now clear, based upon work from a number of research groups worldwide, that **there are bacteria resident in the airways** and, additionally, the bacterial load and proportions of phyla vary under healthy or diseased conditions.
- In a landmark article by Hilty and colleagues, the airway microbiome was shown to be different between healthy individuals and those with asthma or COPD.



5. Medical conditions correlated with microbiome properties

CHEST Translating Basic Research Into Clinical Practice



Not yet clear which is CAUSE and which is EFFECT.

5. Medical conditions correlated with microbiome properties

Human nutrition, the gut microbiome and the immune system

PMID 21677749

Andrew L. Kau[1]*, Philip P. Ahern[1]*, Nicholas W. Griffin[1], Andrew L. Goodman[1]† & Jeffrey I. Gordon[1]

16 JUNE 2011 | VOL 474 | NATURE | 327

Marked changes in socio-economic status, cultural traditions, population growth and agriculture are affecting diets worldwide. Understanding how our diet and nutritional status influence the composition and dynamic operations of our gut microbial communities, and the innate and adaptive arms of our immune system, represents an area of scientific need, opportunity and challenge. The insights gleaned should help to address several pressing global health problems.

- **Short-chain fatty acids** (SCFAs) provide one of the clearest examples of how nutrient processing by the microbiota and host diet combine to shape immune responses.
- SCFAs are the end products of **microbial** fermentation of plant polysaccharides that cannot be digested by humans alone, and the SCFAs affect host **immune responses**.
- **Butyrate** modifies cytokine production by T_H cells, and promotes intestinal epithelial barrier integrity, which in turn limits the exposure of the mucosal immune system to lumenal microbes, and thus prevents aberrant inflammatory responses.
- **Acetate** promotes the resolution of intestinal inflammation by the G-protein-coupled receptor GPR43.
- SCFAs may also regulate the acetylation of lysine residues, a covalent modification that affects proteins involved in a variety of signalling and metabolic processes.

5. Medical conditions correlated with microbiome properties

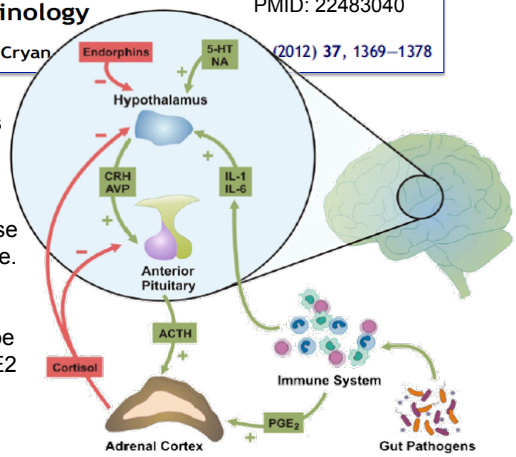
Regulation of the stress response by the gut microbiota: Implications for psychoneuroendocrinology

PMID: 22483040

Timothy G. Dinan *, John F. Cryan

(2012) 37, 1369–1378

- At a hypothalamic level classic neurotransmitters and cytokines regulate corticotrophin releasing hormone (CRH) and vasopressin (AVP) release into the portal vasculature.
- Negative feedback loops control the forward drive.
- The adrenal cortex can be directly activated by PGE2 from immune cells stimulated by gut pathogens.



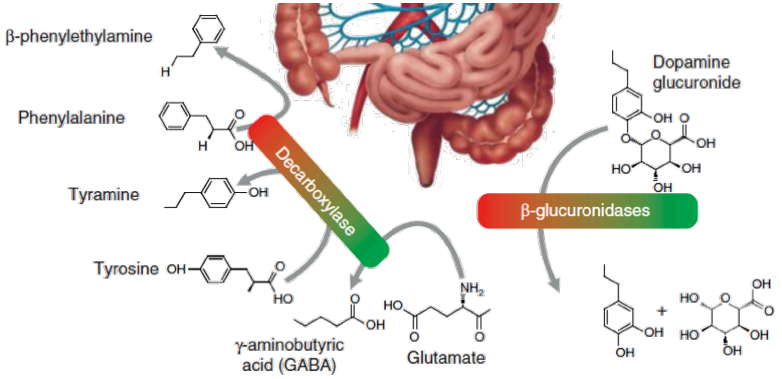
5. Medical conditions correlated with microbiome properties

The gut microbiome: the role of a virtual organ in the endocrinology of the host

Journal of Endocrinology (2013) **218**, R37–R47

James M Evans[1], Laura S Morris' and Julian R Marchesi[1,2]

Plus NO, modulation of serotonin via effects on Trp levels



6. Microbiome properties that can serve as biomarkers

A. Specific genes/organisms

B. Community structure

Detect individual pathogens or genes for functional pathways.

Detect dysbiotic (or abnormal) communities

Biomarkers for:
- Nutritional status
- Immune status
- *etc.*

- *Zobellia galactanivorans* is a marine bacterium that can digest porphyran derived from marine algae.
- Homologs of porphyranase genes from *Z. galactanivorans* are present in the human gut bacterium *Bacteroides plebeius*, and are prominent in the microbiomes of Japanese but not of N. Americans.
- Systematic changes in overall dietary consumption patterns across a population might lead to changes in the microbiome, with consequences for host nutritional status and immune response.

Kau *et al.* PMID: 21677749

7. Manipulation of the microbiome in individualized medicine

- **Diet and pre/pro/syn-biotics**
- **Antibiotics and vaccines**
- **Transplants**

Promote desired microbes

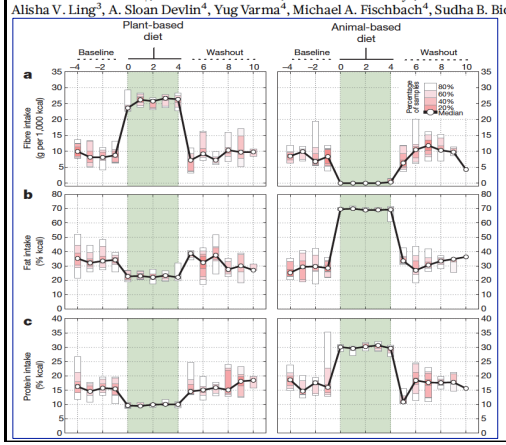
Selectively remove undesired microbes

Replace entire microbial community

7. Manipulation of the microbiome in individualized medicine

Diet rapidly and reproducibly alters the human gut microbiome 23 JANUARY 2014 | VOL 505 | NATURE | 559

Lawrence A. David[1,2]†, Corinne F. Maurice[1], Rachel N. Carmody[1], David B. Gootenberg[1], Julie E. Button[1], Benjamin E. Wolfe[1], Alisha V. Ling[3], A. Sloan Devlin[4], Yug Varma[4], Michael A. Fischbach[4], Sudha B. Biddinger[3], Rachel J. Dutton[1] & Peter J. Turnbaugh[1]

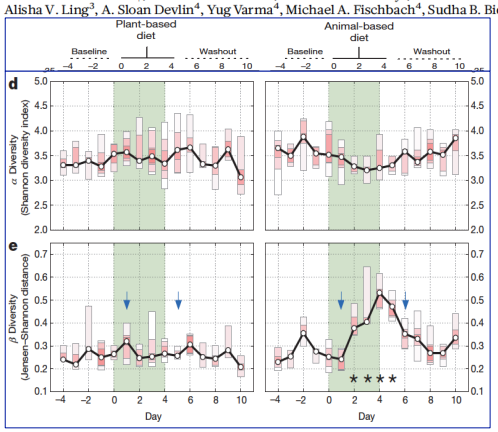


- We examined whether dietary interventions in humans can alter gut microbial communities in a rapid, diet-specific manner.
- Two diets, varied by their primary food source: a 'plant-based diet', rich in grains, legumes, fruits and vegetables; and an 'animal-based diet', which was composed of meats, eggs and cheeses.
- Each diet was consumed *ad libitum* for five consecutive days by six male and four female American volunteers between the ages of 21 and 33, whose body mass indices ranged from19 to 32 kgm.

7. Manipulation of the microbiome in individualized medicine

Diet rapidly and reproducibly alters the human gut microbiome 23 JANUARY 2014 | VOL 505 | NATURE | 559

Lawrence A. David[1,2]†, Corinne F. Maurice[1], Rachel N. Carmody[1], David B. Gootenberg[1], Julie E. Button[1], Benjamin E. Wolfe[1], Alisha V. Ling[3], A. Sloan Devlin[4], Yug Varma[4], Michael A. Fischbach[4], Sudha B. Biddinger[3], Rachel J. Dutton[1] & Peter J. Turnbaugh[1]



- Although no significant differences in α diversity were detected on either diet, we observed a significant increase in β diversity that was unique to the animal-based diet.
- This change occurred only 1 day after the diet reached the distal gut microbiota (as indicated by the food tracking dye).
- Subjects' gut microbiota reverted to their original structure 2 days after the animal-based diet ended.

7. Manipulation of the microbiome in individualized medicine

PRE- PRO- SYN-

Prebiotics: Nutrients that favor growth of particular bacteria

Probiotics: Particular bacteria (or mixes of them)

Synbiotics: Combinations of pre- and pro- biotics meant to work synergistically

- Despite substantial enthusiasm for probiotics to treat conditions that may stem from microbiome disruptions, use of currently-available probiotics alone for these conditions has not proven to be curative.
- This is likely due, in part, to the disparity between the complexity and diversity of the gut microbial ecologic system (mostly unculturable anaerobic microbiota) and the single organism often contained in **OTC** probiotics.

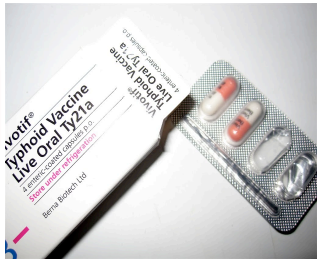
Mayo Clin Proc. 2014;89(1):107-114

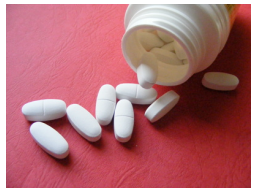
7. Manipulation of the microbiome in individualized medicine

- Antibiotics and vaccines

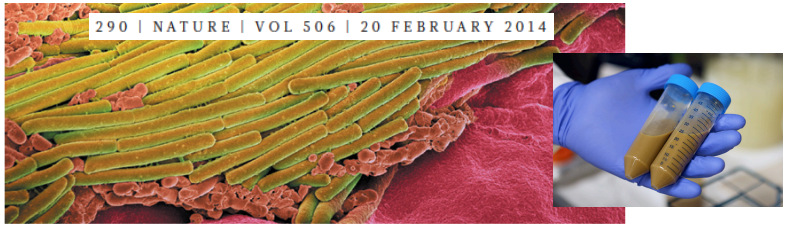
Prevent growth of particular species

Depending on broad vs. narrow spectrum of antibiotics, could eliminate particular groups of bacteria or prepare to replace entire microbial community.





7. Manipulation of the microbiome in individualized medicine



Clostridium difficile (yellow cells) causes an intestinal infection that can be treated with processed stool.

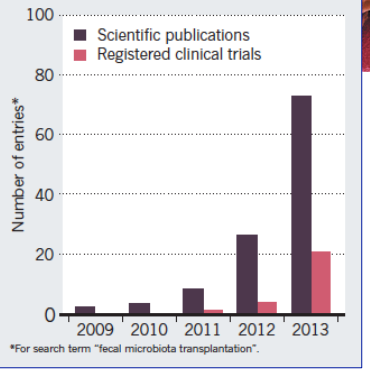
How to regulate faecal transplants

For medical use, human stool should be considered a tissue, not a drug, argue **Mark B. Smith**, **Colleen Kelly** and **Eric J. Alm**.

7. Manipulation of the microbiome in individualized medicine

STOOL TREATMENT

Interest in faecal transplants has surged in the past five years.



*For search term "fecal microbiota transplantation".

In the past decade, our understanding of the microbiome has moved from identifying species to associating them with diseases. The next step is to engineer this system to improve human health. FMT, as it is currently practiced, does not use specific, pure cultures of bacterial isolates; rather, it delivers uncharacterized, minimally processed faecal material into a patient. As this field progresses, we expect that the microbial 'active ingredients' will be elucidated, enabling well-characterized cultures to be used as a second generation of microbiome therapeutics[9,10].

7. Manipulation of the microbiome in individualized medicine

Weight Gain After Fecal Microbiota Transplantation

BRIEF REPORT

Neha Alang[1] **and Colleen R. Kelly**[2]

[1]Department of Internal Medicine, Newport Hospital, and [2]Division of Gastroenterology, Center for Women's Gastrointestinal Medicine at the Women's Medicine Collaborative, The Miriam Hospital, Warren Alpert School of Brown University, Providence, Rhode Island

Fecal microbiota transplantation (FMT) is a promising treatment for recurrent *Clostridium difficile* infection. We report a case of a woman successfully treated with FMT who developed new-onset obesity after receiving stool from a healthy but overweight donor. This case may stimulate further studies on the mechanisms of the nutritional-neural-microbiota axis and reports of outcomes in patients who have used non-ideal donors for FMT.

Winter, 2015

Take-home lessons

- Bacteria typically have several thousand genes, that must be regulated in a coordinated fashion in response to time and to complex environmental changes.
- Very basic questions about bacterial structure and physiology remain to be answered, even in well-studied species such as *Escherichia coli.* Bioinformatics is helping in this (*e.g.*, the discovery of membrane coat proteins in the Planctomycetes).
- Metagenomic approaches are yielding genome sequences for bacteria that cannot yet be grown in the laboratory, and there is great interest in developing robust methods to predict regulation from the DNA sequences.
- Even in *E. coli*, it is surprising how much remains to be learned about basic regulatory processes. So for the rest of the [huge] microbial world…
- Purely bioinformatic approaches to predicting regulation in bacteria will depend on a fuller understanding of transcription factor structure and function than we currently possess.
- The microbiome appears to have much greater impact on human physiology and health than previously appreciated. We are just beginning to learn how to use the microbiome as a biomarker, and how to alter it therapeutically.

BRIM 6200/6800

1. **Choose <u>three</u> or more papers**
   - ✓ that we did NOT use in this lecture
   - ✓ that focus on microbiomics
   - ✓ preferably that focuses on a disease or physiological phenomenon relevant to your research
   - ✓ provide the full author list, title, year, journal, volume, pages, AND PMID for each
   - ✓ provide a 2-3 sentence summary of each paper (except for the one you discuss in detail, per #2)

2. **Discuss <u>one</u> of those papers**
   - ✓ why did you choose this paper to discuss?
   - ✓ what problem were the authors attempting to address?
   - ✓ what microbiomic approaches did they use?
   - ✓ what did they conclude?
   - ✓ why were/weren't their conclusions supported by their results?
   - ✓ what new questions are raised by this work?

3. **Warnings**
   - ✓ I will compare homework from different students.
   - ✓ I will look to see if you're simply quoting from the abstract.
   - ✓ I will only grade homework that has been submitted by email as .rtf, .doc, or .docx
   - ✓ Be sure to put your name in the document itself, not just the file title.

BIPG 6400/8400

1. **Download and read these two papers:**
   - ✓ PMID: 24814145
   - ✓ PMID: 24556726

2. **Answer the following questions:**
   - ✓ What is the relationship between these two studies? How are they complementary?
   - ✓ What are the advantages and disadvantages of targeted, sequence-based, and functional metagenomic approaches?
   - ✓ What is the "resistome", and why are people concerned about it?
   - ✓ What are "ARGD"s, and how was their relative diversity estimated in different environments?

3. **Warnings**
   - ✓ I will compare homework from different students.
   - ✓ I will look to see if you're simply quoting from the abstract.
   - ✓ I will only grade homework that has been submitted by email as .rtf, .doc, or .docx
   - ✓ Be sure to put your name in the document itself, not just the file title.