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# Characterization of the fecal microbiome in different swine groups by high-throughput sequencing

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ABSTRACT

Swine have a complex microbial community within their gastrointestinal tract that plays a critical role in both health and disease. High-throughput 16S rRNA gene-based pyrosequencing was used to identify the possible core microorganisms in the gut of swine groups that differ in meat quality and weight grades (level 1 as higher meat quality and level 2 as lower meat quality). Samples were taken from the rectum and/or stool from ten animals, DNA was extracted, and the V1–V3 regions of the 16S rRNA gene were amplified. Two bacterial populations (*Bacteroidetes* and *Firmicutes*) dominated and were shared between the two groups. Significant differences between the groups were found at the genus level. The genera *Lactobacillus* and *Oscillibacter* were found in slightly higher proportions in the level 2 group (12.6 and 12.4% of the classified reads, respectively) than those of level 1 (9.6 and 7.7%, respectively). By contrast, the proportion of reads assigned to the genus *Roseburia* in the level 1 group (13.0%) was higher than that of level 2 (4.8%). The largest differences were related to the genera *Clostridium*, *Oscillibacter,* and *Roseburia* as core microorganisms. Moreover, two genera, *Roseburia* and *Clostridium*, related to level 1 produced linoleic acid or short chain fatty acids that might contribute to swine health and development. In conclusion, the presence of core bacteria in the swine gut is associated with meat quality with reduced body fat in swine.



## 1. Introduction

The human gut microbiome, which is a complex consortium of 100 trillion ($10^{14}$) microbial cells in the gastrointestinal tract, influences many aspects of life, including metabolic capabilities, protection against pathogens, and the immune system, as a secondary human organ. This complex universe contains bacteria, eukaryotes, viruses, and one archaeon (i.e., methanogen) that interact with the host [1,2].

The animal gut microbiome is also composed of a diverse complex of microorganisms, influencing many aspects of its hosts. Uncovering the taxonomic composition and functional capacity of animal gut microbial consortia is important to identify their roles in host physiology and health [3]. Due to the impact on physiological, nutritional, and immunological processes, the microbial community of the swine gut markedly influences health and performance [4]. The structure and functional role of gut microorganisms have been a significant research focus for decades, although most studies were restricted by culture-based techniques. However, 16S rRNA gene sequence analysis has shed light on the diversity and structure of microbial communities in several animal gut environments due to the development of next-generation sequencing techniques (e.g., massively parallel 454 pyrosequencing) [5–7]. In addition, fosmid-clone-based microarray was developed to analyze the gut microbiome of swine and to compare the gut microbiomes between a healthy and a runt group [8].

The immune responses of the intestinal mucosa are affected by various factors related to the large numbers of commensal microorganisms and their products. These responses regulate and protect the host from both pathogenic and commensal bacteria (non-pathogenic). Changes (i.e., dysbiosis) in this homeostasis may influence susceptibility to or progression of chronic or acute

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inflammatory conditions in the intestine [9,10]. Therefore, the aim of this study was to compare the microbial communities and the potential influence of microorganisms on swine development and/or feeding from two different groups. This information could be fundamental to the development of a diagnostic kit to increase farming productivity.

## 2. Materials and methods

### 2.1. Fecal sample collection and pyrosequencing

Fecal samples were collected from 5–6-month old Yorkshire-Landrace-Duroc crossbred pigs from a livestock farmhouse located in South Korea, which had bred more than 1000 head of swine at the time of collection. Swine were weaned at 3–4 weeks after birth and fed a commercial maize-soybean (WIPig, NongHyup Feed Co., Ltd, South Korea) based diet *ad libitum*. The studied swine were slaughtered at Samkook slaughterhouse (certified by HACCP from Korea Livestock Products HACCP Accreditation Service) in June 2012. Swabbed fecal samples from slaughtered swine rectums were immediately placed into sterile plastic tubes using alcohol-sterilized spatulas. The collected samples were frozen with dry ice on site and carried to the lab and stored at −80 °C until analysis. Fecal DNA was extracted with the Ultra Clean® Fecal DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. Total DNA was quantified using a NanoDrop® ND-1000 UV spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Amplification of fecal DNA was performed using a barcode-tagged primer set for pyrosequencing of the bacterial 16S rRNA gene. This primer set targeted the V1 and V3 hyper-variable regions of the 16S rRNA genes using the 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 338R (5′-TGCTGCCTCCCGTAGGAGT-3′) primer set [11,12]. The polymerase chain reaction (PCR) was performed using 10 μL 2× PCR Master Mix Solution (Intron, Republic of Korea), 1 μM of each primer (final concentration), and *ca.* 10 ng of fecal DNA as the template. Water was added to make a final volume of 20 μL. The following PCR cycles were used: initial denaturation at 94 °C for 5 min, 30 cycles at 94 °C for 50 s, 55 °C for 30 s, and 72 °C for 50 s, and a final extension at 72 °C for 6 min. The amplification products from each sample were purified using a Gel and PCR Clean-up Kit (LaboPass, Cosmogenetech, Republic of Korea). DNA was quantified using a spectrophotometer and was then mixed in equivalent proportions. Sequencing was performed using an FLX Titanium Genome Sequencer (454 Life Sciences, Branford, CT) by a sequencing provider (Chunlab, Republic of Korea) according to the manufacturer's instructions.

### 2.2. Pyrosequencing data analysis

To increase the pyrosequencing result quality, raw reads were processed to remove low-quality sequences according to the following criteria: 1) short reads (<150 bp) and 2) reads longer than the expected PCR product size [13]. CLUSTALW was used to align the DNA sequences. After alignment, the sequences were trimmed to remove non-overlapping ends. Bacterial sequence reads were compared with a reference database of known 16S rRNA genes, which was obtained from the Ribosomal Database Project (RDP) databases, and was assigned taxonomically based on the RDP classifiers [14].

Rarefaction curves, Shannon index, Good's coverage, and the Chao1 nonparametric richness estimators were determined using the Mothur package [15]. The sequences including chimeric sequences that were unassigned and/or related to non-bacteria such as chloroplasts and mitochondria were removed to increase analysis quality. Before comparing the diversity calculations, library size was normalized to the size of the smallest library. Calculations were repeated 100 times using random sub-samples of sequences. A 3% dissimilarity level between sequences was used to calculate the diversity estimators. The microbial community structures in different samples were compared using Fast-UniFrac [16] based on the phylogenetic relationships among representative reads (operational taxonomic units [OTUs]) from different samples. Trees were constructed using the FastTree program [17]. The Fast-UniFrac sample clustering results were used to compare the hierarchical relationships among the samples [16]. The Mann–Whitney *U*-test [18] was performed to compare diversity indices between the two groups. Unless otherwise stated, in this study, we calculated the proportion of total reads that are represented by each taxonomic group.

## 3. Results

### 3.1. Clustering of the swine rectum samples according to microbiome

We used a pyrosequencing approach to better understand and identify the specific microbes in the swine fecal microbial community. Overall, ten fecal samples were immediately collected from ten rectums of the two different groups assigned to “level 1” (designed as lv1) and “level 2” (designed as lv2) after slaughter. A total of 86,305 sequences were used for abundance and diversity analyses, as well as taxonomic comparison. Detailed descriptions of the fecal samples can be found in Table 1.

The bacterial communities of the swine rectum were clearly divided into two groups based on principal components analysis (PCA) and the unweighted pair group method with arithmetic mean (UPGMA) from the Unifrac and Mothur analyses (Fig. 1), with the exception of In35 and In46 swine; the level 1 group was composed of In34, In30, In29, and In24 and the level 2 group was composed of In78, In53, In43, and In55. Pairwise comparisons of bacterial community similarity distances revealed some interesting findings. For example, the bacterial community structures of level 1 In24 (43.2–69.4% similarity) and level 2 In55 (50.7–75.4% similarity) samples showed a high level of distance to other samples in the same level (Fig. 1(b) and Supplementary Fig. 1).

The detailed diversity estimates of the swine rectal microbiome can be found in Table 1. We compared diversity indices from the qualified sequence reads and subsample reads. Diversity in the level 2 swine group was higher than diversity in the other group (Table 1 and Fig. 2) based on the number of OTUs and estimated OTUs from the Chao method and Shannon indices. Particularly, the OTUs and the estimated OTUs from the Chao method were significantly different ($P < 0.05$) (Fig. 2(a) and (b)). Additionally, rarefaction curves of the observed number of phylotypes (OTUs) from the fecal microbiome of the swine rectum did not appear to approach a horizontal asymptote, indicating that the current sequencing effort did not saturate diversity (Supplementary Fig. 2). Nevertheless, the UPGMA tree, diversity indices, and PCA showed that the level 1 group could be clearly distinguished from the level 2 group in terms of bacterial composition at the level of the OTU and genus (Fig. 1(a) and Supplementary Fig. S3).

### 3.2. Comparison of bacterial composition of the swine rectum between levels 1 and 2

The swine fecal microbiomes of the two groups were dominated by the phyla *Bacteriodetes* and *Firmicutes* (Supplementary Fig. S4), as previously shown in several microbial diversity studies of the mammalian gut [19], and were consistent with microbial diversity