Recent Progress of the Research on Microbiome and Probiotics in Japan

* Tamotsu Kuwata, PhD, University and Graduate School of Human Arts and Sciences
* 25 January 2016 ILSI Annual Meeting  St, Petersburg, FL
From Academia
1. Metagenomics of Human Gut Microbiome -profiling of the Japanese Gut Microbiome-
   * (Prof. Mashira Hattori The University of Tokyo)
2. Intestinal bacteria as a symbiont
   (Prof. Phillip Sherman Canadian institute of Health Research)

* From industry
* Importance of Segmented Filamentous Bacteria in intestinal immune system and its application to Gut inflammation Model
  * (Yoshinori Umesaki : Yakult Center Institute)
* Age-related Change of the Gut Microbiota-people Aged 0to over 100 Years old-
  (Toshitaka odamiki :Morinaga Milk Industry Co.,LTD)
* Augmentation of Host Defense Mechanism by Oral Administration of Lactobacillus gasseri SBT2055
  * (Fumihiko Sakai: Milk Science Research Institute, Megumilk Snow Brand Co., LTD)
* Effects of Yogurt on Intestinal Environment and Body Functions
  * (Katsunori Kimura: Food Science Research Laboratories, R&D Division, Meiji Co., LTD)
Metagenomics of Human Gut Microbiome
-Profiling of the Japanese Gut Microbiome-

Masahira HATTORI

Graduate School of Advanced Science and Engineering,
Waseda University, Tokyo, JAPAN
&
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The University of Tokyo, Chiba, JAPAN

Sept. 30, 2015 @ The 7th International Conference on “Nutrition and Aging”, Tokyo, Japan
Methods for bacterial community analysis

**Bacterial culture method (1960’s ~)**
- Presence of unculturable bacteria that cannot be detected.

**Bacterial 16S rRNA gene analysis (1980’s ~)**
- Can detect the unculturable bacteria
- But no functional information
  ⇒ Ecological study

**Metagenomics (2004 ~)**
- Shotgun sequencing of microbiome
- Can get both ecological and functional information
- **Large-scale analysis by NGS (2008 ~)**
  (NGS: Next-generation sequencing)
Outline of human microbiota research

Healthy and disease-affected subjects

Metagenome
Meta 16S rRNA gene
Metabolome

Database
Genome•16S
Isolated bacterial strains

Microbiota
Oral
Skin
Gastrointestinal tract
Respiratory tract
Urinary tract
Genital tract

Metadata of subjects
Human genome

A bacterium or bacterial mixture to be analyzed

Germ-free mice
Gnotobiotic mice

Transcriptome
Metabolome
Proteome
Cell level
Host-microbial interaction

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

“What are they doing?”
Functional study
Targeted metagenomics
Dysbiosis of gut microbiota and disease

### Related diseases
- Obesity
- Metabolic syndrome
- Inflammatory bowel disease (IBD)
- Irritable bowel syndrome
- Atherosclerosis
- Rheumatism
- Type 2 Diabetes
- Allergy
- Asthma
- Celiac disease
- Colon cancer
- Liver cancer
- Autism
- Multiple sclerosis

### Change in bacterial composition
- **IBD**
- **Healthy**

### Decrease in microbial diversity
- **Healthy**
- **IBD**
However, much basic knowledge on the human gut microbiome are still insufficient

Our recent research focuses are:
• To elucidate the population-level diversity in the human gut microbiome by metagenomics.
• To explore environmental factors which profoundly contribute to the diversity in gut microbiomes across countries.
• To characterize Japanese gut microbiome as compared with those of other countries.
• We compared the metagenomic data of the bacterial composition in gut microbiomes of healthy individuals in 9 countries including the Japanese data newly collected.
• The 9 countries included the United States (US), China (CN), Russia (RU), Sweden (SE), Denmark (DK), Spain (ES), Japan (JP), and Venezuela / Malawi natives (VM).
Current status of metagenomics of the human gut microbiome

<table>
<thead>
<tr>
<th>Country</th>
<th>Subject#</th>
<th>Sequencer</th>
<th>Unique gene#</th>
<th>Published year</th>
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<td>USA</td>
<td>2</td>
<td>Sanger</td>
<td>0.05M</td>
<td>2006</td>
</tr>
<tr>
<td>Japan (HMGJ)</td>
<td>13</td>
<td>Sanger</td>
<td>0.7M</td>
<td>2007</td>
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</table>

IHMC launched in 2008

Most researches have been done by metagenomic analysis using NGS after 2010

More than 1,000 subjects
Bacterial composition in gut microbiota of 106 Japanese individuals

High interindividual variability in gut microbiota of the Japanese

Genus level

red: Actinobacteria
blue: Firmicutes
green: Bacteroidetes
Search of factors which profoundly contribute to the population-level diversity in human gut microbiota across the countries

Association study of the microbial composition with:
- Epidemiological data of dietary intake of 119 food items (g/capita/day) from:
  FAOSTAT (Food and Agriculture Organization of the United Nations) database

- Epidemiological data of Antibiotic use from:
  IMS Health MIDAS database (In human)
  A recently published paper (In farm)
# Meal information (FAOSTAT)

(g/capita/day)

Food and Agriculture Organization of the United Nations

http://faostat.fao.org

Data from 245 countries and 35 regional areas from 1961 through the most recent year

<table>
<thead>
<tr>
<th>Food (119 items)</th>
<th>Japan</th>
<th>Russia</th>
<th>Sweden</th>
<th>America</th>
<th>China</th>
<th>Denmark</th>
<th>Venezuela</th>
<th>Malawi</th>
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<td>481</td>
<td>979</td>
<td>700</td>
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<td>766</td>
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<td>Wheat</td>
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<td>222</td>
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<td>350</td>
<td>193</td>
<td>310</td>
<td>75</td>
<td>98</td>
<td>259</td>
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<td>58</td>
<td>312</td>
<td>159</td>
<td>147</td>
<td>101</td>
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<td>297</td>
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<td>Beer</td>
<td>73</td>
<td>211</td>
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<td>229</td>
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<td>195</td>
<td>222</td>
<td>6</td>
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<tr>
<td>Rice (Paddy Equivalent)</td>
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<td>142</td>
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<td>105</td>
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<td>14</td>
<td>16</td>
<td>23</td>
<td>209</td>
<td>14</td>
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<td>16</td>
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<td>Poultry Meat</td>
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<td>62</td>
<td>42</td>
<td>134</td>
<td>35</td>
<td>52</td>
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<td>4</td>
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<td>139</td>
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<td>62</td>
<td>62</td>
<td>79</td>
<td>64</td>
<td>44</td>
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<td>0</td>
<td>5</td>
<td>42</td>
<td>194</td>
<td></td>
</tr>
</tbody>
</table>
Dendrogram of the countries based on FAOSTAT data by hierarchical clustering

Three major nutrient components calculated from FAOSTAT data

(g)

United States_2011
Spain_2011
Sweden_2011
Denmark_2011
Russia_2011
Venezuela_2011
China_2011
Japan_2011
Malawi_2011
The gene for enzyme *Porphyranase* is encoded by marine bacteria, but was found to be also encoded by human intestinal bacteria.

This enzyme can break down specific polysaccharides (porphyran) of aqueous plants such as seaweed.

**5/13 Japanese**
**0/18 Americans**

Horizontal gene transfer of this gene from marine bacteria to intestinal bacteria

*about 70% of the Japanese have
*Only 3.4% of Westerners have

Dietary habits contribute to acquisition of function in human gut microbiota.
Defining a healthy microbiome
ILSI-Japan 7th International Conference on Nutrition and Aging
Wednesday September 30, 2015
Tokyo, Japan

Philip M. Sherman, MD, FRCPC
Professor of Paediatrics, Microbiology, and Dentistry
Hospital for Sick Children, University of Toronto
Scientific Director, CIHR Institute of Nutrition, Metabolism & Diabetes
Canada Research Chair in Gastrointestinal Disease

SickKids
Faculty of Medicine
Great Minds for a Great Future
University of Toronto
Canadian Institutes of Health Research
Institut de recherche en santé du Canada
Learning objectives:

1. Update what is known about the gut microbiome in health & over the life span.
2. Consider the role of prebiotics and probiotics in management of dysbiosis.
Microbiota separates elderly subjects based upon where they live in the community

Green = community
Yellow = day hospital
Orange = rehabilitation
Red = long-stay hospital
Purple = young healthy

Joint Programming Initiative: A Healthy Diet for a Healthy Life (JPI-HDHL)

- 25 member states and associated countries of the European Union
- Strategic research agenda developed in 3 areas:
  1. Determinants of Diet and Physical Activity
  2. Diet and Food Production
  3. Diet-Related Chronic Diseases

- Joint Action: **Biomarkers in Nutrition** and Health - aimed at validation of biomarkers and the investigation of intake/exposure and nutritional status of biomarkers in the area of nutrition and health
  - Funded: FOODBALL consortium, which includes 20 research organizations from 9 EU countries, **Canada**, and New Zealand

- Future Priority Topic: **Intestinal Microbiomics**

For more information: http://www.healthydietforhealthylife.eu
Aging-related changes of gut microbiota composition from new-born to centenarian in Japanese, cross-sectional study

Morinaga Milk Industry Co.,Ltd
Toshitaka Odamaki
Study design

<sample>

- **371 samples**
  (367 healthy Japanese subjects aging 0 to 104 years old of community-dwelling)

<Method>

- DNA extracted from fecal samples by beads beating method.
- V3-V4 region of the 16S rRNA gene were sequenced by Miseq

Study protocols were approved by the ethical committee of a medical institution and written informed consent was obtained from all subjects or their guardian.

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<Sample distribution>

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<tr>
<th>age</th>
<th>number of sample</th>
<th>(M/F)</th>
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<td>14</td>
<td>(7/7)</td>
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<tr>
<td>weaning</td>
<td>12</td>
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<td>weaned-9</td>
<td>32</td>
<td>(16/16)</td>
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<td>10-19</td>
<td>10</td>
<td>(7/3)</td>
</tr>
<tr>
<td>20-29</td>
<td>40</td>
<td>(16/24)</td>
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<tr>
<td>30-39</td>
<td>88</td>
<td>(45/43)</td>
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<td>40-49</td>
<td>34</td>
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<td>50-59</td>
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<td>90-99</td>
<td>19</td>
<td>(4/15)</td>
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<tr>
<td>100-</td>
<td>6</td>
<td>(0/6)</td>
</tr>
<tr>
<td><strong>sum</strong></td>
<td><strong>371</strong></td>
<td>(158/213)</td>
</tr>
</tbody>
</table>
Change of gut microbiota with aging

Phylum (Family/genus):
- Actinobacteria (Bifidobacterium)
- Proteobacteria (Enterobacteriaceae)
- Firmicutes (Clostridiaeceae, Blautia, Faecalibacterium, Lactobacillus)
- Bacteroidetes (Bacteroides)

Inter-and intra-individual variations:
- Genetic background
- Diet-geographical habits
- Health status
- Physiological status

Infancy: New-born  6 mo  3 yr
- Delivery mode
- Feeding (breast vs formula)
- Health status

Adultness

Elderly

Figure 1 Evolution of some representatives of the intestinal microbiota accordingly to age.
(Tojo et. al., World J Gastroenterol. 2014 7;20(41):15163-764)
Three enterotype clustering related with age

(years old)
Take home messages

- It seemed to have some patterns and turning points in the composition change of gut microbiota with aging.

- Higher positive correlations were observed in the elderly-associated CAGs, implying that these genera can grow well in the same environmental condition, or they develop a mutualistic relationship.

- The composition of bifidobacterial species was also changed with aging, especially at weaning and centenarian.

- Factors such as nutrients and drugs existing in the gut might play an important role in shaping the composition of gut microbiota with aging.
Modulation of the colon microflora by the consumption of probiotics foods is increasingly being studied as a potent, cost effective and natural way to improve the health and well-being of elderly people as well as reduce risks for various diseases.
Importance of Segmented Filamentous Bacteria in Intestinal Immune System and Its Application to Gut Inflammation Model

Yoshinori Umesaki
Yakult Central Institute
IgA

Cell

Th17

Villus-Crypt
axis

MHC II

Epithelial
Fucosylation

γδIEL

Recruitment
[αβIEL (CD8αβ)]

SFB

Peyer’s patch

B cell

SFB \(\text{(segmented filamentous bacteria)}\)
IgA Production and Epithelial MHC class II Response to SFB-monocolonization

IgA production

Epithelial MHC class II

(Umesaki et al., Microbiol Immunol, 39, 555, 1995)
Clinical Study for Improvement of UC by *Bifidobacterium*-fermented Milk (BFM)

1: Prevention of relapse of UC
(Osaka Medical Center for Cancer and Cardiovascular Diseases)

2: Improvement of active UC
(Nihon University Itabashi Hospital)

**BFM Placebo**

Augmentation of Host Defense Mechanism by Oral Administration of Lactobacillus gasseri SBT2055

* Fumihiko Sakai
* Milk Science Research Institute, Megmilk Snow Brand
* Co., LTD
Functions of LG2055

Colonization of the intestine

Improvement of the intestinal flora

Ameliorate of colitis

Cholesterol lowering

Anti-obesity

Modulation of mucosal immune system

Effect of LG2055 oral administration on mucosal immune system
LG2055 markedly induced IgA production in vitro by PP cells.
Effect of oral administration of LG2055 on IgA levels in the murine intestine

IgA levels in the small intestine was augmented by oral administration of LG2055.
Effects of Yogurt on Intestinal Environment and Body Functions

* Katsunori Kimura PhD
* Food Science Research Laboratories
* R&D Division Meiji, Co., LTD
Probiotic yogurt (LG21) for Helicobacter pylori infection
Schedule of clinical study (n=31)

8 weeks Yogurt
90g x 2 / day

Urea Breath Test Pepsinogen

1 week

8 weeks LG21 Yogurt
90g x 2 / day (10⁹ cfu of LG21 / 90g)

Urea Breath Test Pepsinogen Biopsy (n=6)

1 week

Urea Breath Test Pepsinogen Biopsy (n=6)
Urea Breath Test

to measure the population of *H. pylori*

(small value = small population)

Pepsinogen I/II Ratio

to evaluate the degree of mucosal inflammation

(large value = low mucosal inflammation)
Urea Breath Test (n=29)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Δ¹³C (‰) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before ingestion</td>
<td>26.2 ± 15.1</td>
</tr>
<tr>
<td>Yogurt</td>
<td>26.6 ± 13.7</td>
</tr>
<tr>
<td>LG21 Yogurt</td>
<td>20.9 ± 11.8</td>
</tr>
</tbody>
</table>

- NS
- *p* < 0.05
Pepsinogen I/II Ratio (n=30)

- Before ingestion: 2.91 ± 1.16
- Yogurt: 2.99 ± 1.05
- LG21 Yogurt: 3.25 ± 1.09

Comparisons:
- NS
- \( p < 0.01 \)
1. *Lactobacillus gasseri* OLL2716 (LG21) was selected from over 200 *Lactobacillus* strains as the most effective probiotic for *H. pylori* infection.

2. LG21 displayed excellent inhibitory activity against *H. pylori* both *in vitro* and in animal studies.

3. Administration of LG21 decreased *H. pylori* in the stomach of Mongolian gerbils and suppressed *H. pylori* induced gastric ulcer.

4. Ingestion of LG21 yogurt decreased *H. pylori* in the stomach of human and alleviated the mucosal inflammation.
Immunomodulatory effects of polysaccharides from *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1
Characterization of EPS produced by OLL1073R-1

Repeating unit

\[
\beta-D-Galp \\
\downarrow \\
\to 2)-\alpha-D-GlcP-(1\to 3)-\beta-D-GlcP-(1\to 3)-\beta-D-Galp-(1\to 4)-\alpha-D-Galp-(1\to)
\]

Acidic EPS

Neutral EPS

Dr. Marie-Rose Van Calsteren, AGR Canada
Effects of ingestion of OLL1073R-1 yogurt on elderly immune function

Funagata Study

Subjects: 57 healthy elderly people (69-80 y)

Diets: Test Yogurt 90 g/day

Milk 100 ml/day

Intake period: 2005/3/13 ~ 2005/5/8 (8 weeks)

Immunological: NK cell activity, Risks of catching common colds or the flu

Arita Study

Subjects: 85 healthy elderly people (59-85 y)

Diets: Test Yogurt 90 g/day

Milk 100 ml/day

Intake period: 2006/11/14 ~ 2007/2/8 (12 weeks)

Immunological: NK cell activity, Risks of catching common colds or the flu
NK cell activity (Funagata Study)

Summary: Effects of EPS and OLL1073R-1 yogurt

1. Oral administration of OLL1073R-1 EPS and OLL1073R-1 yogurt augmented NK cell activity in mice.

2. Oral administration of OLL1073R-1 EPS and OLL1073R-1 yogurt prolonged survival rates following influenza virus infection in mice.

3. Intake of OLL1073R-1 yogurt augmented NK cell activity and reduced risk of catching common colds or the flu in the elderly.

4. OLL1073R-1 EPS may activate NK cells through a pathway mediated by IL-12 from dendritic cells and IFN-\(\gamma\) derived from either CD4 T cells or NK cells.
Conclusion

“Meiji Yogurt Drink R-1” is a probiotic produced using proprietary technology, acting by means of a unique mechanism, showing great potential benefit to consumers.

Last year, yogurt went on sale and this year the line was expanded to drink yogurt, which proved very popular.

Animal studies and human clinical trials both displayed the benefit of Meiji R-1 against H1R1 and the common cold, respectively.
Probioic Dairy Products (yougurt & Milk)

Meiji Bulgaria Yougurt LB81
Morinaga Bifidus Yugurt
Morinaga Caldus Milk
Megumi Yugurt Megumilk Snow

Certification: FOSHU Product
Meiji Bulgaria Yogurt LB81

Meiji Bulgaria Yogurt LB81 fermented by LB81 lactic acid bacteria (L. delbrueckii subsp. bulgaricus 2038 and S. thermophilus 1131) was approved for a FOSHU claim.
Probiotic Dairy Products (Yogurt & Milk)

1. Product name: Morinaga Bifidus Yogurt
2. Dosage: 2 billion *Bifidobacterium longum* BB536/100g,
3. Concept: Nutritional food to support the intestinal environment
4. Certification: FOSHU product

Probiotic yogurt

1. Product name: Morinaga Caldus
2. Dosage: 2 billion *Bifidobacterium longum* BB536/100g, 180ml/bottle
3. Concept: Nutritional food to support the intestinal environment
4. Certification: FOSHU product
<Product name> Bifidus BB536 capsule
<Submission number> No.A153
<Functional component> Bifidobacterium BB536
<Health claim> This product contains Bifidobacterium BB536. The functions of Bifidobacterium BB536 makes good intestinal environment and regulating the intestinal condition has been reported.
<Dosage> 5 billion / capsule, 2-4 capsule / day
The recent years, EFSA has rejected probiotic health claims adducing that there is not sufficient scientific evidence for the declared effects.

This situation obligated food companies to perform new clinical studies trying to generate solid scientific evidence for specific probiotic strains and health benefits.