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Dysbiosis of Salivary Microbiota in Inflammatory Bowel Disease and Its Association With Oral Immunological Biomarkers

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Abstract

Analysis of microbiota in various biological and environmental samples under a variety of conditions has recently become more practical due to remarkable advances in next-generation sequencing. Changes leading to specific biological states including some of the more complex diseases can now be characterized with relative ease. It is known that gut microbiota is involved in the pathogenesis of inflammatory bowel disease (IBD), mainly Crohn's disease and ulcerative colitis, exhibiting symptoms in the gastrointestinal tract. Recent studies also showed increased frequency of oral manifestations among IBD patients, indicating aberrations in the oral microbiota. Based on these observations, we analyzed the composition of salivary microbiota of 35 IBD patients by 454 pyrosequencing of the bacterial 16S rRNA gene and compared it with that of 24 healthy controls (HCs). The results showed that Bacteroidetes was significantly increased with a concurrent decrease in Proteobacteria in the salivary microbiota of IBD patients. The dominant genera, *Streptococcus*, *Prevotella*, *Neisseria*, *Haemophilus*, *Veillonella*, and *Gemella*, were found to largely contribute to dysbiosis (dysbacteriosis) observed in the salivary microbiota of IBD patients. Analysis of immunological biomarkers in the saliva of IBD patients showed elevated levels of many inflammatory cytokines and immunoglobulin A, and a lower lysozyme level. A strong correlation was shown between lysozyme and IL-1β levels and the relative abundance of *Streptococcus*, *Prevotella*, *Haemophilus* and *Veillonella*. Our data demonstrate that dysbiosis of salivary microbiota is associated with inflammatory responses in IBD patients, suggesting that it is possibly linked to dysbiosis of their gut microbiota.

Key words: Crohn's disease; ulcerative colitis; salivary microbiota; 16S rRNA; pyrosequencing

1. Introduction

Current advances of next-generation sequencing technologies (NGS) have enabled us to acquire massive DNA sequence data from any types of samples.[1] In particular, complex bacterial communities composed of numerous species in various environments including human body has become the practically feasible targets, and the analysis has been shifting to the DNA-based approach in conjugation with bioinformatics for enumerated data of metagenome and 16S rRNA gene (16S) produced by NGS.[2–5] Among these approaches, pyrosequencing-based 16S gene analysis is rapid and cost effective to comprehensively evaluate the overall structure of bacterial communities and to identify species present in them, irrespective of the yet-uncultured species.[6] This method includes targeted PCR amplification of 16S rRNA gene variable regions with appropriate primers, followed by sequencing of the 16S amplicons using 454 pyrosequencer.[7–10] We recently developed the improved analytical pipeline for pyrosequencing data of 16S rRNA gene V1–V2 variable region for human gut microbiota, by reassessing a PCR primer sequence, clustering conditions of error-prone 16S reads, and the quality check process to effectively remove low-quality data, and thereby the pipeline provided the high quantitative accuracy to estimation of the bacterial composition and abundance in the community.[10]

In this study, we applied our improved pipeline to the analysis of the human oral microbiota. The oral cavity is a large reservoir of bacteria of >700 species or phylotypes, and is profoundly relevant to host health and disease.[11–14] Current studies reported that various oral symptoms such as aphthous stomatitis, oral ulcer, dry mouth, and pyostomatitis vegetans are frequently observed in inflammatory bowel disease (IBD) patients.[15–20] IBD, including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic, idiopathic, relapsing inflammatory disorder of the gastrointestinal tract.[21,22] The most widely accepted mechanism of IBD pathogenesis includes inflammation due to altered host immune response in association with continuous stimulation from the resident gut microbiota.[23–28] Many studies also revealed that the gut microbiota of IBD patients significantly differed from that of healthy controls (HCs), and is termed dysbiosis.[29–34]

Similarly, oral manifestations observed in IBD patients suggest the association of oral microbiota with such manifestations, yet-limited information exists about the oral microbiota of IBD patients. We characterized the salivary microbiota of IBD patients and HCs by barcoded pyrosequencing analysis of the bacterial 16S rRNA gene. We observed that the salivary microbiota in IBD patients significantly differed from that of HCs, and found particular bacterial species associated with dysbiosis. We also showed that the observed dysbiosis is strongly associated with elevated inflammatory response of several cytokines with depleted lysozyme in the saliva of IBD patients, some of which showed a strong correlation with the relative abundance of certain bacterial species. Thus, the present study demonstrates an association between dysbiosis of the salivary microbiota and change in the host's physiological state in IBD.

2. Material and methods

2.1. *Patients and control groups*

All participants of the CD, UC, and HC groups were informed of the purpose of this study, and written consent was obtained. This project was approved by the ethical committee of University of the Ryukyus. Metadata collected at the time of sampling included various demographics and a medication history for each patient (Supplementary Tables S1 and S2).

2.2. *Sample collection and DNA extraction*

Unstimulated saliva collected from subjects was immediately frozen by liquid nitrogen and stored in −80°C until use. Salivary genomic DNA was prepared according to the literature with minor modifications.[35]

Bacterial cells were harvested from 1 ml of saliva by centrifugation at 3300*g* for 10 min at 4°C. Bacterial pellets were suspended in 10 mM Tris–HCl/10 mM EDTA buffer and incubated with 15 mg/ml lysozyme (Sigma-Aldrich Co. LLC) for 1 h at 37°C. Purified achromopeptidase (Wako Pure Chemical Industries, Ltd.) was added to a final concentration of 2000 units/ml and samples were further incubated for 30 min. Ten percentage of (wt/vol) sodium dodecyl sulphate (SDS) and proteinase K (Merck Japan) were added to the suspension to final concentrations of 1% and 1 mg/ml, respectively, and samples were further incubated at 55°C for 1 h. The lysate was treated with phenol/chloroform/isoamyl alcohol (Life Technologies Japan, Ltd.) and centrifuged at 3300*g* for 10 min. DNA was precipitated by adding 1/10 volume of 3 M sodium acetate (pH 4.5) and 2 volumes of ethanol (Wako Pure Chemical Industries, Ltd.) to the supernatant. DNA was pelleted by centrifugation at 3300*g* for 15 min at 4°C. DNA pellets were rinsed with 75% ethanol, dried and dissolved in 10 mM Tris–HCl/1 mM EDTA (TE) buffer. DNA was further treated with 1 mg/ml RNase A (Wako Pure Chemical Industries, Ltd.) at 37°C for 30 min, and precipitated by adding equal volumes of 20% PEG solution (PEG6000-2.5M NaCl). DNA was pelleted by centrifugation at 8060*g* at 4°C, rinsed twice with 75% ethanol, dried, and dissolved in TE buffer.

2.3. *Bacterial 16S rRNA gene-based analysis*

2.3.1. PCR amplification of the 16S rRNA gene V1–V2 region and barcoded 454 pyrosequencing The hypervariable V1–V2 region of the 16S rRNA gene was amplified by PCR with barcoded 27Fmod and 338R primers.[10] PCR was performed in 50 μl of 1× Ex Taq PCR buffer composed of 10 mM Tris–HCl (pH 8.3), 50 mM KCl, and 1.5 mM $MgCl_2$ in the presence of 250 μM dNTP, 1 U Ex Taq polymerase (Takara Bio, Inc.), forward and reverse primers (0.2 μM) and ~20 ng template DNA. Thermal cycling consisted of initial denaturation at 96°C for 2 min, followed by 25 cycles of denaturation at 96°C for 30 s, annealing at 55°C for 45 s and extension at 72°C for 1 min, and final extension at 72°C on a 9700 PCR system (Life Technologies Japan, Ltd.). Negative controls were treated similarly, except that no template DNA was added to the PCR reactions. PCR products of ~370 bp were visualized by electrophoresis on 2% agarose gels, while negative controls failed to produce visible PCR products and were excluded from further analysis. PCR amplicons were purified by AMPure XP magnetic purification beads (Beckman Coulter, Inc.), and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies Japan, Ltd.). Equal amounts of each PCR amplicon were mixed and then sequenced using either 454 GS FLX Titanium or 454 GS JUNIOR (Roche Applied Science).

2.3.2 Analysis pipeline for 16S data We developed and used an analysis pipeline for pyrosequencing data of the 16S rRNA gene V1–V2 region generated from oral microbiota. Based on sample specific barcodes, reads were assigned to each sample followed by the removal of reads lacking both forward and reverse primer sequences. Data were further denoised by removal of reads with average quality values <25 and possible chimeric sequences. For chimera checking and taxonomy assignment of the 16S rRNA data, we constructed our own databases from three publically available databases: Ribosomal Database Project (RDP) v. 10.27, CORE (http://microbiome.osu.edu/), and a reference genome sequence database obtained from the NCBI FTP site (ftp://ftp.ncbi.nih.gov/genbank/, December 2011). Reads having BLAST match lengths <90% with the representative sequence in the three databases were considered as chimeras and removed. Finally, filter-passed reads were used for further analysis after trimming off both primer sequences.

All of the 16S rRNA sequence data used in this study were deposited in DDBJ/GenBank/EMBL under accession numbers: DRA000984–DRA000986.

2.3.3. Operational taxonomic unit clustering and UniFrac analysis From the filter-passed reads, 3000 high-quality reads/sample were randomly chosen. The total reads (59 × 3000 reads) were then sorted according to average quality value and grouped into operational taxonomic units (OTUs) using UCLUST (http://www.drive5.com/) with a sequence identity threshold of 96%. Taxonomic assignments were made according to the best BLAST-hit phylotype. Weighted and unweighted UniFrac metrics[36] were used to assess the diversity of the salivary microbiota between the CD, UC, and HC groups. UniFrac distances were based on the fraction of branch length shared between two communities within a phylogenetic tree constructed from the 16S rRNA gene sequences from all communities being compared.

2.4. *Immunoassays*

The centrifugal supernatant of unstimulated saliva was analyzed by the Luminex fluorescence technique, using the Bio-Plex Pro Human cytokine 27-Plex Assay (Bio-Rad Laboratories, Inc.) according to the manufacturer's instructions. LL-37 (cathelicidin, hCAP-18) levels were measured by ELISA using the Human LL-37 ELISA Kit (Hycult Biotech, Uden, The Netherlands). IgA levels were measured using the EIA-sIgA Test (MBL, Nagoya, Japan). Salivary lysozyme levels were measured using turbidimetric technique (SRL Inc., Japan). Total protein concentrations were measured by the Bradford protein assay using bovine serum albumin as the standard. In this study, saliva samples of only 15 HC, 14 CD, and 10 UC subjects were used for the assay of biomarkers, because the saliva from the other subjects was insufficient for measurement of all the indicated biomarkers.

2.5. *Statistical analysis*

All statistical analyses were conducted with R version 2.15.2. Microbial richness, evenness, and diversity were assessed using the R Vegan package. Depending on the normality of the data, the Student's *t*-test or Mann-Whitney's U-test was used to perform statistical analysis. *P*-values were corrected for multiple testing using the Benjamini–Hochberg method. Correlations between relative abundance of genera and immunological markers in saliva were calculated by Pearson correlation coefficients.

3. Results

3.1. *Collection of 16S data*

We surveyed the salivary microbiota of 21 CD patients, 14 UC patients, and 24 HCs, all of whom (including their relatives) are residents, lasting at least three generations, of the Okinawa area in Japan. The general and clinical parameters of the study populations are given in Supplementary Table S1, and individual details are shown in Supplementary Table S2.

Sample-assigned pyrosequencing reads having both forward and reverse primer sequences accounted for ~60% of the total number of reads. The 16S reads having average quality values <25 and possibly chimeric sequences represented 0.75 and 0.46% of the selected dataset, respectively. Finally, 506 133 high-quality 16S reads were obtained from 59 salivary samples. Sorting of the 16S reads by average quality value prior to clustering enabled selection of the representative sequence with the highest quality value among the 16S reads grouped in each OTU. On the other hand, the primer check step for removing reads lacking both primer sequences[10] had the possibility to incorrectly remove reads containing V1–V2 regions longer than the maximum length of 431 bp in the filter-passed reads. This is because there are a few species with a V1–V2 region >431 bp (e.g. *Campylobacter rectus* has a length of 493 bp). Our primer check step did not significantly affect the present results because only one of the 177 000 raw reads examined hit to Campylobacter. However, to avoid the incorrect filtration of reads, we modified the primer check step so as not to remove reads having a length of >400 bp, even though they may not have both primer sequences.

3.2. *Overall composition of the salivary bacterial communities*

We evaluated the ecological features of the salivary bacterial communities of the CD, UC, and HC groups by a variety of indices at the OTU level.[37,38] The results are summarized in Table 1. Species richness is the observed number of bacterial species assigned by OTUs detected in the samples. Richness estimates were obtained from the observed number of species by the extrapolation method using estimators such as the Chao1 and ACE indices. Evenness is the degree of homogeneity of abundance of the species detected in the samples. Diversity estimates were obtained from species richness and evenness by using several different indices, which exhibit different sensitivities to given factors, to confirm our results. The results suggested that there were no significant differences in the overall configuration of the salivary microbiota among the three groups (Table 1).

Table 1. OTU-based microbial richness and diversity across the HC, CD and UC groups

	HC	CD	UC
Diversity estimates			
Shannon Index	3.4 ± 0.1	3.4 ± 0.1	3.4 ± 0.1
Simpson Index	0.93 ± 0.01	0.93 ± 0.01	0.94 ± 0.01
Invsimpson Index	16.7 ± 1.1	16.7 ± 1.1	17.1 ± 1.4
Fisher alpha Index	26.8 ± 1.4	26.3 ± 1.4	24.8 ± 1.8
Evenness estimate			
Pielou's Index	0.7 ± 0.01	0.7 ± 0.01	0.71 ± 0.01
Richness estimates			
Number of OTUs	126 ± 5	124 ± 5	118 ± 7
chao1 Index	183 ± 8	183 ± 9	164 ± 13
ACE Index	182 ± 8	177 ± 8	165 ± 11

We then compared the overall bacterial community composition using the UniFrac distance metric, a phylogenetic tree-based metric ranging from 0 (distance between identical communities) to 1 (distance between totally different communities). A principal coordinate analysis (PCoA) plot based on the weighted UniFrac metric revealed clear clustering of most IBD samples apart from the HC samples, indicating the difference in microbial communities between the two groups (Fig. 1A). A bar chart more clearly shows the significant difference in microbiota composition between the IBD and HC groups (Fig. 1B). Comparison of the salivary microbiota of HCs with that of the CD and UC groups indicated that the microbiota of HCs significantly differs from both of them, and no significant difference was found between the UC and CD groups (Fig. 1C). Similar results were obtained using the unweighted UniFrac metric with lower statistical significance than that of the weighted UniFrac metric (Supplementary Fig. S1). These data suggest that species abundance, rather than species diversity, largely contributes to the observed differences in salivary microbiota between the HC and IBD groups.

Although the average age was considerably different between HCs and the IBD patients, weighted UniFrac distance analysis of 10 selected healthy subjects (average age 25.0 yr), 10 IBD patients (average age 28.7 yr, which matched the selected HC group), and the remaining 25 IBD patients (average age 54.6 yr) showed results similar to that of the total samples (Supplementary Fig. S2). Moreover, there was no significant difference between the two IBD subgroups. These data suggest that age might not affect the observed dysbiosis of the salivary microbiota of the IBD patients.

3.3. *Differences in salivary microbiota composition between the HC, CD, and UC groups*

The final dataset of the examined CD, UC, and HC groups ($n = 59$) consisted of 177 000 reads and included representatives of 12 bacterial phyla (Fig. 2; Supplementary Fig. S3 and Table S3). The majority of the 16S reads were classified into only five phyla: Firmicutes (46.5%), Bacteroidetes (22.3%), Actinobacteria (13.7%), Proteobacteria (12.5%), and Fusobacteria (4.2%). TM7, SR1, Spirochaetes, Synergistetes, Tenericutes, and Cyanobacteria were also detected and collectively represented <1% of the total reads analyzed. Analysis at the phylum level showed that the relative abundance of Bacteroidetes was significantly higher in both the CD

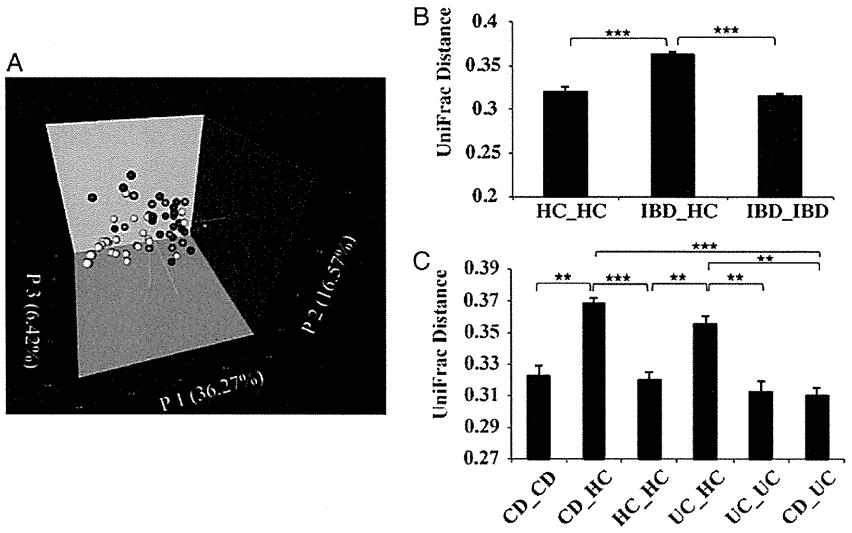


Figure 1. Analysis of the salivary microbiota of the HC, CD, and UC groups based on 16S data. (A) PCoA plot generated using weighted UniFrac metric. The three components explained 59.26% of the variance. White, grey, and black dots indicate HCs, UC, and CD samples, respectively. (B) Weighted UniFrac distance metric (a measure of differences in bacterial community structure) between HCs and the IBD (CD and UC) groups. (C) Weighted UniFrac distance metric between the HC, CD, and UC groups. Student's *t*-test was used; $*P < 0.01$, $**P < 10^{-5}$, and $***P < 10^{-10}$; mean ± S.E.M.

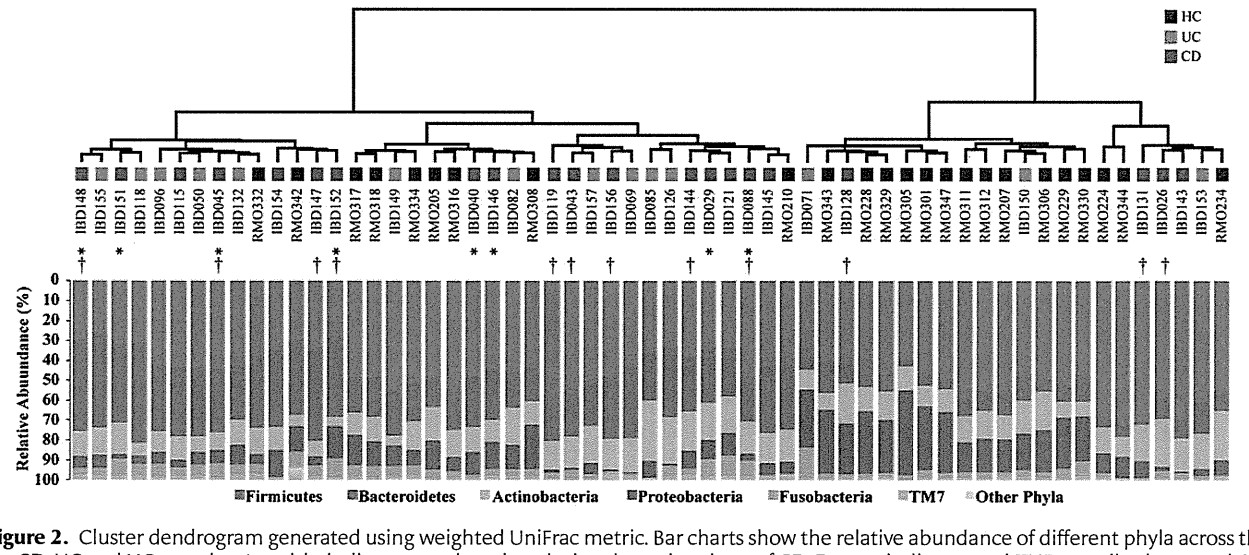


Figure 2. Cluster dendrogram generated using weighted UniFrac metric. Bar charts show the relative abundance of different phyla across the CD, UC and HC samples. Asterisks indicate samples taken during the active phase of CD. Dagger indicates anti-TNF-α antibody treated CD.

and UC groups as compared with HCs ($P < 0.01$), while that of Proteobacteria was significantly lower in both the CD and UC groups as compared with HCs ($P < 0.01$). No significant difference at the phylum level was observed between the UC and CD groups, which was consistent with the results of the UniFrac distance analysis.

In total, 107 bacterial genera were identified (at 95% identity), accounting for 97.8% of the total dataset. The remaining unclassified sequences (2.2%) were assigned to higher level taxa. Fourteen genera, including *Streptococcus*, *Prevotella*, *Rothia*, *Neisseria*, *Granulicatella*, *Actinomyces*, *Haemophilus*, *Veillonella*, *Gemella*, *Leptotrichia*, *Fusobacterium*, *Porphyromonas*, *Uncultured Lachnospiraceae*, and *Oribacterium*, predominated accounting for 92.7% of the total dataset. Other genera represented <0.5% each (Fig. 3; Supplementary Table S3). Two genera, *Prevotella* (phy. Bact.) and *Veillonella* (phy. Firm.), were significantly higher in both the CD and UC groups compared with HCs ($P < 0.01$). Two genera, *Streptococcus* (phy. Firm.) and *Haemophilus* (phy. Prot.), were significantly lower in both the CD and UC groups as compared with HCs ($P < 0.05$ and

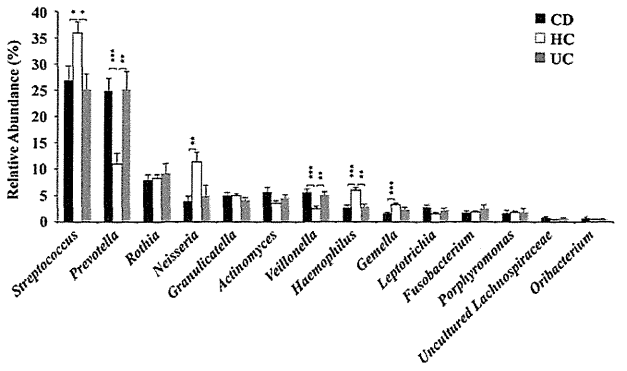


Figure 3. Mean genus abundance in the CD, UC and HC groups. Plotted values are the mean abundance of the 14 most abundant genera in each group. Welch's test with BH adjustment was used; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$; mean ± S.E.M.

0.01, respectively). Two other genera, *Neisseria* (phy. Prot.) and *Gemella* (phy. Firm.), were also found to be significantly lower only in the CD group as compared with HCs ($P < 0.01$ and 0.001, respectively). These results indicate that the relative increase of Bacteroidetes in IBD patients was mainly due to the increase of *Prevotella*, and the relative decrease of Proteobacteria in IBD patients was mainly due to the decrease of *Neisseria* and *Haemophilus*. No significant difference in the relative abundance of either Gram-positive or Gram-negative bacteria was observed among the three groups (Supplementary Table S3).

Clustering of all reads using a 96% pairwise-identity cutoff generated 1257 OTUs, of which only 40 OTUs represented 67.2% of the total reads analyzed. The remaining OTUs were present at relative abundance levels <0.5% of the total dataset (Supplementary Table S4). The relative abundance of several OTUs belonging to the genera *Streptococcus*, *Prevotella*, *Veillonella*, *Nesisseria*, *Haemophilus*, and *Gemella* showed significant differences in IBD patients as compared with HCs. These results were concordant with those detected at the genus level. Among the abundant OTUs, those most closely assigned to *Prevotella melaninogenica*, *Veillonella* sp. oral taxon 158, *Streptococcus mitis*, *Gemella sanguinis*, *Neisseria mucosa*, and *Haemophilus parainfluenzae* showed significant differences in relative abundance between the HC and IBD groups (Supplementary Table S4).

3.4. *Salivary immunological biomarkers in the HC, CD, and UC groups*

We evaluated the inflammatory state, considering its influence on shaping the salivary microbiota, in saliva of the CD and UC patients as compared with that of HCs. The analysis was performed by measuring secretory IgA, cytokines, and enzymes including lysozyme in unstimulated saliva of 15 HC, 14 CD, and 10 UC individuals (Supplementary Table S5 and Fig. S4). There was no significant difference in the total protein concentration in saliva of the CD and UC patients as compared with that of HCs ($P = 0.112$ and 0.192, respectively). The lysozyme level was significantly lower in saliva of both the CD and UC groups as compared with HCs ($P < 0.01$). On the other hand, the levels of IgA and LL37 in both CD and UC groups were higher than that of HCs with statistical significance. The use of Luminex technology was highly sensitive in measuring cytokines from small volumes of saliva samples. In saliva of the CD and UC groups, the level of IL-1β was significantly higher as compared with HCs ($P < 0.05$ and <0.01, respectively). The levels of IL-6, IL-8, and MCP-1 were significantly higher only in saliva of the UC group, while elevated TNF-α level was found only in the CD group with statistical significance. The levels of IgA and MCP-1 in the UC group were significantly higher than those in the CD group. These data indicate that the oral cavity of IBD patients is usually in the inflammatory state, and the levels tend to be slightly higher in the UC group than the CD group.

3.5. *Composition of the salivary microbiota in relation to immunological biomarkers*

We searched for correlations between the relative abundance of dominant bacterial genera and the measured biomarkers in the saliva of 39 subjects (Supplementary Table S5). The results are shown in Fig. 4. The relative abundance of *Streptococcus* negatively correlates with IL-1β and IL-8 ($r = -0.54$ and -0.51, respectively, $P < 0.001$), while it positively correlates with lysozyme ($r = 0.63$, $P < 0.001$). On the other hand, the abundance of *Prevotella* positively correlates with IL-1β ($r = 0.58$, $P < 0.001$) but negatively correlates with lysozyme ($r = -0.54$, $P < 0.01$). The relative abundance of *Veillonella* negatively correlates with lysozyme ($r = -0.54$, $P < 0.001$), while *Haemophilus* positively correlates with lysozyme ($r = 0.58$, $P < 0.001$). Linear regressions also validated correlations between the relative abundance of *Streptococcus* and *Prevotella* and the levels of lysozyme and IL-1β, and between the relative abundance of *Veillonella* and *Haemophilus* and the level of lysozyme (Supplementary Fig. S5). On the whole, *Prevotella*, *Actinomyces*, *Veillonella*, and *Lachnospiracea* tended to positively correlate, while *Streptococcus*, *Rothia*, *Neisseria*, *Haemophilus*, and *Gemella* tended to negatively correlate with elevated cytokines in saliva of IBD patients.

3.6. *Validation of 16S pyrosequencing data by targeted quantitative PCR*

We designed specific PCR primers for quantitative PCR (qPCR) targeting genomes of *P. melaninogenica* and *H. parainfluenzae*, which showed significant differences

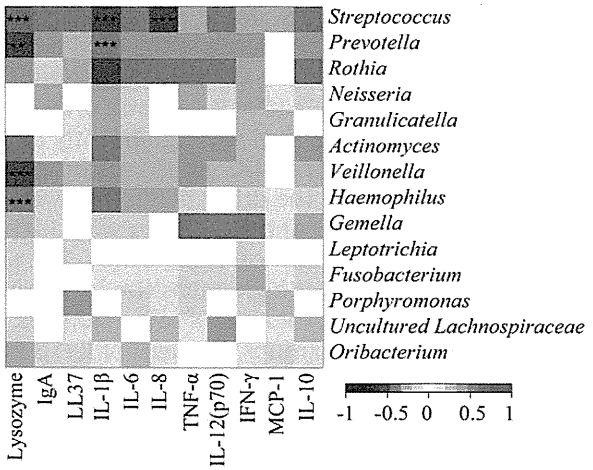


Figure 4. Correlation between the relative abundance of predominant genera and the level of immunological biomarkers in the saliva of IBD patients. Pearson product moment correlation coefficients are represented by colour ranging from blue, negative correlation (−1), to red, positive correlation (1). Normalized values of immunological biomarkers by total protein amount were used in this analysis. Significant correlations after *P*-value adjustment are marked by $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

between HCs and IBD patients by 16S pyrosequencing analysis (Supplementary Table S4). Using these primers, we found strong correlations between 16S-based and qPCR data for the quantification of *P. melaninogenica* ($r = 0.87$, $P < 0.001$) and *H. parainfluenzae* ($r = 0.86$, $P < 0.001$), indicating the quantitative accuracy of our 16S pyrosequencing-based results (Fig. 5).

4. Discussion

4.1. *Bacterial 16S rRNA-based pyrosequencing analysis*

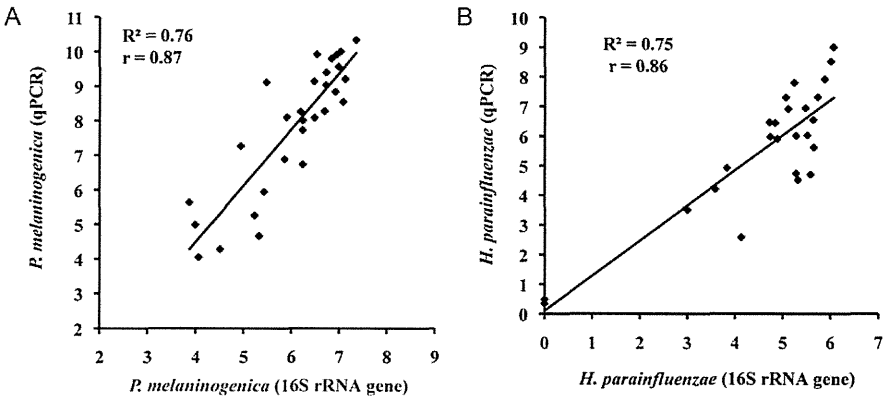
In this study, we used targeted amplicon sequencing of the 16S rRNA gene hypervariable V1–V2 region to evaluate bacterial composition at finer taxonomic levels. The use of primer 27Fmod enabled us to reduce underestimation of the relative abundance of *Bifidobacterium* species that predominate human microbiota, and thus the quantitative accuracy of the overall bacterial composition was greatly improved.[10,39] One limitation of clustering the 16S reads using the UCLUST program is selection of the representative sequence for each OTU. The quality of the representative sequence is not always the highest in the OTU, which affects the BLAST identity, *E*-value and score, sometimes providing inappropriate results for taxonomic assignment of the OTUs. We overcame this limitation by sorting the 16S reads by their average quality values prior to clustering, leading to 16S reads with the highest quality being selected as the representative sequence for each OTU. Our 16S-based results were also validated by strongly correlating with the qPCR data targeting bacterial species showing significant changes between HC and IBD samples (Fig. 5). In addition, clustering of the reads was performed with a 96% pairwise-identity cutoff to reduce overestimation of the number of bacterial species (or OTUs) largely due to 454 pyrosequencing errors.[10,40] Clustering with a 96% pairwise-identity cutoff should be applied for pyrosequencing reads obtained from other types of human microbiota.

4.2. *Salivary microbiota composition in IBD patients*

The abundant bacterial groups in the salivary microbiota detected in this study were similar to those previously reported,[41–44] but the compositions differed from those observed in plaque microbiota.[44] Our data clearly showed a significant difference in salivary microbiota composition between HCs and IBD patients. Shifts in oral microbiota composition were also observed in several oral manifestations such as dental caries,[45] periodontitis,[46] and oral squamous cell carcinoma.[47] Moreover, various components of the oral microbiota have been implicated in systemic diseases such as pancreatic disease including pancreatic cancer,[48] atherosclerosis,[49] bacteremia,[50] and endocarditis.[51]

Altered bacterial community structure in the gut microbiota of IBD patients is a common finding in comparison with that of healthy subjects. Previous studies showed overall structural changes as well as reduced species richness of the gut microbiota in IBD patients.[29–33] It is likely that the high microbial richness and diversity characterizing healthy microbiota may have a protective effect on humans. Unlike the gut microbiota of IBD patients, our estimates using several metrics revealed that microbial richness and diversity in the salivary microbiota of IBD patients was similar to that of HCs, despite significant changes in community structure (Fig. 1). These data suggest that the extent of the changes in the salivary microbiota is less than that in the gut microbiota of IBD patients.

Our data indicated a significant increase of the genus *Prevotella* in the salivary microbiota of IBD patients, in which its relative abundance was almost equivalent to that of reduced *Streptococcus*, which is most abundant in healthy salivary microbiota (Fig. 3). *Prevotella* is a Gram-negative, obligate anaerobe, and a member of the prevalent genera in the human microbiome.[52] Some *Prevotella* species were similarly increased, distinguishable from opportunistic infections, in bacterial vaginosis,[53] esophagitis,[54] antral gastritis,[55] and saliva of caries-active subjects.[45] These data suggest that the increase of *Prevotella*, with concurrently decreased *Streptococcus*, is clearly related with abnormal physiologies in IBD patients. The relative abundance of total Gram-positive and Gram-negative bacteria showed no significant difference between HCs and IBD patients



C. Bacterial genome specific primers for quantitative PCR.

Targeted species	Primer	Sequence (5'-3')	Tm (°C)	Product size (bp)	Annealing temp (°C)
Prevotella melaninogenica	Fw	GCTTTTGGAGCAAACAGAGG	60	234	55
	Rv	GTTGTGAATACGCGGTCCTT	60		
Haemophilus parainfluenzae	Fw	ACCGTGGTCGTTTAGCAATC	60	252	55
	Rv	GTCCGGGTTTACGTTTAGCA	60		

Figure 5. Correlation between the 16S rRNA pyrosequencing and qPCR data. The results are shown in (A) for *P. melaninogenica* and (B) for *H. parainfleuenzae*. The *y*-axis represents the copy number per nanogram of bacterial DNA obtained from qPCR data, transformed by the inverse hyperbolic sine method. The *x*-axis represents the number of reads assigned as bacterial spp. obtained from the pyrosequencing data, transformed by inverse hyperbolic sine method. Pearson product moment correlation coefficient (*r*) on transformed data (using inverse hyperbolic sine transformation) is shown. (C) Primer sequences and PCR conditions used for qPCR experiments are shown.

(Supplementary Table S3). From these results, Gram-stain properties of bacterial surface structures may not be related with dysbiosis of IBD salivary microbiota, unlike the association of Gram-negative oral bacteria with dysbiosis observed in subgingival microbiota in periodontitis.[56]

4.3. *Salivary microbiota associated with immunological biomarkers*

Saliva contains a variety of components such as cytokines, immunoglobulins, and antimicrobial proteins involved in host defence mechanisms for maintaining oral and systemic health.[57] Alteration of the salivary microbiota in IBD patients suggests the occurrence of inflammatory immune responses in the oral cavity of IBD patients as intestinal inflammation associated with aberrant gut microbiota of IBD.[23–26] Our data showed that the levels of many salivary cytokines and IgA were significantly higher in both CD and UC patients than those observed in HCs, indicating that inflammatory responses are elicited in the oral cavity of the patients. Similarly, elevated salivary IL-1β, IL-6, and TNF-α levels in CD patients and an elevated IL-8 level in the saliva of patients with bowel disease were also reported.[58,59] Unexpectedly, the elevated level of inflammatory biomarkers in UC patients was similar to or slightly higher than that observed in CD patients, regardless of differences in disease states between IBD patients (Supplementary Fig. S4 and Table S5). Salivary IgA induction was observed in CD patients with oral symptoms but not in those without oral symptoms.[60] The elevated level of IgA in most IBD patients' saliva examined suggests that those patients may have oral manifestations, however, we did not have access to their oral health clinical records.

Salivary lysozyme levels were significantly reduced in both CD and UC patients as compared with that of HCs. Lysozyme is an antimicrobial protein, expressed by various cells including neutrophils, macrophages, and epithelial cells. It is abundant in saliva and plays an important role in the host constitutive defence system.[61] It has been reported that salivary lysozyme was significantly lower in patients with gingivitis and periodontitis as compared with healthy subjects.[62] In contrast, faecal lysozyme levels were significantly elevated in IBD patients.[63] Further analysis will be required to elucidate the difference in lysozyme levels between saliva and the intestine.

Lysozyme exclusively catalyses hydrolysis of Gram-positive bacterial cell wall. However, lysozyme can also be bactericidal for Gram-negative bacteria *in vivo* through synergistic action with salivary lactoferrin in the normal state.[64] Therefore, this *in vitro* specificity of lysozyme activity may not be largely involved in the dysbiosis of salivary microbiota in IBD patients, in which the abundance of Gram-positive bacteria was not significantly different as compared with HCs (Supplementary Table S3).

There were several subgroups of patients dependent on different medical treatments, and patients with different states of disease (Supplementary Tables S1 and S2). In addition, Infliximab (anti-TNF-α antibody) therapy is commonly used for IBD patients, but up to one-third of the patients have been shown not to respond.[65] Therefore, it was very difficult to precisely evaluate the differences in microbiota structure and biomarker levels between the subgroups. Nevertheless, phylogenetic analysis based on the weighted UniFrac distance metric did not show discrete clustering of particular subgroups, such as CD patients with or without Infliximab treatment and active CD, or CD in remission, suggesting limited contributions from the patients' disease state or medical treatment to the overall microbiota structure (Fig. 2).

Strong correlations between some inflammatory biomarkers and salivary microbiota compositions were revealed (Fig. 4). The lower lysozyme and elevated IL-1β, IL-8, IgA and several other biomarkers were likely to be synergistically or interactively associated with the abundance of the four dominant genera, *Streptococcus*, *Prevotella*, *Veillonella*, and *Haemophilus*. Interactions between these microbes and other species may also be involved in the dysbiosis of salivary microbiota of IBD patients.

Finally, it is still unknown whether the inflammatory state in the oral cavity of IBD patients is the cause or a consequence of imbalances in the salivary microbiota, and which local (the oral cavity) or systemic (the gut) immune response is more responsible for the observed dysbiosis of salivary microbiota. Our results strongly suggest the existence of certain defined mechanisms by which aberrant, but similar, salivary microbiota among IBD patients is formed. The human gut microbiota is gradually shaped to its matured assemblage in a few years after birth, with temporal changes in the diversity and rank of dominant species largely dependent on diet and host physiological state.[66] Salivary microbiota may also be established similar to gut microbiota. Since >1000 ml of saliva is produced per day in the average adult and it always flows into the gastrointestinal tract, bacteria in saliva also have many opportunities to reach the intestine. Therefore, it can be postulated that salivary microbiota affects the development of gut microbiota to some extent. To evaluate this hypothesis, it is necessary to investigate the progression of infant salivary microbiota and the oral inflammatory state. Additionally, further studies such as comparison of the salivary microbiota between IBD and other diseases will provide informative sources for discovering non-invasive salivary biomarkers specific to IBD.

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Supplementary data: Supplementary data are available at www.dnaresearch.oxfordjournals.org.

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ヒト腸内細菌研究の進歩

服部正平*

ヒト腸内細菌叢研究は，近年における細菌叢を網羅的に解析するメタゲノム解析，国際プロジェクトの進展や次世代シークエンサーの進歩などにより，ヒトマイクロバイオーム研究としてこの約10年間で大きく前進した．とくに，健常者の腸内細菌叢の基本的な実態解明やさまざまな疾患患者の腸内細菌叢の異常が明らかになり，また，宿主に作用する機能細菌種が同定されるなど，これまでの想像を超えて，腸内細菌叢が腸管にとどまらず宿主の免疫系，代謝系，あるいは脳の機能などの全身に影響することが明らかとなってきた．さらには，糞便そのものが治療に用いられようとしている．

はじめに

わが国の腸内細菌研究は，光岡知足博士らによる腸内細菌の系統的な分離培養が進められた1960年代にはじまる．1980年代には，細菌の16Sリボソーム RNA(16S)遺伝子を指標とした培養を介さない菌種の特定や菌種組成の解析が可能となった．このほか，無菌マウスなどに分離菌や特定の細菌叢を投与したノトバイオート動物を用いた機能研究が進められた．一方，分離株を凌駕する細菌種が16S解析で検出され，これらの大部分は未知の「難培養性細菌」として今日まで手つかずのままとなった．しかし，細菌叢の構成細菌種の集合ゲノムである「メタゲノム」の遺伝子群を枚挙するメタゲノム解析法が2004年に発表され，難培養性細菌も含めた細菌叢全体の代謝系などの機能に関する研究が可能となった．さらに，近年のシークエンス技術の進歩とヒト常在菌叢メタゲノム(ヒトマイクロバイオーム)研究の国際コンソーシアムの立ち上げにより，腸内細菌(叢)研究は世界的にもアトラクティブなテーマへと大きく変貌した．本稿では，この約10年間における腸内細菌叢とその関連研究について解説する．

〔キーワード〕
マイクロバイオーム
メタゲノム
腸内細菌
16S
次世代シークエンサー

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1．ヒト腸内細菌叢研究の推移

この約10年間におけるヒト腸内細菌叢研究に関する論文数の推移を**図1**に，主な論文やできごとを**表1**に示す．わが国では，2005年に筆者らが中心となったヒト腸内細菌叢をメタゲノム解析する Human MetaGenome Consortium Japan (HMGJ)が発足し，同年パリでヒトマイクロバイオーム研究の最初の国際会議が開催された．そして，2006年に米国グループが，2007年にHMGJがヒト腸内細菌叢のメタゲノム解析を世界に先駆けて論文発表した[1)2)]．これらの論文では，腸内細菌叢がヒト代謝系を補完する多くの代謝系を有することやヒト腸内細菌叢に特徴的な機能遺伝子の

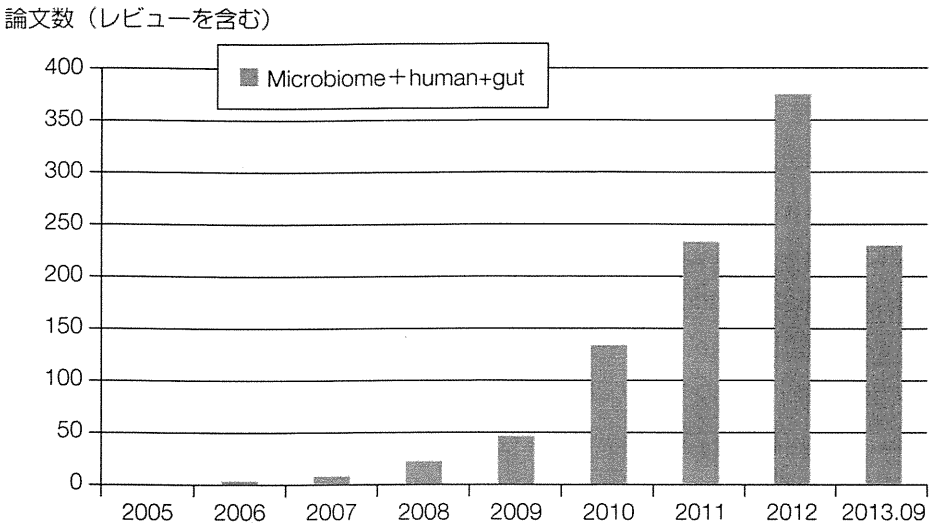

図 1. ヒト腸内細菌(叢)研究に関する年間論文数の推移
PubMed で Microbiome＋human＋gut のキーワードをそれぞれ検索した結果を示す(2013 年 9 月現在).

表 1. 腸内細菌(叢)研究の主要な論文と国際動向

年	論文または動向	文献
2005	日本 HMGJ の発足 ヒトマイクロバイオーム研究の国際会議開催	
2006	アメリカ人(3 名)の腸内細菌叢メタゲノム解析	1
2007	日本人(13 名)の腸内細菌叢メタゲノム解析 炎症性腸疾患の腸内細菌叢の 16S 遺伝子解析	2 17
2008	欧州 MetaHIT プロジェクトと米国 HMP の始動 国際コンソーシアム IHMC の発足	
2009	ヒト腸内細菌叢と肥満の関係解明 セグメント細菌による Th17 細胞の誘導(マウス)	14 31
2010	皮膚細菌叢による法医学での個人識別の可能性 *Bacteroides fragilis*(PSA)による T_{reg}細胞の分化誘導 欧州人(124 名)腸内細菌叢のメタゲノム解析	12 30 4
2011	*Bifidobacterium* による大腸菌 O157 感染死の防御機構の解明 ヒト腸内細菌叢エンテロタイプの発見	29 13
2012	中国人(345 名)のメタゲノムから 2 型糖尿病のマーカーの探索 米国 HMP：139 名アメリカ人(18 部位)常在菌叢のメタゲノム解析 アイルランド高齢者(178 名)腸内細菌叢のメタゲノム解析 大腸がんに相関する *Fusobacterium* の同定	6 5 7 18, 19
2013	T_{reg}を誘導するヒト *Clostridium* 菌の発見 糖尿病を含むスウェーデン人(145 名)腸内細菌叢のメタゲノム解析 メタゲノムから肥満のマーカーの探索 ロシア人(96 名)腸内細菌叢メタゲノム解析 腸内細菌叢と肝臓がんの関係解明 健常者糞便を用いた感染症治療の可能性	32 8 16 9 24 34

特定などがおこなわれた．2008年には日米欧中などからなるInternational Human Microbiome Consortium(IHMC)が設立された[3]，それと同時に，米国National Institutes of Health(NIH)のHuman Microbiome Project(HMP)，フランスを中心とした欧州連合(EU)＋中国Beijing Genomics Institute(BGI)のMetagenomics of the Human Intestinal Tract(MetaHIT)Projectが開始された．HMPは口腔や皮膚，腸内などの全身の常在菌叢をターゲットにし，MetaHITは腸内細菌叢に特化したプロジェクトである．

この世界的なマイクロバイオーム研究の始動後，関連論文は毎年急増し，2012年には2008年時の約10倍の論文が発表され，この5年間での総論文数は1,000を超えている．その後，次世代シークエンサー(next generation sequencers：NGS)の普及もあり，大量データの収集がNGS解析を通して加速的に進められている．2010年にはMetaHITが124名の炎症性腸疾患(inflammatory bowel disease：IBD)を含む欧州人(スペイン人＋デンマーク人)の腸内細菌叢メタゲノム解析結果を発表した[4]．2012年にはHMPによる139名のアメリカ人の腸内や皮膚，口腔などの18部位の常在菌叢[5]，MetaHITによる糖尿病を含む345名の中国人[6]，178名の平均78歳のアイルランド人[7]の腸内細菌叢がそれぞれ論文となった．さらに，2013年には糖尿病を含む145名のスウェーデン人[8]と96名のロシア人[9]の腸内細菌叢が論文となった．現在，トータルで1,000人以上の腸内細菌叢データが蓄積されている(**表1**)．筆者らも100名以上の日本人腸内細菌叢メタゲノムデータの解析を進めている[未発表]．

2．ヒト腸内細菌叢の解析概略

上述した大型プロジェクトでは多数の被験者のメタゲノムと16Sデータを用いて，ヒトマイクロバイオームの実態(細菌と遺伝子組成など)の基本的特徴の解明，それらと人種や食事，年齢などの各メタデータとの相関解析がおこなわれている．また，IBDなどの疾患患者の細菌叢データも収集し，健常者データとの比較による疾患細菌叢の特徴解明も進められている．

NGSを用いたヒト腸内細菌叢の解析戦略の全体像を**図2**に示す．基本はメタゲノムとメタ16Sデータの収集と解析である．また，解析データの解釈に必要な被験者の年齢や既往症，日頃の食事内容などのメタデータも収集する．分離株の個別ゲノムデータは，得られたメタゲノムやメタ16Sデータの正確な菌種帰属や菌種組成の定量解析にきわめて有効であり，3,000株以上が現在収集されている(http://www.hmpdacc.org/)．

メタゲノム解析で枚挙された遺伝子群は細菌叢の代謝系や機能プロファイルの解明に有効である．また，上述したように，メタゲノムリードのリファレンスゲノムへのマッピングは高い定量性をもった菌種特定と菌種組成の解析法でもある．異なった複数の細菌叢または被験者集団のマッピング解析を主成分分析(principal component analysis：PCA)すればそれらの相違を知ることができる．現在，70%程度のデータがリファレンスゲノムにヒットする．少なくとも90%はヒットするゲノムデータベースになるまで個別ゲノムデータの収集は今後も進められるであろう．

メタ16S解析はoperational taxonomic unit(OTU)をベースにした菌種特定と菌種組成の解析ならびにUniFrac-主座標分析による菌叢間の相違を調べる方法である[10]．メタゲノム解析よりもサンプルあたりのコストが1/10程度と安価であるので多サンプル処理も容易で，また，皮膚細菌叢のような微量の細菌叢DNAにも対応できるという利点をもつ．しかし，polymerase chain reaction(PCR)増幅がそのプロセスにあるため，メタゲノム解析にくらべ定量性に問題があった．最近，筆者らはPCRプライマーの改良やNGSのシークエンスエラーを考慮した従来よりも高い定量性をもった腸内細菌叢のメタ16S解析法を開

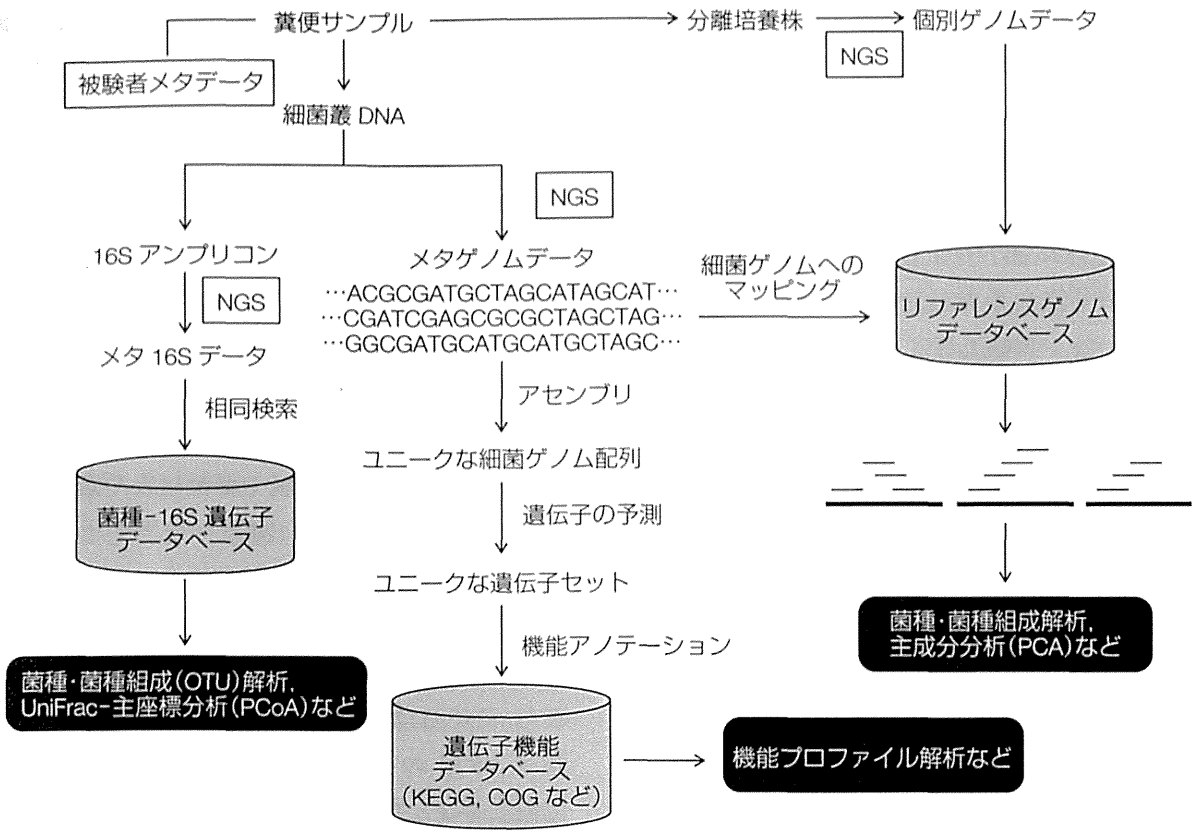

図 2. NGS を用いた腸内細菌叢解析法の概略
KEGG：Kyoto Encyclopedia of Genes and Genomes，COG：Clusters of Orthologous Groups

発した[11]．なお，マウスなどの他の動物細菌叢ではリファレンスゲノムが充実していないため，メタ 16S 解析が最も有効な菌種解析法である．

3．ヒト腸内細菌叢の全体像

ヒト常在菌叢の少なくとも 95%は 4 つの門(Firmicutes，Bacteroidetes，Actinobacteria，Proteobacteria)の菌種で占められている．これら菌種の相対組成は個人間や棲息部位ごとで大きく異なる[5]．Firmicutes 門は腸内の最優占菌種であり，Actinobacteria 門や Proteobacteria 門は口腔や皮膚，鼻腔でその組成比が高くなる．マイナー菌種である Fusobacteria 門は口腔細菌叢で相対的に多くなる．TM7 門のような口腔内でしか検出できない菌種もいる．個人間の多様性はこれら菌種の有無や組成比の違いに起因する．たとえば，124 名の欧州人の腸内細菌叢メタゲノム解析では，検出された少なくとも 1,000 種(種レベル)の細菌種のうち，124 名全員に共通した菌種はわずか 18 菌種であった[4]．よって，常在菌叢にはヒトゲノムのような血縁同士の高い遺伝性はほとんどなく，きわめて個人に特異的である．この特徴を利用して皮膚細菌叢プロファイルを法医学分野での個人識別に使う研究もある[12]．一方，ヒト腸内細菌叢は大きく 3 つのタイプ(エンテロタイプ)に分類できる[13]．*Bacteroides*(Type 1)，*Prevotella*(Type 2)，*Ruminococcus*(Type 3)の 3 属がそれぞれのタイプで優占菌種となる特徴をもつ．エンテロタイプは人種や地域に関係なく，一様に分布していると考えられているが，最近の筆者らの解析から，日本人の多くは Type 3 であり，欧米人の多くは Type 1 であることがわかった．なお，Type 2 はベネズエラやアフリカの原住民に多いタイプである．エンテロタイプは人種(？)，

食習慣(？)，地域(？)によって，その分布状態が偏っているらしい[未発表]．

腸内細菌叢の遺伝子数については，約50万/個人で，欧米の全データからは約500万のユニークな遺伝子が見つかっている[5]．これはヒト遺伝子数(約2.2万)の200倍以上であり，腸内細菌叢がヒトよりはるかに多様な遺伝子をもっていることを示している．興味あることに，腸内細菌叢の菌種組成は各個人間で大きく異なるが(上述)，遺伝子(機能)組成はほとんど同じである[14]．この事実は，菌種よりも，その菌種が有する遺伝子の機能が腸内細菌叢形成に大きく影響することを示唆している．上述した4門の優占菌種は，とくに人体での棲息に適した遺伝子(機能)を獲得し，長い進化の中で選択されてきたものと考えられる．

ヒト腸内細菌叢を特徴づける遺伝子(機能)は，豊富な炭水化物代謝系の機能群である[2]．腸内細菌の主なエネルギー源は，宿主が消化できずに腸内へたどり着く食事由来の多糖類であり，腸内細菌はこれらを代謝することでエネルギーを得ている．また，その代謝産物は酢酸や酪酸，ビタミンなどのヒト細胞に有用なものである．つまり，ヒトと腸内細菌は相互扶助的な関係にある．もうひとつの特徴は，腸内細菌叢には鞭毛や化学走性などの運動にかかわる遺伝子群がきわめて少ないことである[2]．腸内ではその蠕動運動のために自ら餌に向かって移動する必要がなく，宿主免疫のターゲットとなる鞭毛をもつ多くの病原菌との識別など，これらをもたない細菌種の選択と優占化は，宿主と腸内細菌の両方にとって不利な炎症反応を最小限にする方向での進化があるようだ．

4．ヒト腸内細菌叢と疾患

ヒト腸内細菌(叢)はさまざまな疾患と密接に関係する(**表2**)．これらの疾患のうち，ヒトにおいてその関連が明確なのは肥満[14)~16)]，IBD[17)]，糖尿病[6)]，大腸がん[18)19)]，自閉症[20)]，アテローム性動脈硬化症[21)]などである．その根拠として，これら患者の腸内細菌叢の異常，病巣などに特異的な細菌種が高頻度に検出されることなどが挙げられる．自閉症と関連して腸内細菌が脳機能に及ぼす影響を示したマウスの研究もある[22)]．なお，多発性硬化症[23)]，肥満と関連した肝臓がん[24)]，メタボリック症候群[25)]などはモデルマウスのデータから示唆されている．このほか，疾患ではないが，乳児・幼児の成長期や女性の妊娠時では腸内細菌叢が大きく変動する[26)27)]．これらのデータは，腸内細菌叢がこれまでの想像をはるかに超えて，宿主の生理状態への影響が全身的で多岐にわたることを強く示唆するものである(**図3**)．まさしく，ヒトはLederberg博士のいう「超有機体」である[28)]．

疾患細菌叢の研究では，疾患マーカーの探索もひとつの目的である．たとえば，BMI>30(肥満)とBMI<25(健康)の被験者群間で有意に増減する9菌種のreceiver operating characteristic(ROC)解析でのベストarea under ROC curve(AUC)値が0.78となった．この値はヒトゲノムの32の肥満関連遺伝子座でのAUC値(0.58)よりも高く，ヒト遺伝子よりも高い予測能・診断能があることを示す[16)]．

上述したように，疾患患者群の細菌叢の多くはその菌種組成や菌種数が健常者群と有意に異なる細菌叢異常(dysbiosis)を示す．たとえば，IBDの腸内細菌叢では*Firmicutes*と*Bacteroidetes*が健常者群にくらべて有意に減少し，*Proteobacteria*が相対的に増加する．また，菌種数も有意に減少する(多様性の減少)．肥満では*Firmicutes*が増加し，*Bacteroidetes*が相対的に減少する．これらの細菌叢異常と免疫系などの宿主の生理状態の変化はたがいに影響しあうようだ．遺伝子欠損でメタボリック症候群になったマウスの腸内細菌叢はdysbiosisを起こす．ところが，このdysbiosisした腸内細菌叢を遺伝的に健全な無菌マウスに移植すると，そのマウスもメタボリック症候群となった[25)]．同様のことが肥満マウスなどでも観察されている[14)]．

表 2. 常在菌と関連する疾患例

肥満
メタボリック症候群
炎症性腸疾患(IBD)
過敏性大腸炎
アテローム性動脈硬化症
関節リウマチ
糖尿病(1型，2型)
アレルギー
喘息
セリアック病
大腸がん
肝臓がん
湿疹
歯周病
多発性硬化症
自閉症

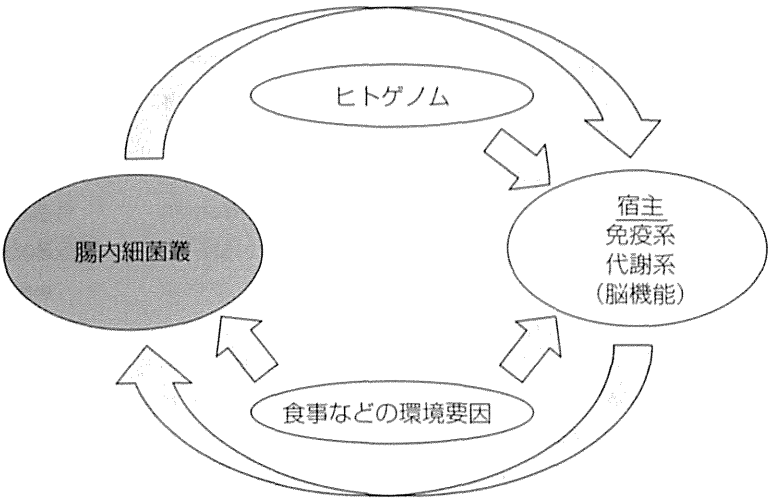

図 3. 腸内細菌叢，ヒトゲノム，宿主，環境要因の関係
腸内細菌叢と宿主の生理機能はたがいに影響し合う．宿主の生理機能はヒトゲノム(遺伝要因)とその他環境要因の影響を受ける．腸内細菌叢は食習慣などのその他環境要因の影響を受ける．

5．腸内細菌の機能と宿主への作用機構

宿主に作用する特定の細菌種の実験的探索や宿主—細菌間相互作用のメカニズムの解明に関する研究も活発である．これらの研究には宿主側の細胞や遺伝子発現などのさまざまな実験データの取得が可能なマウスを利用している．宿主の遺伝子発現や腸管内代謝物などの各種オーミクスデータをベースにした *Bifidobacterium* による大腸菌O157感染死の防御機構の解明[29]，大腸がんと関連するヒト *Fusobacterium* の特定[18][19]，T細胞の分化を誘導するヒト *Bacteroides*[30]，マウスのセグメント細菌(segmented filamentous bacteria：SFB)[31]，ヒト *Clostridium*[32]の同定などがある．上述のメタゲノムからの疾患関連の菌種や遺伝子の探索はデータ駆動型のトップダウン戦略であるのに対して，これらの研究はボトムアップ戦略である．同定された機能細菌(とその遺伝子や代謝物など)は，疾患の発症メカニズムの解明やその寛解・治療法の開発につながると期待される．また，これらの情報はメタゲノムデータをより生物学的に解釈する上で貴重である．

おわりに

健常者の糞便そのものを治療に使用する研究が話題となっている[33]．従来の治療法ではほとんど完治しない感染症に健常者の新鮮な糞便を投与したところ治癒率が格段に向上した[34]．この治療では，抗生物質の投与と腸管洗浄，糞便の腸注をくり返し，患者の dysbiosis した腸内細菌叢を健常者の細菌叢に総替えしたのである．しかし，健常者の糞便だといっても，その安全性やまれな病原菌やウイルスの存在がわからないため，健常者から分離した既知細菌種の培養混合物からなる人工細菌叢の作成も試行されている[35]．一方，移植した細菌叢が治療後どれだけ長く安定的に患者腸内に定着するかは不明である．しかし，ヒトゲノムは一生不動であるが，マイクロバイオームは理論的にリセット可能である．Dysbiosis した疾患細菌叢が疾患を発症するのが事実なら，健康な細菌叢が宿主を健康にするのも理屈である．

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