Note

Gut microbial composition of elderly women born in the Japanese longevity village Ogimi

Hiroto MORITA1*, Mutsuki ICHISHIMA1, Ipputa TADA2,3, Hirotugu SHIROMA2,4, Makoto MIYAGI2, Tepepi NAKAMURA1, Hiroshi TANAKA2 and Shinya IKEMATSU2

1Department of Microbiological Flora Technology, Core Technology Laboratories, Asahi Quality & Innovations, Ltd., 11-10-5 Fuchinobe, Sagamihara, Kanagawa 252-0206, Japan
2Department of Bioresources Engineering, National Institute of Technology, Okinawa College, 905 Henoko, Nago, Okinawa 905-2192, Japan
3Present address: Department of Genetics, The Graduate University for Advanced Studies, SOKENDAI, 1111 Yata, Mishima, Shizuoka 411-8540, Japan
4Present address: School of Life Science and Technology, Tokyo Institute of Technology, Tokyo, Japan

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Ogimi is one of Japan’s longevity villages and is located in Okinawa Prefecture. In this study, we focused on the elderly women living in the village, classified them into two groups based on whether or not they lived in Ogimi during the first 3 years of their lives, and compared the gut microbiota between the two groups. There were no differences in alpha and beta diversity; however, we found that the elderly women who lived in Ogimi during the first 3 years of their lives had a higher rate of Akkermansia muciniphila colonization in their guts.

Key words: microbiota, Akkermansia muciniphila, longevity, early-life environment
5 years of life, we classified the women into two groups based on whether they were living in Ogimi during the first 3 years of their lives (group OG, n=40) or not (group NOG, n=20) and compared the gut microbiotas between the two groups. The areas where they lived from their birth to the present are shown in Supplementary Table 1. The mean age ± standard deviation (SD) of group OG was 83.8 ± 6.8 years, and that of group NOG was 81.3 ± 6.1 years (p=0.16, student’s t-test). The gut microbiota of all participants were characterized by Illumina MiSeq sequencing of the V4 region of the bacterial 16S rRNA gene using DNA extracted from fecal samples. These analyses were carried out as previously described [14]. In total, 3,558,508 high-quality filtered sequences were obtained, and we first compared the Chao1, Shannon diversity index, and phylogenetic distance (PD) whole tree between groups OG and NOG. All data were expressed as the mean ± SD, and p-values were calculated by the two-sample t-test using Monte Carlo permutations. The results revealed no significant differences (Chao1, 1,468 ± 495 for group OG and 1,563 ± 452 for group NOG, p=0.62; Shannon diversity index, 5.81 ± 1.00 for group OG and 5.99 ± 0.97 for group NOG, p=0.50; PD whole tree, 38.68 ± 11.94 for group OG and 40.39 ± 11.64 for group NOG, p=0.62). Then, principal coordinate analysis based on the unweighted or weighted UniFrac distance was performed, and no significant differences were observed between the two groups (p=0.36 and 0.39, respectively, with p-values determined by permutational multivariate analysis of variance). These results showed that there were no differences between groups OG and NOG in the overall gut microbiota. In studies which focused on longevity areas, differences were observed in the diversity of the gut microbiota of centenarians or elderly people living in longevity areas, differences were observed in the diversity of the gut microbiota, and this conflicts with our results [15–18]. The previous studies grouped subjects according to age and/or living area, which may have contributed to the differences found in their gut microbiotas. However, our study compared two groups that differed in terms of their early-life environments but matched in terms of age, gender, and current living area. This might explain why no differences were observed in our microbial diversity analysis.

The microbial compositions of both groups at the genus level and adjusted p-values of the Mann-Whitney U test with Benjamin-Hochberg false discovery rate correction (q-value) are shown in Table 1. No differences were observed in the relative abundances of each genus between two groups if q-value <0.05 was considered to indicate statistical significance. However, when our microbiota data were analyzed with the linear discriminant analysis effect size algorithm (LEfSe) (http://huttenhower.sph.harvard.edu/galaxy/), 5 genera were found to be significantly different between the groups (Fig. 1). Akkermansia was more abundant in group OG, and Lachnospiraceae_r._, Collinsella, Peptococcus, and f.S24_7;g_ were more abundant in group NOG. Akkermansia muciniphila, which was isolated from human feces in 2004 [19], is expected to be a next-generation probiotic. The administration of live A. muciniphila partially protected against high-fat diet-induced obesity and insulin resistance in mice [20], and oral administration of pasteurized A. muciniphila significantly improved insulin sensitivity and reduced plasma total cholesterol in humans [21]. To determine the cell counts and detection rate of A. muciniphila in groups OG and NOG, we calculated the cell counts of A. muciniphila in fecal samples by quantitative PCR using A. muciniphila-specific primers [22]. Quantitative PCR amplification and detection were performed on a LightCycler 480 Instrument II (Roche Diagnostics K.K., Tokyo, Japan) using SYBR Premix Ex Taq II (Takara Bio Inc., Kusatsu, Japan). The standard curve was generated using a synthesized partial 16S rRNA gene of A. muciniphila and serial 10-fold diluents. The partial 16S rRNA gene was a 429 bp fragment that contained the region from the forward primer annealing position to the reverse primer annealing position (329 bp) and 50 bp up- and downstream of that region. The fragment was synthesized by Thermo Fisher Scientific K.K. (Tokyo, Japan). Due to A. muciniphila having 3 copies of the 16S rRNA gene, copy numbers measured by the quantitative PCR were divided by 3 to calculate the cell count of A. muciniphila. A. muciniphila was detected in 27 of 40 group OG fecal samples and in 8 of 20 group NOG fecal samples. The detection rate of A. muciniphila in group OG was significantly higher than that in group NOG (Table 2). Also, the cell count of A. muciniphila was higher in group OG than in group NOG (Table 2). However, when the cell counts of A. muciniphila were compared using only the data from participants in which A. muciniphila was detected, no significant difference was observed between the groups (Table 2). These results indicated that the elderly women who lived in Ogimi during the first 3 years of their lives had a higher rate of A. muciniphila colonization in their guts than those who were born in other regions and moved to Ogimi.

In the Japanese elderly, the relative abundance of Akkermansia has been reported to be in the range of 0 to 0.79% [23]. The relative abundance of this bacteria in the group OG ranged from 0 to 13.6%, and the mean was 1.2%. For the group NOG, relative abundance ranged from 0 to 2.3%, and the mean was 0.2%. Due to differences in methodology of microbiota analysis,

![Fig. 1. Most likely bacterial genera to explain differences between groups.](Image)
Table 1. Relative abundance of genera in feces from groups OG and NOG

| Taxa | Relative abundance (mean ± SD, %) | q-valueb) |
|------|----------------------------------|-----------|
|      | Group OG                         | Group NOG |          |
| f_Lachnospiraceae;g_Blautia        | 14.9 ± 11.8 | 18.3 ± 15.1 | 0.91 |
| f_Streptococcaceae;g_Streptococcus| 13.9 ± 13.4 | 8.3 ± 9.4  | 0.73 |
| f_Bifidobacteriaceae;g_Bifidobacterium| 9.6 ± 12.4 | 9.7 ± 12.2 | 0.94 |
| f_Ruminococcaceae;g_Faecalibacterium| 8.4 ± 7.4  | 8.6 ± 9.4  | 0.94 |
| f_Lactobacillaceae;g_Lactobacillus| 26.1 ± 13.7| 7.6 ± 13.2 | 0.91 |
| f_Lachnospiraceae;g_Coprococcus  | 6.9 ± 5.8  | 7.7 ± 5.7  | 0.91 |
| f_Enterobacteriaceae;g_Escherichia| 4.1 ± 10.1 | 1.1 ± 2.7  | 0.84 |
| f_Lachnospiraceae;g_[Ruminococcus]| 3.0 ± 2.5  | 3.4 ± 2.4  | 0.87 |
| f_Ruminococcaceae;g_Ruminococcus | 2.4 ± 2.7  | 3.0 ± 3.4  | 0.94 |
| f_Enterobacteriaceae;Other       | 2.3 ± 4.6  | 3.1 ± 4.8  | 0.91 |
| f_Lachnospiraceae;g              | 2.0 ± 2.3  | 3.5 ± 2.6  | 0.21 |
| f_Coriobacteriaceae;g_Collinsella| 1.8 ± 2.6  | 2.5 ± 2.0  | 0.29 |
| f_Lachnospiraceae;g_Dorea        | 1.7 ± 1.7  | 1.9 ± 1.3  | 0.84 |
| f_Erysipelotrichaceae;g_[Eubacterium]| 1.7 ± 3.4 | 2.5 ± 3.4  | 0.73 |
| o_Clostridiales;f_g              | 15.3 ± 3.2 | 2.3 ± 4.9  | 0.91 |
| f_Lachnospiraceae;g_Roseburia     | 1.4 ± 2.0  | 1.6 ± 2.1  | 0.84 |
| f_Bacteroidaceae;g_Bacteroides    | 1.4 ± 3.0  | 0.7 ± 0.7  | 0.94 |
| f_Clostridiales;g_SMB53          | 1.3 ± 2.2  | 1.2 ± 1.8  | 0.91 |
| f_Clostridiales;g_Clostridium    | 1.3 ± 2.6  | 0.9 ± 0.9  | 0.91 |
| f_Verrucomicrobiaceae;g_Akkermansia| 1.2 ± 2.7 | 0.2 ± 0.5  | 0.21 |
| f_Lachnospiraceae;Other          | 1.0 ± 1.2  | 1.0 ± 0.9  | 0.91 |
| f_Ruminococcaceae;g_Oscillospira  | 0.8 ± 1.0  | 1.0 ± 1.2  | 0.98 |
| f_Ruminococcaceae;g              | 0.8 ± 1.2  | 0.9 ± 0.9  | 0.84 |
| f_Methanobacteriaceae;g_Methanobrevibacter| 0.7 ± 2.2 | 0.5 ± 1.1  | 0.84 |
| f_Erysipelotrichaceae;g           | 0.6 ± 1.0  | 1.1 ± 2.1  | 0.84 |
| f_Enterobacteriaceae;g_Enterococcus| 0.5 ± 1.5  | 0.2 ± 0.4  | 0.94 |
| f_Ruminococcaceae;g              | 0.5 ± 0.5  | 0.4 ± 0.3  | 1.00 |
| f_Erysipelotrichaceae;g_Catenibacterium| 0.5 ± 1.4 | 0.6 ± 1.4  | 0.91 |
| f_Leuconostocaceae;g              | 0.5 ± 1.6  | 0.7 ± 2.1  | 0.85 |
| f_Coriobacteriaceae;g             | 0.4 ± 0.5  | 0.6 ± 0.5  | 0.53 |
| o_Clostridiales;Other;Other       | 0.4 ± 1.0  | 0.4 ± 0.7  | 0.91 |
| f_Ruminococcaceae;g_Butyricoccus | 0.3 ± 0.7  | 0.5 ± 0.5  | 0.53 |
| o_RF39;f_g                        | 0.3 ± 3.2  | 0.4 ± 4.9  | 0.91 |
| f_[Mogibacteriaceae];g            | 0.3 ± 0.3  | 0.3 ± 0.3  | 0.94 |
| f_Lachnospiraceae;g_Annaerostipes| 0.3 ± 0.5  | 0.2 ± 0.4  | 1.00 |
| f_Lachnospiraceae;g_Clostridium   | 0.2 ± 0.6  | 0.1 ± 0.3  | 0.73 |
| f_Turicibacteriaceae;g_Turicibacter| 0.2 ± 0.6  | 0.1 ± 0.2  | 0.91 |
| f_[Mogibacteriaceae];g_Mogibacterium| 0.2 ± 0.7 | 0.2 ± 0.5  | 0.91 |
| f_Rikenellaceae;g                 | 0.2 ± 0.4  | 0.1 ± 0.1  | 1.00 |
| f_Porphyrmonadaceae;g_Parabacteroides| 0.2 ± 0.5 | 0.1 ± 0.1  | 0.91 |
| f_Peptostreptococcaceae;g_Clostridium| 0.2 ± 0.7 | 0.6 ± 2.6  | 0.94 |
| f_Prevotellaceae;g_Prevotella     | 0.1 ± 0.6  | 0.1 ± 0.2  | 0.53 |
| f_Erysipelotrichaceae;g_Bulleidia| 0.1 ± 0.8  | 0.1 ± 0.1  | 0.73 |
| f_Christensenellaceae;g           | 0.1 ± 0.3  | 0.1 ± 0.2  | 0.91 |
| f_Veillonellaceae;g_Dialister     | 0.1 ± 0.5  | 0.0 ± 0.0  | 0.97 |
| f_Veillonellaceae;g_Megamonas     | 0.1 ± 0.7  | 0.0 ± 0.1  | 1.00 |
| f_Veillonellaceae;g_Veillonella   | 0.1 ± 0.4  | 0.0 ± 0.1  | 0.80 |
| f_Methanobacteriaceae;g_Methanosphaera| 0.1 ± 0.4 | 0.1 ± 0.4  | 0.84 |
| f_Lachnospiraceae;g_Lachnospira   | 0.1 ± 0.3  | 0.3 ± 1.0  | 0.84 |
| f_Coriobacteriaceae;g_Eggerthella| 0.1 ± 0.2  | 0.1 ± 0.2  | 0.85 |
| f_Enterobacteriaceae;g            | 0.1 ± 0.2  | 0.0 ± 0.1  | 0.84 |
| f_Erysipelotrichaceae;g_Clostridium| 0.1 ± 0.2  | 0.1 ± 0.2  | 0.84 |
| f_Desulfovibrionaceae;g_Desulfovibrio| 0.1 ± 0.2 | 0.0 ± 0.1  | 0.84 |
| f_Coriobacteriaceae;g_Adlercreutzia| 0.1 ± 0.1  | 0.1 ± 0.1  | 0.84 |
| f_Enterobacteriaceae;g_Citrobacter| 0.1 ± 0.1  | 0.1 ± 0.3  | 0.84 |
| f_Coriobacteriaceae;Other         | 0.1 ± 0.2  | 0.0 ± 0.0  | 0.84 |
| o_Lactobacillales;Other;Other     | 0.1 ± 1.0  | 0.0 ± 0.7  | 0.91 |
| f_Veillonellaceae;g_Megaposphaera | 0.1 ± 0.2  | 0.0 ± 0.2  | 1.00 |
| f_Leuconostocaceae;g_Leuconostoc| 0.1 ± 0.2  | 0.1 ± 0.2  | 0.91 |
| f_Streptococcaceae;g_Lactococcus  | 0.1 ± 0.1  | 0.3 ± 0.8  | 0.84 |
| f_Erysipelotrichaceae;g_Coprobacillus| 0.1 ± 0.1 | 0.0 ± 0.0  | 0.98 |
| f_Clostridiales;g                | 0.0 ± 0.0  | 0.1 ± 0.4  | 0.84 |
| f_Peptococcaceae;g_Peptococcus    | 0.0 ± 0.0  | 0.1 ± 0.3  | 0.21 |

a) One hundred five genera with mean relative abundances over 0.01% in at least one group were compared. The table shows 63 genera having mean relative abundances of over 0.1% in at least one group.

b) q-values indicate adjusted p-values of the Mann-Whitney U test with Benjamini-Hochberg false discovery rate correction.
such as differences in sample processing, DNA extraction, and subsequent processing, it is difficult to compare previously reported results with our results. However, elderly women who lived in Ogimi during the first 3 years of their lives might have more Akkermansia in their guts than Japanese elderly living in other locations.

A recent study showed that the composition of the gut microbiota is different between people living in the same city due to differences in ethnic background affecting their genetics, cultural habits, and early-life environments [24]. We did not investigate the genetic background of our participants; however, their family names indicated that more than 90% of them were Ryukyuan, suggesting that genetic background would not play a part in the differences between the two groups in this study. In particular, A. muciniphila colonizes the human gut within a year of birth [22], suggesting that the area where a person lives in early life contributes to the differences in colonization rate of this bacteria. We concluded that the differences in A. muciniphila colonization rate between groups OG and NOG may have been due to whether the participants lived in Ogimi during the first few years of their lives. We attempted to determine the factors affecting the gut microbiota in our participants, but the living environment, lifestyle, and food in the village have changed in the past 70 to 90 years. Therefore, the reasons behind the high rate of A. muciniphila colonization in the women who lived in Ogimi in early life are unclear.

In a Chinese study, the relative abundance of Akkermansia was significantly lower in a centenarian group than in an 85 to 99 years old group [16]. In contrast, an Italian study and a South Korean study both showed that the abundance of Akkermansia was higher in a centenarian group than in an elderly group [15, 17]. Whether Akkermansia is related to longevity is unclear; however, it is notable that the elderly women who were born in Ogimi have a higher rate of A. muciniphila colonization than other women. A follow-up study is needed to determine whether our participants who had A. muciniphila in their guts live longer.

### DATA AVAILABILITY

16S rRNA gene sequence data have been deposited into the DNA Data Bank of Japan (DDBJ) under the accession no. DRA010457.

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