A metabolomic analysis of the mammalian gut microbiota

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Humans are constantly engaging in complex interactions with microbes
The mammalian gut microbiota

- Consortium of commensal microorganisms
  - 100 trillion cells
  - 10x the number of human cells, 100x the number of human genes
  - >200 genera, >1000 species, >7000 strains
  - Collectively >35,000 species

- Development of the gut-associated immune system

- Energy balance
  - Degradation of complex carbohydrates
  - Activation of nutrient assimilation
  - Synthesis of vitamins

- Protection against pathogens – “Colonization resistance”
  - Competition for nutrients and colonization sites
  - Production of antibacterial molecules
'Omics' technologies provide a powerful way of probing host-microbe interactions

Small molecules play important roles in the lifestyle of all organisms

- Endocrine signaling in mammals
  - Homeostasis
  - Response to insult

- Microbial communication
  - Quorum sensing
  - Competition
  - Cooperation

- Metabolic interrelationships
  - Microbial consortia
  - Secondary metabolites

What are the roles played by small molecules in commensal host-microbe interactions?

What is the impact of the microbiota on the chemical composition of the gastrointestinal tract?

- Collect feces
- Acetonitrile extract
- Fourier Transform Ion Cyclotron Resonance Mass Spectrometry
- Metabolic profiling

DATABASE SEARCHES
The levels of several hundred fecal metabolites are affected by antibiotic treatment

<table>
<thead>
<tr>
<th>Metabolites detected</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative ionization</td>
<td>1043</td>
</tr>
<tr>
<td>Positive ionization</td>
<td>1386</td>
</tr>
<tr>
<td>Overlap</td>
<td>199</td>
</tr>
<tr>
<td>Total</td>
<td>2230</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metabolites changed</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated &gt; Treated</td>
<td>793</td>
</tr>
<tr>
<td>Treated &gt; Untreated</td>
<td>1165</td>
</tr>
<tr>
<td>Total changed</td>
<td>1958</td>
</tr>
<tr>
<td>% total</td>
<td>87.8</td>
</tr>
</tbody>
</table>

Multiple host metabolic pathways are affected by antibiotic treatment

**Decreased after antibiotic treatment**
- Alpha-linoleic acid metabolism
- Androgen and estrogen metabolism
- Arachidonic acid metabolism
- Biosynthesis of unsaturated fatty acids
- C21-steroid hormone metabolism
- Fatty acid metabolism
- Linoleic acid metabolism
- Porphyrin and chlorophyll metabolism
- Primary bile acid biosynthesis
- Sphingolipid metabolism
- Steroid biosynthesis
- Ubiquinone and other terpenoid-quinone biosynthesis

**Increased after antibiotic treatment**
- Alpha-linoleic acid metabolism
- Amino sugar and nucleotide sugar metabolism
- Androgen and estrogen metabolism
- Arachidonic acid metabolism
- Ascorbate and aldarate metabolism
- Biosynthesis of unsaturated fatty acids
- C21-steroid hormone metabolism
- Cyanaminic acid metabolism
- Cystathionine and methionine metabolism
- Fatty acid metabolism
- Fructose and mannose metabolism
- Galactose metabolism
- Glycerolipid metabolism
- Glycolysis and gluconeogenesis
- Inositol phosphate metabolism
- Inositol phosphate metabolism
- Linoleic acid metabolism
- Lysine biosynthesis
- Metabolism of xenobiotics by cytochrome P450
- Pentose phosphate pathway
- Porphyrin and chlorophyll metabolism
- Primary bile acid biosynthesis
- Purine metabolism
- Pyrimidine metabolism
- Retinol metabolism
- Riboflavin metabolism
- Sphingolipid metabolism
- Starch and sucrose metabolism
- Sulfur metabolism
- Taurine and hypotaurine metabolism
- Tryptophan metabolism
- Tyrosine metabolism
- Ubiquinone and other terpenoid-quinone biosynthesis

**Steroids**
- Androgen and estrogen metabolism
- C21-steroid hormone metabolism
- Steroid biosynthesis

**Eicosanoids**
- Alpha-linoleic acid metabolism
- Linoleic acid metabolism
- Arachidonic acid metabolism

Steroid hormone metabolism is affected by antibiotic treatment

Steroid hormone metabolism is affected by antibiotic treatment

Eicosanoid hormone metabolism is affected by antibiotic treatment
Eicosanoid hormone metabolism is affected by antibiotic treatment

Leukotriene B4 levels are highly impacted by antibiotic treatment

Leukotriene B4 levels correlate with the number of bacteria colonizing the gastrointestinal tract.
Leukotriene B4 levels are highly impacted by antibiotic treatment
Clinically-relevant doses of antibiotics can impact fecal levels of leukotriene B4

Streptomycin: 450 mg/L
Tetracycline: 50 mg/L
Metronidazole: 750 mg/L
How does the gut microbiota affect leukotriene B4 production?

- Transcriptional regulation
- Phospholipase activity
- Lipoxygenase activity
- Transport
Transcriptional regulation alone does not explain the effect of antibiotic treatment on leukotriene B4 metabolism
Eicosanoids are involved in host responses to infection

Phagocytosis and bactericidal action of mouse peritoneal macrophages treated with leukotriene B4.

Signal transduction and invasion of epithelial cells by S. typhimurium.

Salmonella infection induces a hypersecretory phenotype in human intestinal xenografts by inducing cyclooxygenase 2.

Salmonella enterica serovar Typhimurium infection induces cyclooxygenase 2 expression in macrophages: involvement of Salmonella pathogenicity island 2.
Antibiotic treatment increases mouse susceptibility to *Salmonella* infection

*The gut microbiota confers resistance to colonization by pathogens*

**20 mg streptomycin**

24 hours

*Salmonella* Typhimurium

1-5 days

*Salmonella* pathogenesis
- Bacterial loads
- Inflammation
- Transmission
Leukotriene B4 can partially rescue resistance to Salmonella infection in antibiotic-treated mice

20 mg streptomycin + 24 hours $\rightarrow$ Salmonella Typhimurium $\rightarrow$ 1-5 days $\pm$ LTB4 orally $\rightarrow$ Intestinal colonization by Salmonella

$p=0.0002$
What are the roles of the other 1000’s of small molecules present in the mammalian gut?

- Ethyl acetate extraction ≈16 hours
- Centrifuge
- Supernatant -20 °C

Growth
Gene expression
The gut metabolome is a potential source of small molecules with antibiotic activity.
The gut metabolome contains small molecules that control Salmonella gene expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Common Name of Primary Target</th>
<th>Fold-change</th>
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<tbody>
<tr>
<td>fliC</td>
<td>flagellin</td>
<td>7.1</td>
</tr>
<tr>
<td>flgL</td>
<td>flagellar hook-associated protein FlgL</td>
<td>3.8</td>
</tr>
<tr>
<td>spoT</td>
<td>bifunctional (p)ppGpp synthetase/hydrolase</td>
<td>3.8</td>
</tr>
<tr>
<td>flgD</td>
<td>flagellar basal body rod modification protein</td>
<td>3.6</td>
</tr>
<tr>
<td>flgN</td>
<td>putative FlgK/FlgL export chaperone</td>
<td>3.4</td>
</tr>
<tr>
<td>flgM</td>
<td>anti-sigma28 factor FlgM</td>
<td>3.3</td>
</tr>
<tr>
<td>lon</td>
<td>DNA-binding ATP-dependent protease La</td>
<td>2.8</td>
</tr>
<tr>
<td>cheY</td>
<td>chemotaxis regulatory protein CheY</td>
<td>2.7</td>
</tr>
<tr>
<td>flgB</td>
<td>flagellar basal body rod protein FlgB</td>
<td>2.3</td>
</tr>
<tr>
<td>iagB</td>
<td>invasion protein precursor</td>
<td>2.3</td>
</tr>
<tr>
<td>hiiD</td>
<td>invasion protein regulatory protein</td>
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</tr>
<tr>
<td>fljB</td>
<td>flagellin</td>
<td>-7.1</td>
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<tr>
<td>invB</td>
<td>secretion chaperone</td>
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<tr>
<td>sopA</td>
<td>secreted effector protein</td>
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<tr>
<td>prgK</td>
<td>needle complex inner membrane lipoprotein</td>
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<tr>
<td>orgB</td>
<td>needle complex export protein</td>
<td>-3.7</td>
</tr>
<tr>
<td>invH</td>
<td>needle complex outer membrane lipoprotein precursor</td>
<td>-2.2</td>
</tr>
<tr>
<td>fis</td>
<td>DNA-binding protein Fis</td>
<td>-2.1</td>
</tr>
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</table>
The gut metabolome is a potential source of small molecules with anti-virulence activity
The Salmonella Pathogenicity Island 1 is repressed by small molecules from human feces
The bioactive molecule from human feces binds C₁₈ resin and elutes at 60% methanol.
The bioactive molecule from human feces can be purified through $C_{18}$ reverse-phase HPLC.
UPLC-ESI MS profiles can be used to identify potential candidates with bioactivity

Fractions

Retention time

Relative intensity

Fraction 3

Fraction 5

Fraction 7

HIV inhibition (% control)

3 5 7 Fractions
UPLC-ESI MS profiles can be used to identify potential candidates with bioactivity

Accurate mass determination
Database searches

<table>
<thead>
<tr>
<th>Mass</th>
<th>Compound</th>
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<tbody>
<tr>
<td>327.086843</td>
<td>2-Hydroxycinnamic acid</td>
</tr>
<tr>
<td></td>
<td>Enol-phenylpyruvate</td>
</tr>
<tr>
<td></td>
<td>Phenylpyruvic acid</td>
</tr>
<tr>
<td></td>
<td>m-Coumaric acid</td>
</tr>
<tr>
<td></td>
<td>4-Hydroxycinnamic acid</td>
</tr>
<tr>
<td>329.103062</td>
<td>Hydroquinone</td>
</tr>
<tr>
<td></td>
<td>Pyrocatechol</td>
</tr>
<tr>
<td>283.097583</td>
<td>Heme A</td>
</tr>
<tr>
<td>299.128867</td>
<td>Hydrocinnamic acid</td>
</tr>
<tr>
<td></td>
<td>2-Phenylpropionate</td>
</tr>
<tr>
<td></td>
<td>4-Coumaryl alcohol</td>
</tr>
<tr>
<td>359.113457</td>
<td>Galactinol dihydrate</td>
</tr>
</tbody>
</table>
The gut microbiota is required for the production of the bioactive molecule from human feces.
What’s next?

- What are the molecular mechanisms involved in microbe interactions with the mammalian endocrine system?
- What are the molecular details of signaling between the intestinal microbiota and incoming pathogens?
- Can we use this systems biology approach to identify the molecular determinants of interactions between humans and other microbiomes?
- What are the roles of the other 1000’s of small molecules present in the mammalian gut?
- Can the intestinal metabolome be explored as a source of bioactive molecules?
  - Antibiotic
  - Anti-virulence
  - Anti-inflammatory
  - Prebiotic
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