論文の内容の要旨

論文題目　A study on human gut microbiota using improved methods of 16S ribosomal RNA gene analysis　(改良した 16S リボソーム RNA 遺伝子解析法を用いたヒト腸内細菌叢に関する研究)

氏　　名　　金　錫元

It is known that there are about 10^{14} indigenous bacteria in various sites of human body, and the gastrointestinal tract is the major habitat of gut microbes. 16S ribosomal RNA (rRNA) gene is an essential gene in prokaryote and has nine hypervariable regions, which are used as a phylogeny marker. Recently, next-generation sequencing (NGS)-based 16S rRNA gene analysis became more popular due to culture-independent high-throughput approach to comprehensively evaluate the overall structure and change in relative abundance of species at the operational taxonomic unit (OTU) level. Based on these backgrounds, I performed three studies in this dissertation.

In the first study, I constructed genomic-based 16S ribosomal database called GRD. GRD is the highly curated 16S rRNA gene database, and was constructed from full-length 16S rRNA genes in sequenced bacterial genomes using various informatics tools and by my own curated editing. From this database, I revealed that the quality of annotation by GRD was higher than that by GenBank, and GRD was composed of high-quality 16S rRNA genes as compared with other three publically available databases. Moreover, I could know that the average 16S copy number was 3.58 in prokaryote. In addition, I estimated 16S rRNA sequence similarity that determined the boundary between each taxonomic level by using GRD. In this result, I found boundary identity of 16S rRNA genes between each taxonomic level, which is useful and reliable for 16S-based taxonomical assignment of species.

In the second study, I developed an analytical pipeline for 454 pyrosequencing data of 16S rRNA gene V1-V2 region, by which the quantitative accuracy in 16S-based bacterial composition analysis was greatly improved. First, I estimated the error rate of 454 pyrosequencing data to be ~0.6% from 16S rRNA sequence data obtained from two artificial bacterial communities, Mock 1 and Mock 2, prepared by mixing

genomic DNAs of known bacteria with appropriate ratio. Second, I found that the error in the 454 pyrosequencing data was the primary cause of overestimation of species richness based on the number of OTUs generated from clustering of 16S rRNA sequence reads. This overestimation was improved by using clustering with a 96% identity cutoff instead of the conventional 97% identity cutoff. Finally, I developed the modified primer (27Fmod) used for PCR amplification of V1-V2 region in 16S rRNA genes, which provided higher quantitative accuracy than the conventional primer 27F for the analysis of the bacterial composition in human gut microbiota.

In the third study, I analyzed the overall structure of gut microbiota of healthy adults in response to probiotic intervention by using GRD, UniFrac distance metrics, and the improved analytical pipeline for 454 pyrosequencing data of 16S rRNA V1-V2 region. I obtained 158 faecal samples from 18 healthy adult Japanese who were subjected to intervention with 6 commercially available probiotics containing either Bifidobacterium or Lactobacillus strains. I then analyzed and compared bacterial composition of the faecal samples collected before, during, and after probiotic intervention by OTUs and UniFrac distances. The results showed no significant changes in the overall structure of gut microbiota in the samples with and without probiotic administration regardless of groups and types of the probiotics used. I noticed that 32 OTUs (2.7% of all analyzed OTUs) assigned to the indigenous species showed a significant increase or decrease of ≥10-fold or a quantity difference in >150 reads on probiotic administration. Such OTUs were found to be individual specific and tend to be unevenly distributed in the subjects. This study using large datasets enabled us to evaluate the effect of probiotics on gut microbiota of healthy adults more comprehensively and precisely than the previous probiotic intervention researches in which the analysis exclusively focused on only several limited bacterial species by using conventional methods. This data further supports the high inter-subject variability and the high intra-subject stability that is the current common view for the feature of adult gut microbiota.