Who are they?
Gut microbiota, a crowded kingdom
Population, diversity, and distribution

Our gastrointestinal tract represents one of the largest interfaces (250–400 m²)

A total number of 30-400 trillion microbe species resides in our GI tract
More abundant than our cells of the body!

Gut microbiota, a crowded kingdom

Population, diversity, and distribution

**GI tract**

- Stomach
- Small intestine
- Large intestine
- Rectum

**Bacteria**

- Archaea

**Eukarya, mostly yeast**

- Viruses, mostly phage
- Parasites
- ...

Gut microbiota, a crowded kingdom

Population, diversity, and distribution

Over 1000 microbe species resides in our GI tract
Great genomic and functional diversity

Gut microbiota, a crowded kingdom

Population, diversity, and distribution

Diverse environment within our GI tracts leads to distribution of microbiota
Gut microbiota, a crowded kingdom

Population, diversity, and distribution

Diverse environment within our GI tracts leads to distribution of microbiota
Gut microbiota, a crowded kingdom

Population, diversity, and distribution

Diverse environment within our GI tracts leads to distribution of microbiota
Tools to interrogate gut microbiota

Culture-dependent

pre-incubation

blood culture
rumen fluid

Culture

Different types of agar media

builds up fundamental knowledge of gut microbiota

Uncultivable

Enhancing bias

1843-1910

Robert Koch

1890s

Tools to interrogate gut microbiota

Culture-independent

Robert Koch
1843-1910

1990s-2010

Metagenomic analysis
Advancing of PCR, 16s RNA, NGS…

Reconstitute diversity

Tools to interrogate gut microbiota

Evolution of our opinions

Gut microbiome

1,000,000+ genes

Human genome

23,000 genes

NIH Human Microbiome Project

variability among groups
age, geographic...

HMP1
2008 - 2013

iHMP
2014 - Now

Tools to interrogate gut microbiota

Multiomics - functional focus

Metagenomic analysis
Advancing of PCR, 16s RNA, NGS…

Reconstitute diversity

3.3 million non-redundant genes from fecal microbes

Tools to interrogate gut microbiota

Multiomics - functional focus

Germ-free mice
Humanized germ-free mice

Model systems in validation

16S / 18S / ITS Amplicon Sequencing

Metagenomic analysis
Advancing of PCR, 16s RNA, NGS...

Reconstitute diversity
3.3 million non-redundant genes from fecal microbes

How do we encounter?

an evolution lasting one’s lifespan
Pioneer species first establish the community, followed by rapid changes in one’s childhood until a stable, climax community is finally reached.
Gut microbiota evolution

Primary succession

Delivery methods give fetus exposure to different sets of microbiota

Gut microbiota evolution

Primary succession

When does the first exposure take place?
Gut microbiota evolution
First colonization debate

Fetal environment is sterile
- No sign of live bacteria being cultivated
- Physiological and immunological barriers prevents microbial invasion
- Live bacteria / bacteria DNA contamination in commercial reagents
- Low biomass
  - Statistical insignificance
  - Batch effects…

Fetal environment is not sterile
- Molecular techniques suggest bacterial communities in the placenta, amniotic fluid, and meconium from healthy pregnancies
- Ensuring fresh sample and germ-free lab environment
- Decontaminating commercial reagents
- Multiple detections beyond 16s rRNA sequencing efforts
  - Immune-modulatory metabolites and fetal immunity response
Primary succession

Gut microbiota evolution

Mounting evidence showing womb as non-sterile place
The debate is still on…

Gut microbiota evolution

Primary succession

Dominated by keystone species through adulthood

Rebound or rebuild of the community after the relatively stable microbiota being perturbed and pushed away from climax

Gut microbiota stability and volatility

Gut microbiota composition is relatively stable across time for the same individual

Jaccard index(sample A, sample B) = \frac{\text{sample A } \cap \text{ sample B}}{\text{sample A } \cup \text{ sample B}}

Gut microbiota stability and volatility

Gut microbiota stability and volatility

Volatility

**Short-term perturbation**
Nearly two-fold increase in the Bacteroidetes to Firmicutes ratio, which reversed upon return

**Long-term perturbation**
Permanent decline of most gut bacterial taxa, which were replaced by genetically similar species

Gut microbiota stability and volatility

Daily diet and microbiota change

Composition responds quickly to diet change

humanized germ-free mice

Rich in fat and sugar but low in plant polysaccharides

Rich in plant polysaccharides but low in fat or sugar

Gut microbiota stability and volatility

Daily diet and microbiota change

We are feeding our gut microbiota with what we eat
Nutrients can directly interact with microorganisms to promote or inhibit their growth

Rich in fat and sugar but low in plant polysaccharides
Rich in plant polysaccharides but low in fat or sugar

Gut microbiota stability and volatility

Daily diet and microbiota change

Gut microbiota stability and volatility
Daily diet and microbiota change

Indigestible carbohydrates
resistant starch, cellulose…

Primary degradation with carbohydrate-active enzymes
Secondary fermentation

Indigestible carbohydrates

SCFA
acetate, butyrate, lactate…
cross-feeding metabolic network

Prudent Diet

Phytochemicals
Omega-3 fatty acids

High dietary fiber, MACs, Prebiotics

SCFAs
Enhanced mucus secretion
Increased antimicrobial peptides

Healthy gut microbiota
intact barrier function

Microbiota dysbiosis
barrier dysfunction

Western Diet

High sugar
Emulsifiers

High fat
Hyperglycemia

High meat protein
Low dietary fiber

Toxic metabolites, BCFAs
Toxic bile acids

Enhanced mucus degradation
Reduced antimicrobial peptides

Gut microbiota stability and volatility

Drugs and antibiotics

Killing pathogens

Killing probiotics

Double sword

Triggering antibiotics resistance
Gut microbiota stability and volatility

Sweetener vs added sugar

Aspartame  Saccharin  Sucralose  Stevia
Gut microbiota stability and volatility

Sweetener vs added sugar

Non-nutritive = biologically inert

Aspartame
Saccharin
Sucralose
Stevia
Gut microbiota stability and volatility

Sweetener vs added sugar

Supplementing specific sweeteners (8%-75% of daily intake guideline)

Gut microbiota change

Glucose intolerance

Gut microbiota stability and volatility

Sweetener vs added sugar

Supplementing specific sweeteners (8%-75% of daily intake guideline)

Germ-free mice conventionalized with participants gut microbial

Glucose intolerance linked with sweetener-altered gut microbiota

How do they affect us?
How do they affect us?

Locally…
How do they affect us?
Locally… and globally
Gut microbiota and our health
Gut microbiota and our health

A biological rheostat

A healthy diet

after fermentation…

Secondary bile acid
Lipid metabolism, bile acid recycling and homeostasis

Short-chain fatty acids
Powering both microbiota diversity and mucosa integrity
Immune and inflammatory responses

Branched-chain amino acids
Essential amino acids

Tryptophan and indole-derivative
Intestinal 5-HT production, AHR ligands

Trimethylamine N-oxide
Imidazole propionate

...
Gut microbiota and our health

Cross talk with host cells

Healthy intestinal functioning
Diverse microbiota habitat

Metabolic syndrome
Depression and neurological disease
Inflammation and allergy
Gut microbiota and our health

*Inflammatory bowel disease (IBD)*

Chronic inflammation of the GI tract associate with mucus damage and ulcers

**0.3%–0.5% of the global population**

Gut microbiota and our health

Inflammatory bowel disease (IBD)

*Imbalanced population of bacteria*

Beneficial bacteria
- Bifidobacterium longum, Roseburia intestinalis...

Harmful bacteria
- Bacteroides fragilis, Ruminococcus torques...

*Consumed mucus glycan coating*

*Intensified immune response*

Inflammation

Th1
- IFNγ, TNFα

Th17
- IL-17, IL-22

Gut microbiota and our health

**Obesity**

*Obese* gut microbiota utilized more energy from food

Gut microbiota and our health

Obesity


fed with LF-HPP chow diet

germs-free mice

“Obese” phenotype ameliorated
Gut microbiota and our health

Obesity

Reduced obesity phenotype due to “Lean” microbiota invasion

SCFAs (increased in Ln), branched-chain amino acids (increased in Ob), bile acid species (increased in Ln)

Gut microbiota and our health

Depression and anxiety

Gut microbiota and our health

Depression and anxiety

Gut microbiota and our health
Allergic responses

Genetic, environmental, social… Microbial!
Gut microbiota and our health

Allergic responses

Healthy infants’ gut microbiota associates with allergy prevention

Gut microbiota and our health

Allergic responses

Clostridia-containing microbiota associates with allergy prevention

Gut microbiota and our health

Hygiene hypothesis

Delayed exposure and insufficient colonization

Normal exposure and sufficient colonization

Gut microbiota and our health

Hygiene hypothesis

Associated with allergic response

Gut microbiota and our health

Maturation during infancy
Gut microbiota and our health

Maturation during infancy

Tolerance-promoting factors enabling a window of opportunity for intervention
Manage, manipulate and design
better gut flora for better living
Targeting gut microbiota as bio-therapeutics

Early intervention

Combination of live bacteria that are isolated from healthy human microbiome and manufactured as pharmaceutical biologics

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>DISCOVERY</th>
<th>PRE-CLINICAL</th>
<th>PHASE 1B</th>
<th>PHASE 2</th>
<th>PHASE 3</th>
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<tbody>
<tr>
<td>STMC-103H</td>
<td>Atopic Diseases Prevention</td>
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<td>STMC-103H</td>
<td>Atopic Dermatitis Treatment</td>
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<td>STMC-105</td>
<td>Bacterial Vaginosis</td>
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<td>STMC-106</td>
<td>Necrotizing Enterocolitis</td>
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</tbody>
</table>

allergic disease, bacterial vaginosis and necrotizing enterocolitis

Any methods/treatments for adults?
Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

A well-established treatment for *Clostridioides difficile* infection
Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

A well-established treatment for *Clostridioides difficile* infection

Traditional antibiotic treatment fails to eradicate *C. difficile*

The infusion of donor feces was significantly more effective

How to carry out a fecal transplant?
Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

Step 1
Bowel preparation

3-7 days of antibiotic before FMT

Oral polyethylene glycol with electrolyte purgative

Step 2
FMT delivery methods

Stool suspension via endoscope, naso-enteric tube, or capsules by ingestion

Donor stool

Stool suspension duodenal tube, rectal tube, colonoscopy

Capsules

Suspensions

Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

The routes of delivery matters as contradictory outcomes been seen in the FMT attempts to cure ulcerative colitis (IBD)

<table>
<thead>
<tr>
<th>Author</th>
<th># patients</th>
<th>Mode and frequency of delivery</th>
<th>Outcome</th>
<th>Lesson Learned</th>
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<tbody>
<tr>
<td>Rossen Gastro 2015</td>
<td>37</td>
<td>Nasoduodenal x 2</td>
<td>30% v. 20% clinical remission (SCAI≤2 + ≥1 decrease in Mayo endo)</td>
<td>Negative study, perhaps lower delivery preferable</td>
</tr>
<tr>
<td>Moayyedi Gastro 2015</td>
<td>70</td>
<td>Enema q week x 6 weeks</td>
<td>24% v. 5% remission (Mayo score ≤2, endo=0)</td>
<td>Superdonor effect</td>
</tr>
<tr>
<td>Paramsothy Lancet 2017</td>
<td>85</td>
<td>Enema q day (M-F) x 8 weeks</td>
<td>27% v. 8% steroid-free clinical remission</td>
<td>Batched donors</td>
</tr>
<tr>
<td>Costello JAMA 2019</td>
<td>73</td>
<td>Colonoscopy + enema x 2 weeks</td>
<td>32% v. 9% achieved steroid free remission</td>
<td>Numerous donors</td>
</tr>
<tr>
<td>Haifer Lancet 2021</td>
<td>35</td>
<td>Abx + Capsule FMT</td>
<td>53% v. 15% steroid-free clinical remission</td>
<td>Antibiotic pretreatment</td>
</tr>
</tbody>
</table>

A robust delivery frequency

Anaerobic preparation

Antibiotics pretreatment

Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

**Stool banks**

*high-quality, ready-to-use* donor feces suspensions from a *prescreened, well-defined* donor pool

**MTP-101LR**
- **Manufacturer:** University of Minnesota
- **Modality:** Lower Delivery (colonoscopy, sigmoidoscopy, or enema) or Upper Delivery (Nasoenteric/gastric tube or EGD)
- **Pricing:** $1,695 per dose (1 cryobag)

Information on storage, thawing, and administering MTP-101LR is located in our Clinician’s Checklist.

**FMP CapDE**
- **Manufacturer:** OpenBiome
- **Modality:** Oral administration
- **Pricing:** $2,050 per dose (1 bottle=30 capsules)
  - Includes 2 inert test capsules to assess patient’s swallowing abilities

Physician orientation is required before first order. Information on storage, thawing, and administering FMP CapDE is located in our Clinician’s Checklist.

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Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

You are an ideal recipient!

You are an ideal donor!

serious adverse events
abdominal pain, followed by diarrhea

A fecal transplant led to a patient's death.

Oct. 30, 2019, 5:02 PM EDT

Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

A fecal transplant led to a patient's death.

MDR E. coli

Intravenous antibiotics

with severe liver disease

MDR E. coli cured

73-year-old man

leukemia patients, received chemotherapy and stem cell transplants

Unable to save his life

**Gut microbiota, new target for bio-therapeutics**

**Precision engineering**

*First example of precision editing of microbiome inside GI tract*

*(Though mice colonized with a single E. coli strain)*

Gut microbiota, new target for bio-therapeutics

Precision engineering

Native E. coli from the murine and human gut can be engineered for transgene delivery

Single oral treatment in non-sterile conditions

Persisting improvement of bile acid metabolism

Gut microbiota, new target for bio-therapeutics

Modeling and designing

Can we design and synthesize a defined community?
that mimics phenotypes of a healthy native microbiota
Gut microbiota, new target for bio-therapeutics

Modeling and designing

From late 2000s, separated low-complexity (<20 strains) gut microbiota have been used to study specific impacts on host

Gut microbiota, new target for bio-therapeutics

Modeling and designing

A defined community (stains=104) phenotypically similar to human fecal community

Build your own gut microbiota!
Gut microbiota, new target for bio-therapeutics

Daily care with healthy lifestyle

Be a good gardener if you want it flourish
Gut microbiota, new target for bio-therapeutics

Daily care with healthy lifestyle

Probiotics in fermented food

Probiotics supplements

One take-home massage…
Thank you!