The Human Microbiome

David N. Fredricks, MD
Vaccine and Infectious Disease Division
Fred Hutchinson Cancer Research Center
Division of Allergy and Infectious Diseases
Department of Medicine &
Department of Microbiology, Univ. of Washington
Outline

• Role of the indigenous microbiota in human health and disease
  – The human microbiota and microbiome: definitions

• Introduction to molecular methods for characterizing the microbial inhabitants of humans

• The human vaginal microbiota
  – Diversity: What is the bacterial census of the human vagina?
    • Species richness, composition, and concentration
  – Dynamism: how stable are vaginal bacterial communities and what factors influence the composition and concentrations of bacteria?
  – Dysbiosis: What changes ensue with the onset of bacterial vaginosis (BV) and what is the impact of antibiotic treatment for BV?

• Use of “omics” approaches to characterize the genetic and functional capabilities of microbial communities
  • Single cell genomics and metagenomics (genes)
  • Metatranscriptomics (mRNA and rRNA)
  • Proteomics and metabolomics (proteins and metabolites)
• 3.16 billion base pairs of DNA in genome; cost of HGP: $2.7 billion
• Anticipated number of human genes at initiation of project: >100,000
  — Fruit fly ~ 14,000 genes, Chicken ~23,000 genes, Corn ~59,000 genes
• Humans ~25,000 genes!
Humans as “Super-organisms”

The microorganisms that live on and inside humans (the microbiota) are estimated to outnumber human somatic and germ cells by a factor of ten.

Together, the genomes of these microbial symbionts provide traits that humans did not need to evolve on their own.

- Microbiome #1: collection of microbial genes associated with humans
- Microbiome #2: collection of microbes within the human biome

We are a genetic and metabolic composite of microbial and human cells, leading to the concept of the human super-organism.

- More than 3,000,000 genes provided by our gut microbiome!
The core human microbiome (red) is the set of genes present in a given habitat in all or the vast majority of humans. Habitat can be defined over a range of scales, from the entire body to a specific surface area, such as the gut or a region within the gut. The variable human microbiome (blue) is the set of genes present in a given habitat in a smaller subset of humans.
Cultivation vs. Molecular Analyses of the Human Microbiome

• Cultivation of microbes
  – Description of species (phenotypic or genotypic)
  – Sequence genomes from isolates

• Cultivation-independent analysis of microbial populations and their genes (molecular)
  – PCR of 16S rRNA genes from bacteria to detect and identify species; no information on other elements of the microbiome
  – Metagenomic analysis: extract nucleic acid directly from a sample and perform high throughput sequencing to catalog the microbes and genes represented
Metagenomic Analysis of the Human Distal Gut Microbiome


The human intestinal microbiota is composed of $10^{13}$ to $10^{14}$ microorganisms whose collective genome (“microbiome”) contains at least 100 times as many genes as our own genome. We analyzed $\sim$78 million base pairs of unique DNA sequence and 2062 polymerase chain reaction–amplified 16S ribosomal DNA sequences obtained from the fecal DNAs of two healthy adults. Using metabolic function analyses of identified genes, we compared our human genome with the average content of previously sequenced microbial genomes. Our microbiome has significantly enriched metabolism of glycans, amino acids, and xenobiotics; methanogenesis; and 2-methyl-d-erythritol 4-phosphate pathway–mediated biosynthesis of vitamins and isoprenoids. Thus, humans are superorganisms whose metabolism represents an amalgamation of microbial and human attributes.
Fig. 2. COG analysis reveals metabolic functions that are enriched or underrepresented in the human distal gut microbiome (relative to all sequenced microbes). Color code: black, subject 7; gray, subject 8. Bars above both dashed lines indicate enrichment, and bars below both lines indicate underrepresentation ($P < 0.05$). Asterisks indicate categories that are significantly different between the two subjects ($P < 0.05$). Secondary metabolites biosynthesis includes antibiotics, pigments, and nonribosomal peptides. Inorganic ion transport and metabolism includes phosphate, sulfate, and various cation transporters.
• 3.3 million non-redundant microbial genes
• >1000 gut bacterial species in cohort of 124
  – But only ~160 bacterial species/individual
• About 500,000 microbial genes/individual
  – 40% of genes present in at least half of cohort
The gut microbiome affects...

- Vitamin production (vitamin K)
- Development of innate and adaptive immunity
- Turnover of gut epithelial cells (malignancy?)
- Metabolism of xenobiotics (drugs)
- Harvest of nutrients/energy metabolism (physiology)
  - Propensity to develop obesity
- Organ size: Heart, intestine
  - Anatomy and development
- Locomotor activity (behavior)

*Nature* 449, 804-810 (18 October 2007)
The 16S rRNA gene

- Present in all bacteria (essential: codes for small subunit of ribosomal RNA complex, necessary for protein synthesis)
- Has properties of a molecular clock
  - rDNA sequence similarities between species correlate with evolutionary relatedness (time to common ancestor)
  - Little evidence of horizontal gene transfer or recombination
- Conserved regions: useful for broad range PCR
- Variable regions: useful for species identification

www.bioinformatics-toolkit.org
The bacterial 16S rRNA gene

Conserved
Present in all bacteria
Little evidence of horizontal gene transfer
Accurate phylogenies

Variable

Conserved
Why Study the Vaginal Microbiota?

• The vaginal microbiota affects the health of women and impacts the success of pregnancy
  – *E. coli* colonization of the vagina may precede UTI
  – Group B streptococcus and neonatal sepsis

• The vagina hosts unique consortia of microbes suggesting selection for these key organisms

• Bacterial vaginosis (BV) is a condition linked to numerous health problems, including:
   Preterm birth
   Pelvic inflammatory disease (infection of upper tract)
   HIV acquisition and shedding
   Increased risk of other sexually transmitted diseases (GC, CT, Trich, HSV, HPV)
   Post hysterectomy vaginal cuff cellulitis and other surgical infections


Bacterial Vaginosis

The most prevalent cause of vaginal symptoms among women of childbearing age

~ 4 million doctor visits/year in U.S.

- >10% of women experience BV
- NHANES survey in US: overall prevalence 29%
- Prevalence >50% in settings with high HIV burden (SS Africa)

- Abnormal vaginal discharge in ~50% of women
  - Increased amount -glycosidase activity of GNR on vaginal mucous
  - Odor from volatilization of amines produced by anaerobic metabolism → trimethylamine

- High rate of relapse: causes unknown
Bacterial Vaginosis (BV)

Gram stain of normal vaginal fluid with many GPR (lactobacilli), normal epithelial cells

Gram stain of BV with few GPR, greater diversity of morphotypes, and clue cells
Schematic for Pyrosequencing Approach

16S rRNA Gene

Fusion primer A: broad range Fw primer

Fusion primer B: Bar code: Broad range Rev

PCR Products w/ Fusion Primers

Broad-range PCR

Alignment/Data Analysis

Sequencing by synthesis

emPCR

Attachment to Bead

Beads to Picotiter Plate
Remove Roche Primers

Bin based on barcodes

Remove barcodes & Linkers

Determine if sequence originates from gene-specific primers

Alignment & placement on reference tree

Taxonomic assignment using tree topology

Database – curated reference sequences from Fredricks Lab

Rank Abundance Plots
Phylogenetic Trees
Diversity indices

If no match with reference sequences, BLAST tool - GenBank

Computational Biologists: Erick Matsen, Noah Hoffman, Martin Morgan
BACTERIAL DIVERSITY – PYROSEQUENCING

BV Negative Subject
4 Phylotypes

BV Positive Subject
23 Phylotypes

1000 sequences analyzed

Species richness increased in BV
Species diversity increased in BV
Molecular Identification of Bacteria Associated with Bacterial Vaginosis
Fredricks DN et al. NEJM 2005;353:1899-911

Clostridium Cluster XIVa

BVAB1

BVAB2

BVAB3

AF407407 Uncultured bacterium
AJ278163 Uncultured bacterium
AJ249110 Uncultured bacterium

Clostridium staminisolvens
Acetivibrio cellulosolvens
Clostridium species AB093546

BVAB2

Lachnobacterium bovis
Roseburia intestinalis
Eubacterium ramulus
AB034121 Uncultured rumen bacterium

BVAB1

Gardnerella vaginalis

0.1
Table 3. Multivariable Analysis of Factors Associated with Persistence of Bacterial Vaginosis (BV) in 113 Women, Adjusted for Nonadherence to Treatment

<table>
<thead>
<tr>
<th>BVAB Detected at Baseline</th>
<th>Risk Ratio (95% CI)*</th>
<th>Expected Risk for BV Persistence among Adherent Participants (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVAB3</td>
<td>2.6 (1.4–5.45)</td>
<td>0.20 (0.04–0.44)</td>
</tr>
<tr>
<td><em>Peptoniphilus lacrimalis</em></td>
<td>2.8 (1.2–13.3)</td>
<td>0.22 (0.10–0.36)</td>
</tr>
<tr>
<td>Neither BVAB nor <em>P. lacrimalis</em></td>
<td>Referent</td>
<td>0.08 (0.02–0.15)</td>
</tr>
</tbody>
</table>

BVAB = bacterial vaginosis–associated bacteria.
* Risk ratios and 95% CIs were obtained by using Poisson regression with bootstrap CIs.
• Levels of human 18S rRNA gene: indicator of amount of vaginal fluid loaded on swab
• *Lactobacillus* species profiles can be different in healthy women

**Fluctuation of bacteria in women without BV**

- Gardnerella vaginalis
- Megasphaera sp.
- BVAB1
- BVAB2
- BVAB3
- *Lactobacillus crispatus*
- *Lactobacillus iners*
- Atopobium vaginae
- Mobiluncus sp.
- Leptotrichia & Sneathia spp.
- *Lactobacillus jensenii*

**16S rRNA gene copies/swab**

- Subject A: *L. crispatus*
- Subject B: *L. crispatus, L. jensenii* & *L. iners*
Differences in levels of bacteria by qPCR during menstruation

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>mean log$_{10}$ adjusted difference (95% CI), p-value* during menstruation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus crispatus</td>
<td>-0.60 (-0.94, -0.25), p=0.001</td>
</tr>
<tr>
<td>Lactobacillus jensenii</td>
<td>-0.39 (-0.79, 0.01), p=0.06</td>
</tr>
<tr>
<td>Lactobacillus iners</td>
<td>0.10 (-0.23, 0.43), p=0.56</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>1.38 (0.83, 1.93), p&lt;0.001</td>
</tr>
</tbody>
</table>

Recurrent BV

16S rRNA gene copies/swab

Sample Collection Day

Antibiotic
Menses

Gardnerella vaginalis
Megasphaera sp.
BVAB1
BVAB2
BVAB3
Lactobacillus crispatus
Lactobacillus iners
Atopobium vaginae
Mobiluncus sp.
Leptotrichia & Sneathia spp.
Lactobacillus jensenii

16S rRNA gene copies/swab

Sample Collection Day

Episode 1
Episode 2
Episode 3

pH

1.00E+10
1.00E+9
1.00E+8
1.00E+7
1.00E+6
1.00E+5
1.00E+4
1.00E+3
1.00E+2
1.00E+1
1.00E+0
0

1.00E+10
1.00E+9
1.00E+8
1.00E+7
1.00E+6
1.00E+5
1.00E+4
1.00E+3
1.00E+2
1.00E+1
1.00E+0
0
Summary: Vaginal Microbiota

• The human vagina harbors communities of bacteria that are very different from other human body sites.
• Bacterial diversity in subjects without BV is limited, whereas subjects with BV have a high degree of species richness that includes many novel and fastidious bacteria.
• Treatment of BV with antibiotics results in a rapid decline of anaerobic bacteria, though relapse is common.
• The nature of the interactions among BV-associated bacteria is poorly understood.
  – Functional redundancy to explain heterogeneity?
  – Are there syntrophic metabolic interactions?
The Human Microbiome and Omics

- Single cell genomics: NIH sequencing initiative
  - What are the functional capabilities of individual microbes?
- Metagenomics: assessing community gene content by high throughput sequencing
  - What are the functional capabilities of microbial communities as assessed by gene representation?
- Metatranscriptomics: mRNA and rRNA
  - Which genes are expressed in certain communities under defined conditions?
- Proteomics: Which proteins are present and how do they change with host factors or community composition?
- Metabolomics: Which small molecule metabolites are present in a given habitat and how do fluxes illuminate the biochemistry of the community?
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