Outline of human microbiome research

Healthy subjects and disease-afflicted patients

Metagenome
Meta 16S rRNA gene
Metabolome
Proteome

Germ-free mice
Gnotobiotic mice

Microbiota
Oral
Skin
Gastrointestinal tract
Respiratory tract
Urinary tract
Genital tract

Transcriptome
Metabolome
Proteome
Host-microbial interaction

Isolated bacterial strains

Database
Genome × 16S

Metadata of subjects
Human genome

“Who’s there?”
Data-driven study
(Technology development, Database construction)

“What are they doing?”
“What are they making?”
Functional study
Multi-omics approach

Analytical pipeline for human microbiomes

Wet process

Feces
Saliva
Skin...

DNA
PCR
16S amplicon

1) 16S data
Microbial 16S db

NGS

2) Metagenomic data

Unique contigs/sequences
Gene prediction
Gene catalog
Functional DB (KEGG/COG)

Functional analysis (strain-level)

Mapping

Table: Taxonomic analysis (strain-level)

Cultured strains

3) Genomic data
Ref genome DB >3,000 genomes

NGS

NGS: Next-generation sequencers
Human microbes

The four major phyla account for >99% of the total abundance.
Additional 18 phyla are detected with <1% abundance.

>70 phyla have been detected on the earth.
⇒ A strong selective pressure may be involved in colonization by the human microbes.

### Metagenomic data of healthy human gut microbiomes

<table>
<thead>
<tr>
<th>Country</th>
<th>Subject#</th>
<th>Sequencing</th>
<th>Unique gene#</th>
<th>Published year</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>2</td>
<td>Sanger</td>
<td>0.05M</td>
<td>2006</td>
</tr>
<tr>
<td>Japan</td>
<td>13</td>
<td>Sanger</td>
<td>0.7M</td>
<td>2007</td>
</tr>
<tr>
<td>---</td>
<td></td>
<td></td>
<td></td>
<td>IHMC launched in 2008</td>
</tr>
</tbody>
</table>

Metagenomic sequencing by NGS after 2010

861 healthy subjects from 12 countries

- Denmark N=121
- Sweden N=36
- Russia N=83
- USA N=126
- Austria N=41
- France N=55
- Spain N=54
- China N=187
- Japan N=106
- Peru N=31
- Malawi N=5
- Venezuela N=10
**Microbial composition of 106 Japanese gut microbiomes**

At the genus level (a high inter-individual diversity in the microbial composition)

- Bifidobacterium: most abundant
- Power law

**Comparison of gut microbial compositions between individuals**

The overall gut microbiome structure of individuals in the same country is significantly more similar than those between different countries, indicating existence of the population (country)-level diversity in the human gut microbiome. The observed diversity is not significantly affected by methodologies (sequencers, fecal storage conditions, and DNA extraction methods).

**Variations in human gut microbiomes across the 12 countries**

Ave. 3.4 Gb/individual (350 Gb/106 Japanese)/MiSeq, IonPGM/Proton, 454

- Mapped 1 M reads to reference genomes
- 21 phyla
- 425 genera

**機械学習プログラムRandomForestを用いた国間の違いに大きく寄与する菌種の特定とそれらによる国の識別**

A set of ≥14 genera or ≥18 species has the ability to significantly differentiate the countries

- N=14
- Average accuracy rate of the prediction: 83% (Genus)

**MDS of all 861 subjects at the genus level**


**Average accuracy rate of the prediction: 83% (Genus)**

<table>
<thead>
<tr>
<th>Predicted country</th>
<th>CN</th>
<th>DK</th>
<th>JP</th>
<th>RU</th>
<th>ES</th>
<th>SE</th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>87%</td>
<td>1%</td>
<td>2%</td>
<td>4%</td>
<td>4%</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>DK</td>
<td>3%</td>
<td>78%</td>
<td>0%</td>
<td>3%</td>
<td>2%</td>
<td>8%</td>
<td>3%</td>
</tr>
<tr>
<td>JP</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>RU</td>
<td>4%</td>
<td>1%</td>
<td>0%</td>
<td>55%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>ES</td>
<td>14%</td>
<td>13%</td>
<td>0%</td>
<td>56%</td>
<td>7%</td>
<td>9%</td>
<td>9%</td>
</tr>
<tr>
<td>SE</td>
<td>1%</td>
<td>0%</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>US</td>
<td>2%</td>
<td>17%</td>
<td>0%</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>79%</td>
</tr>
</tbody>
</table>
Hierarchical clustering of the 12 countries based on the average microbial abundance

Food seems not to be the primary factor affecting the human gut microbiome

Effect of traditional vegetable dishes (VD) on the human gut microbiome

Meal information of the 12 countries from FAOSTAT

<table>
<thead>
<tr>
<th>119 food items</th>
<th>Japan</th>
<th>Russia</th>
<th>Sweden</th>
<th>USA</th>
<th>China</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk - Excluding Butter</td>
<td>203</td>
<td>481</td>
<td>979</td>
<td>704</td>
<td>82</td>
</tr>
<tr>
<td>Vegetables, Other</td>
<td>223</td>
<td>240</td>
<td>170</td>
<td>186</td>
<td>766</td>
</tr>
<tr>
<td>Wheat</td>
<td>121</td>
<td>453</td>
<td>218</td>
<td>212</td>
<td>185</td>
</tr>
<tr>
<td>Milk, Whole</td>
<td>127</td>
<td>350</td>
<td>193</td>
<td>310</td>
<td>75</td>
</tr>
<tr>
<td>Potatoes</td>
<td>58</td>
<td>312</td>
<td>159</td>
<td>147</td>
<td>10</td>
</tr>
<tr>
<td>Beer</td>
<td>73</td>
<td>211</td>
<td>113</td>
<td>229</td>
<td>73</td>
</tr>
<tr>
<td>Rice (Pastry Equivalent)</td>
<td>73</td>
<td>97</td>
<td>109</td>
<td>175</td>
<td>7</td>
</tr>
<tr>
<td>Sugar, Raw Equivalent</td>
<td>73</td>
<td>97</td>
<td>109</td>
<td>175</td>
<td>7</td>
</tr>
<tr>
<td>Maize</td>
<td>31</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>Sugar (Raw Equivalent)</td>
<td>31</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>Pig meat</td>
<td>54</td>
<td>54</td>
<td>99</td>
<td>82</td>
<td>103</td>
</tr>
<tr>
<td>Sugar, Refined Eqv</td>
<td>43</td>
<td>89</td>
<td>93</td>
<td>84</td>
<td>15</td>
</tr>
<tr>
<td>Rice (Milled Equivalent)</td>
<td>148</td>
<td>14</td>
<td>16</td>
<td>23</td>
<td>209</td>
</tr>
<tr>
<td>Poultry Meat</td>
<td>46</td>
<td>63</td>
<td>42</td>
<td>134</td>
<td>35</td>
</tr>
<tr>
<td>Oranges, Mandarines</td>
<td>28</td>
<td>22</td>
<td>139</td>
<td>85</td>
<td>7</td>
</tr>
<tr>
<td>Fruits, Other</td>
<td>28</td>
<td>60</td>
<td>62</td>
<td>62</td>
<td>79</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>23</td>
<td>64</td>
<td>61</td>
<td>121</td>
<td>78</td>
</tr>
<tr>
<td>Bovine Meat</td>
<td>24</td>
<td>48</td>
<td>68</td>
<td>109</td>
<td>13</td>
</tr>
<tr>
<td>Cassava</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

---

*Average of FAOSTAT data in 2003-2012*
Dietary restrictions changed the gut microbiome of obesity patients. In American people, the ratio of Firmicutes/Bacteroidetes is significantly higher in obesity patients than that of leans.

Change to lean-type from obese-type gut microbiome with low-calories diets (low carbohydrates) for 52 weeks

Reduction of obesity

Obese gut microbiome

Dysbiotic (obese) gut microbiome is more sensitive (unstable) to change in diet than healthy gut microbiome.

Association between dysbiosis of gut microbiome and various diseases

Metabolism system, immune system, and nervous system (brain function)

Change in overall microbial abundance

Dysbiosis of gut microbiome** including mouse data

Change in species richness

*Our collaborative work

Change of overall microbial abundance

Cluster of gut microbiomes of healthy subjects in 12 countries with those of Swedish and Chinese T2D, and Japanese and Spanish IBD patients

Inter-country variations in the gut microbiome are higher than variations between disease and healthy gut microbiomes in the same country

Schematic view explaining “dysbiosis” of gut microbiome
Schematic view explaining “population-level variations between different countries are higher than those between healthy individuals and patients with diseases in the same country”

Evaluation of gut microbiomes of patients with diseases requires data of healthy controls in the same country.


More salivary species in fecal samples of patients with various diseases than those of healthy control.

Human salivary microbiome-derived gnotobiotic mice (HSM)

Human saliva

HSM mice

Gut microbiome analysis

Immunological analysis

C57BL/6

Saliva of healthy adults and IBD patients

Sacrifice

5 weeks

6-8 weeks

6-9 weeks

Input salivary microbiome

Number of species

0 1 2 3 4 5 6 7 8

0 30 60 90 120 150 180 210

Feces

Saliva

Human salivary species that stably persist in the mouse gut
Intestinal inflammation in mice colonized by salivary species

Possible mechanism for TH1 induction by a Klebsiella strain in SPF mice with or without antibiotics administration

疾患の(異常)腸内細菌叢は病気発症の原因

腸内細菌は腸免疫細胞のTh17とTreg分化誘導を制御する

制御性T細胞（Treg）を誘導するヒト腸内細菌の同定と分離


ヒト腸内細菌17株のTreg分化能

腸炎を抑制する

下痢を抑制する

腸内細菌叢/盲腸

常在菌は単独ではフルに機能しない。
一方、多くの病原菌は単独で機能する。

常在菌はチームで最大活性をもつ？⇒常在菌の特徴？

17菌株のカクテルをマウスに投与

17菌株の同定

→240億円で導出された
⇒抗炎症微生物製剤の開発
Genome sequencing of 17 Treg-inducing strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total contig length in Mb</th>
<th>Number of predicted genes</th>
<th>Most similar strain</th>
<th>Genome size of similar strain in Mb</th>
<th>16S identity in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain_01</td>
<td>3.28</td>
<td>1,171</td>
<td>Clostridium ramosum DSM 1402</td>
<td>3.23</td>
<td>96.6</td>
</tr>
<tr>
<td>Strain_03</td>
<td>5.16</td>
<td>4,897</td>
<td>Flavonifractor plautii ATCC 29863</td>
<td>3.81</td>
<td>99.8</td>
</tr>
<tr>
<td>Strain_04</td>
<td>7.40</td>
<td>6,717</td>
<td>Clostridium histolyticum DSM 13479</td>
<td>6.63</td>
<td>95.3</td>
</tr>
<tr>
<td>Strain_06</td>
<td>6.21</td>
<td>5,662</td>
<td>Lachnospiraceae bacterium 6_1_EF6AA</td>
<td>2.72</td>
<td>96.5</td>
</tr>
<tr>
<td>Strain_07</td>
<td>6.06</td>
<td>5,968</td>
<td>Clostridium boletae ATCC BAA-613</td>
<td>6.56</td>
<td>99.5</td>
</tr>
<tr>
<td>Strain_08</td>
<td>3.39</td>
<td>3,312</td>
<td>Erysipelotrichaceae bacterium 2_2_44A</td>
<td>4.97</td>
<td>92.9</td>
</tr>
<tr>
<td>Strain_09</td>
<td>3.51</td>
<td>3,619</td>
<td>Anaerostipes caccae DSM 14662</td>
<td>3.61</td>
<td>97.7</td>
</tr>
<tr>
<td>Strain_10</td>
<td>3.56</td>
<td>3,660</td>
<td>Anaerotruncus cohlix DSM 17241</td>
<td>3.72</td>
<td>100.0</td>
</tr>
<tr>
<td>Strain_12</td>
<td>3.16</td>
<td>3,251</td>
<td>Coprococcus coecis ATCC 27758</td>
<td>3.24</td>
<td>93.3</td>
</tr>
<tr>
<td>Strain_14</td>
<td>6.27</td>
<td>6,076</td>
<td>Clostridium asparagiforme DSM 15981</td>
<td>6.22</td>
<td>99.7</td>
</tr>
<tr>
<td>Strain_16</td>
<td>5.44</td>
<td>5,275</td>
<td>Clostridium symbiosum WAL-1416d</td>
<td>5.35</td>
<td>99.9</td>
</tr>
<tr>
<td>Strain_18</td>
<td>3.60</td>
<td>3,692</td>
<td>Clostridium ramosum DSM 1402</td>
<td>3.23</td>
<td>100.0</td>
</tr>
<tr>
<td>Strain_24</td>
<td>5.40</td>
<td>5,139</td>
<td>Clostridium sp. D5</td>
<td>5.35</td>
<td>98.9</td>
</tr>
<tr>
<td>Strain_26</td>
<td>3.93</td>
<td>3,395</td>
<td>Clostridium scindens ATCC 35704</td>
<td>3.62</td>
<td>99.6</td>
</tr>
<tr>
<td>Strain_27</td>
<td>7.05</td>
<td>6,724</td>
<td>Lachnospiraceae bacterium 3_3_S7FA_C1</td>
<td>7.69</td>
<td>97.5</td>
</tr>
<tr>
<td>Strain_28</td>
<td>6.96</td>
<td>6,786</td>
<td>Clostridiales bacterium 1_7_S7FAA</td>
<td>6.51</td>
<td>99.7</td>
</tr>
<tr>
<td>Strain_29</td>
<td>7.55</td>
<td>6,912</td>
<td>Lachnospiraceae bacterium 3_3_S7FAA_C1</td>
<td>7.69</td>
<td>99.6</td>
</tr>
</tbody>
</table>

VE202-01 (Clostridium sachiorgarum DSM 17460)
VE202-06 (Clostridiales bacterium VE202-06)
VE202-09 (Clostridiales bacterium VE202-09)
VE202-13 (Anaerotruncus cohlii DSM 17241)
VE202-14 (Clostridiales bacterium VE202-14)
VE202-15 (Clostridium asparagiforme DSM 15981)
VE202-21 (Clostridiales bacterium VE202-21)
VE202-26 (Clostridium scindens ATCC 35704)
VE202-27 (Clostridiales bacterium VE202-27)
VE202-28 (Clostridiales bacterium VE202-28)

2866, VE202-18 (Erysipelotrichus intestinalis DSM 1402)
2811, VE202-03 (Flavonifractor plautii ATCC 29863)
1F8, VE202-04 (Clostridium histolyticum DSM 1248931)
1F7, VE202-07 (Clostridium boletae 90B8)
1C12, VE202-09 (Deltella furtidossa)
1A9, VE202-16 (Clostridium symbiosum WAL-14163)
204 (Clostridium innocuum 3959)
2G11 (Bacteroides dorei CI0321206)
2F7 (Clostridiales bacterium 2F7)
2E3 (Clostridiales bacterium 2E3)
2E1 (Anaerostipes caccae DSM 14662)
2D9 (Ruminiclostridium gryseum CC55_001C)
1E3 (Coprococcus sp. D6)
1E11 (Clostridiales bacterium 1E11)
1D4 (Clostridiales bacterium 1D4)
1D2 (Clostridiales bacterium 1D2)
1D0 (Clostridiales bacterium 1D0)
1C2 (Bifidobacterium breve DSM 20213 = SCM 1192)
1B11 (Bifidobacterium pseudolongum 1B11)

Six strains are overlapping

**Treg-inducing 17 strains**
Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota.

**Th17-inducing 20 strains**
Th17 Cell Induction by Adhesion of Microbes to Intestinal Epithelial Cells.
Translocation of a gut pathobiont drives autoimmunity in mice and humans.


- Translocation of Enterococcus gallinarum to the liver and other systemic tissues triggers autoimmune responses in a genetic background predisposing to autoimmunity.
- E. gallinarum-specific DNA was recovered from liver biopsies of autoimmune patients.

Klebsiella pneumoniae strains enriched in primary sclerosing cholangitis (PSC) trigger bacterial translocation and pathologic Th17 priming in the liver

Under revision

* The epithelial-damaging effect of KP was strain-specific and associated with the presence of Type VI secretion system.

Feces of UC patients → Gnotobiotic mice → no liver inflammatory response
Feces of PSC patients → Gnotobiotic mice → liver inflammatory response (Th17)

PSC-fecal gnotobiotic mice
→ Cultured bacterial in the mesenteric lymph nodes
→ Isolated Klebsiella pneumoniae, Proteus mirabilis, and Enterococcus gallinarum.

* The epithelial-damaging effect of KP was strain-specific and associated with the presence of Type VI secretion system.

---

**Precision Medicine Initiative (PMI)**

[YouTube video about PMI, including microbiome](https://www.youtube.com/watch?v=RlsqBtlcsw)

PMI announced by President Obama

January 30, 2015


---

**Translocation of a gut pathobiont drives autoimmunity in mice and humans.**


- Translocation of Enterococcus gallinarum to the liver and other systemic tissues triggers autoimmune responses in a genetic background predisposing to autoimmunity.
- E. gallinarum-specific DNA was recovered from liver biopsies of autoimmune patients.

Klebsiella pneumoniae strains enriched in primary sclerosing cholangitis (PSC) trigger bacterial translocation and pathologic Th17 priming in the liver

Under revision

* The epithelial-damaging effect of KP was strain-specific and associated with the presence of Type VI secretion system.

Feces of UC patients → Gnotobiotic mice → no liver inflammatory response
Feces of PSC patients → Gnotobiotic mice → liver inflammatory response (Th17)

PSC-fecal gnotobiotic mice
→ Cultured bacterial in the mesenteric lymph nodes
→ Isolated Klebsiella pneumoniae, Proteus mirabilis, and Enterococcus gallinarum.

* The epithelial-damaging effect of KP was strain-specific and associated with the presence of Type VI secretion system.
Thank you for your attention

All in a day's catch!