More than a gut feeling: How the microbiota improves health

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You and your gut microbes

100 trillion microbial cells in your body

~200 times as many microbial genes in your body than human genes

Two-thirds of the species of gut bacteria in your body are unique to you

1,000 species of gut bacteria identified to date

6 pounds is the combined weight of all the microbes in your body (twice that of your brain)
Why are gut microbes important?

Holobiont

You + Microbes = Holobiont

Digestion
Vitamin Production
Immune System Development
Gut Barrier Integrity
Colonization Resistance
What do we call our gut microbes?

What’s the difference between the microbiota, the metagenome and the microbiome?

**Microbiota**
- The community of **microbes** in an environment

**Metagenome**
- **Genes and genomes of the microbiota** (the genetic potential of the population)

**Microbiome**
- Total collection of **genes**, **genomes** and **products** of the **microbiota**
How do we know who’s there?
How do we know who’s there?
Can we culture them?

Known Bacterial Phylogenetic Divisions

**1987**
(12 divisions; 12 cultured / 0 uncultured)

**1997**
(36 divisions; 24 cultured / 12 uncultured)

**2003**
(53 divisions; 26 cultured / 27 uncultured)

**2006**
(∼100 divisions; 30 cultured / ∼70 uncultured)
So what do we do?

Metagenomics: the study of the genes and genomes present in the microbiota
What do we do with the DNA data?

ATGGCACTTA...

TCCGAAAGCC.

GGGTCTAGCG...

Microbiome A

Microbiome B
How do we visualize microbiome differences?

- **Microbiome A**
- **Microbiome B**
- **Microbiome C**

**Beta diversity**
When does our gut microbiota develop?
When does our gut microbiota develop?

Mother’s body habitat:
- Oral mucosa
- Vagina
- Skin

Baby’s delivery mode:
- Vaginal
- Cesarean

$n = 2$
Infant (Fecal)

Oral

Vaginal

Skin

Fecal

Fecal

Courtesy Rob Knight
Does our gut microbiota change throughout our lives?

Does our gut microbiota change throughout our lives?

David et. al. 2014. Genome Biology. 15:R89.
Are changes in the gut microbiota linked to disease?

A need exists to causally link the identified differences in the microbiota to a physiological function or disease state.
How can we establish causation?

Gnotobiotic: from Greek gnōtos “known” + biotē “life”

The status of the microbial community is known

Germ-free: No microbes
Mono-associated: Germ-free mouse colonized with one bacterial species
Conventional: Complex microbiota from birth—NOT gnotobiotic

University of Nebraska Gnotobiotic Mouse Facility
Obesity: A connection to the gut microbiota

Prevalence of Self-reported Obesity in the United States (2016)
Obesity: A connection to the gut microbiota

Observations in people…

Observations in conventional mice…

Ley et al. PNAS. 2005.
Obesity: A connection to the gut microbiota

…and studies in germ-free mice.

Gut microbes and obesity: What else can we learn from *germ-free* mice?
Gut microbes from twins discordant for obesity modulate mouse metabolism
How well do “lean” and “obese” microbiomes compete with each other?
How well do “lean” and “obese” microbiomes compete with each other?

Ridaura et al. Science 2013;341:1241214
"Lean" microbiomes outcompete "obese" microbiomes

Ridaura et al. Science 2013;341:1241214
How does the microbiota contribute to obesity and metabolic disease?

Adapted from Gravitz, Nature 2012
Immunity, inflammation and the gut microbiota

Figure 12.5 Janeway's Immunobiology, 8th ed. (© Garland Science 2012)
Gut microbes and the immune system: a delicate balance

Microbes

Single-cell layer of epithelial cells

Immune tissue

Can individual microbiota members modulate immunity?

Health (probiotics)

Lactobacillus species
Bifidobacterium species
Faecalibacterium prauznitsii

Disease (colitogenic)

Klebsiella pneumoniae
Proteus mirabilis
Adherent and invasive E. coli
Helicobacter spp.
Some gut microbes promote the development of Regulatory T Cells

- Helps *initiate* immune responses and *promotes* inflammation
- Stops immune responses and *limits* inflammation
**Clostridium species: promoting protection**

- Colonized germ-free mice with 46 *Clostridium* species
- Expanded the numbers of Foxp3$^+$ Regulatory T Cells
- Induced TGF-β1 secretion from intestinal epithelial cells

Clostridium species: promoting protection

- Subsets of Clostridium species thought to protect against inflammation are reduced in patients with Inflammatory Bowel Diseases
- Can providing a cocktail of Clostridium species to mice provide protection against colitis?

How do gut microbes provide protection against inflammation?

By producing metabolites and by-products of fermentation called short-chain fatty acids (butyrate)

Baas, T. SciBX 6(46); 10.1038/scibx.2013.1310
Should we change the composition of the gut microbiota? How?

Antibiotics
Bacteriophages (viruses)
Fecal transplantation

Diet

Probiotics
Prebiotics
Fiber and whole grains
Other food components
Probiotics

dietary supplements containing potentially beneficial bacteria or yeasts

According to the currently adopted definition by FAO/WHO, probiotics are:

“Live microorganisms which when administered in adequate amounts confer a health benefit on the host.”

Role of probiotics in gut health

1. SCFA (Short Chain Fatty Acids) influence colonocytes and induce trophic and anti-neoplastic effects.
2. Increased mineral absorption leads to better serum lipids and cholesterol control.
3. Antagonism of pathogens and putrefactive bacteria reduces toxic bacterial metabolites.
4. Allergy prevention:
   - Th2 cells suppress IBD inflammation, IL-10, TGF-β.
5. Reduced cancer risk:
   - M cell immunomodulation suppresses IBD inflammation, anti-inflammatory.
6. DC (Dendritic Cells) contribute to allergy prevention through IL-10, TGF-β.

Adapted from Crittenden, 2006.
Challenges along the way

**salivary enzymes:** amylase, lipase, kallikrein, lysozyme
**mineral salts:** calcium and phosphate ions
**pH 6.5 - 7.5**

**gastric enzymes:** pepsinogen, lipase, mucin
**pH 1.5 - 3**

**microbial products:** SCFA, anaerobic environment, competitive exclusion, mucus
**pH 5 - 7**

**pancreatic enzymes:** trypsinogen, lipase, carboxypeptidase, amylase, mucus, bile salts
**pH 4 - 7**

Kok and Hutkins, 2018
Challenges: colonization resistance

- Food associated microbes
  - Attachment site restriction
- Commensal microbiota
- Mucus layer
- Intestinal epithelium
  - Nutrient limitation
  - pH changes (SCFAs)
  - Bacteriocins
  - Antimicrobial peptides

Adapted from Sassone-Corsi and Raffatellu, 2015.
How do we overcome these challenges?

Consume probiotics regularly at high doses

Regular yogurt consumption maintains bifidobacteria in the gut

David et. al. 2014. Genome Biology. 15:R89.
Probiotic consumption does not routinely alter microbiota composition...
…but probiotic consumption does alter microbiota function

Urinary metabolites change in mice after consuming a fermented milk product:

- *Bifidobacterium animalis* subsp. *lactis*
- Two strains of *Lactobacillus delbrueckii* subsp. *bulgaricus*
- *Lactococcus lactis* subsp. *cremoris*
- *Streptococcus thermophilus*
...or probiotic consumption *may* promote gut microbiota homeostasis *(and maybe minimize the effects of microbiota disturbances)*
Probiotics Everywhere!
Dairy Foods as Sources of Probiotics
Plant-based yogurt products + probiotics
Nutrition Bars
Beverages
Fruit-and Veggie-based Beverages
Breakfast Cereals
The Kambucha Kraze
Gaga for Gummies
Probiotics for Kids
Babies and Infants
Probiotics Are Just About Everywhere
Probiotic Supplements Galore
Up close and personal: The science of naming microbes and why it matters
What’s in a name?

Why does it matter?

HELLO
MY NAME IS

*Bifidobacterium lactis*
Different species and even strains can deliver different benefits.

Genus: *Lactobacillus*
Species: *acidophilus* LA-5

My name is HELLO.
Probiotic strains that have been evaluated in clinical trials

- *Lactobacillus fermentum* RC-14 (Urex Biotech)
- *Lactobacillus acidophilus* SBT (Snow Brand)
- *Lactobacillus acidophilus* ADH (Dupont)
- *Lactobacillus rhamnosus* GG (Valio)
- *Lactobacillus rhamnosus* Lcr35 (Lycocentre)
- *Lactobacillus reuteri* DS2112 (Biogaia)
- *Lactobacillus casei* strain Shirota (Yakult)
- *Lactobacillus casei* F19 (Alra Foods)
- *Lactobacillus johnsoni* LA1 (Nestle)
- *Lactobacillus casei* DN-114001 (Dannon)
- *Lactobacillus plantarum* 299v (Probi Foods)
- *Lactobacillus acidophilus* NCFM (Danisco)
- *Lactobacillus crispatus* CTV05 (Osel, Inc.)
Lactobacillus

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Bifidobacterium

Commercial strains that have been evaluated in clinical trials

- *Bifidobacterium longum* BB536 (Moringa)
- *Bifidobacterium bifidum* (Yakult)
- *Bifidobacterium infantis* (Shirota)
- *Bifidobacterium breve* (Yakult)
- *Bifidobacterium lactis* DS 920 (DSM Foods)
- *Bifidobacterium animalis* DN 173010 (Dannon)
- *Bifidobacterium lactis* Bb12 (Chr Hansen)
- *Bifidobacterium essensis* (Danone)
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- *Bifidobacterium longum* BB536 (Moringa)
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- *Bifidobacterium lactis* Bb12 (Chr Hansen)
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Probiotics Q and A

Q. How to know what’s in the yogurt, kefir or capsule?
   A. Read the label and look for species and strains

Q. What is an appropriate dose? And in what units?
   A. Usually, 100 million cells per g is the minimum.
      Many products will contain 1 - 5 billion cells per g

Q. Food versus supplement?
   A. Foods components may protect cells from digestion but
      most supplements are now well protected
### Which probiotic should I choose?

Clinical guides summarize the scientific literature [here](https://isappscience.org).

![AEProbio Clinical Guide to Probiotic Products Available in USA](https://isappscience.org)

**Clinical Guide to Probiotic Products Available in USA**

**Indications, Dosage Forms and Clinical Evidence to Date - 2018 Edition**

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Probiotic Strain</th>
<th>Dosage Form</th>
<th>CFU/Dose</th>
<th>No of Doses/Day</th>
<th>Indications (Level of Evidence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activia®</td>
<td>B. (animalis) lactis CNCM I-2494</td>
<td>Ferm. milk lq.</td>
<td>1B/serving</td>
<td>1-3 servings</td>
<td>C - Constipation (I) IBS - Irritable bowel syndrome (I)</td>
</tr>
<tr>
<td>Align®</td>
<td>B. longum 35624</td>
<td>Capsules</td>
<td>1B/capsule</td>
<td>1 capsule</td>
<td>IBS - Irritable bowel syndrome (I)</td>
</tr>
<tr>
<td>Align® Chewables</td>
<td>B. longum 35624</td>
<td>Tablet</td>
<td>1B/Tablet</td>
<td>1 tablet</td>
<td>IBS - Irritable bowel syndrome (I)</td>
</tr>
<tr>
<td>Align® Extra Strength</td>
<td>B. longum 35624</td>
<td>Capsules</td>
<td>5B/capsule</td>
<td>1 capsule</td>
<td>IBS - Irritable bowel syndrome (I)</td>
</tr>
<tr>
<td>Alter-Aid™ L-928®</td>
<td>L. acidophilus L-928® 30B heat-killed cells = 21mg</td>
<td>Capsule</td>
<td>11mg per cap</td>
<td>2 capsule</td>
<td>E/AD - Eczema/Atopic Dermatitis (as adjunct to standard therapy) (I)</td>
</tr>
<tr>
<td>Bio-K® CL1285</td>
<td>L. acidophilus CL 1285 L. casei LBC80R L. rhamnosus CL2</td>
<td>Ferm. rice lq.</td>
<td>Travel Protection</td>
<td></td>
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</tr>
<tr>
<td>BIOMED® ProTect® Drops with Vitamin D</td>
<td>L. reuteri DSM 17938</td>
<td>Drops</td>
<td>100M/5drops</td>
<td>5 drops</td>
<td>AAD - Antibiotic associated diarrhea - Prevention (II)</td>
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<td>BIOMED® ProTect®</td>
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<td>100M/5drops</td>
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<td></td>
<td></td>
<td>Chew. tabs</td>
<td>100M/5drops</td>
<td>1 tab</td>
<td>C - Constipation (I) HP - Helicobacter pylori - Adjunct to standard eradication therapy (I)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ID - Infectious diarrhea (II)</td>
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<tr>
<td>Jamo-Dophilus® Women</td>
<td>L. crispatus LbV 88</td>
<td>Oral Capsule</td>
<td>5B CFU/cap</td>
<td>2 capsules</td>
<td>BV - Bacterial vaginosis (II)</td>
</tr>
<tr>
<td></td>
<td>L. jensenii LbV 116</td>
<td></td>
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<tr>
<td></td>
<td>L. gasseri LbV 150N</td>
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<tr>
<td></td>
<td>L. rhamnosus LbV 96</td>
<td></td>
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</tr>
<tr>
<td>Jarrow Formulas® Fem-Dophilus</td>
<td>L. rhamnosus GR-1</td>
<td>Oral capsule</td>
<td>2.5B each/capsule</td>
<td>1 capsule</td>
<td>BV - Bacterial vaginosis (I)</td>
</tr>
<tr>
<td></td>
<td>L. reuteri RC-14</td>
<td></td>
<td></td>
<td></td>
<td>VC - Vulvovaginal candidiasis (I)</td>
</tr>
<tr>
<td>ProB™ (ReFlresh ProB)</td>
<td>L. rhamnosus GR-1</td>
<td>Oral capsule</td>
<td>2.5B each/capsule</td>
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<td>UltraFlora® Women's</td>
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Indication    Level of Evidence References

The *prebiotic* concept

Manipulating the gut microbiota through better eating

- Prebiotics are usually non-digestible oligosaccharides that survive gastric digestion and reach the colon intact.

- In the colon, these oligosaccharides are fermented, but only by *select members* of the GI tract.

- The population of *oligosaccharide-fermenting strains* is *enriched*, at the expense of OS non-fermenting organisms.

- Among the bacteria capable of metabolizing prebiotics are bifidobacteria and lactobacilli.
The prebiotic concept

Diverse microbiota + prebiotic → microbiota enriched with bifidobacteria and lactobacilli
Role of prebiotics in gut health

Adapted from Crittenden, 2006.
Prebiotic oligosaccharides are normal dietary components

- Inulin, fructooligosaccharides, galactooligosaccharides + others
- Extracted or synthesized and added as ingredients
- Naturally present in wide variety of fiber-rich foods
Synbiotics

- **Complementary**: components are chosen independently of one another, with each responsible for a particular effect or health benefit

- **Synergistic**: combination is specifically designed with a prebiotic substrate synergistically supporting the competitiveness, survival or metabolic activity of a cognate probiotic strain

How synbiotics are formulated can have considerable influence on their potential effectiveness!
We think diet is the best tool available for manipulating the gut microbiome AND influencing health!
Mission: Identify food molecules with clinically proven effects

Audacious Goal: Crops and Foods with Real Health Claims

https://foodforhealth.unl.edu
Thank you!