

단 보

Differences in swine gut microbiota in southern region of Republic of Korea

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한국 남부 지역별 돼지 장내 미생물생태 비교분석

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ABSTRACT: Since the banning of antibiotic growth promoters (AGPs), the death of livestock has been increased, thus there is a strong demand for AGP-alternatives. Modulation of gut microbiota has been reported to affect host physiological functions and suggested to be a novel approach for developing AGP-alternatives. However, little has been understood about livestock gut microbiota compared to that of humans. We conducted preliminary study provide fundamental information regarding to regional differences in swine gut microbiota. Swine fecal samples were obtained from farms in Jeju (n=40), Gwangju (n=28), and Haenam (n=30). MiSeq was used to sequence 16S rRNA V4 region, and Mothur pipeline [Schloss *et al.*, 2009] was used for data processing. A total of 5,642,125 reads were obtained and 3,868,143 reads were remained after removing erroneous reads. Analysis of taxonomic composition at the phylum level indicated greater abundance of Firmicutes among Jeju swine, and cluster analysis of distribution of operational taxonomic units also showed regional differences among swine gut microbiota. In addition, correlation analysis between non-metric multidimensional scaling and abundance of phyla suggested that the phyla Actinobacter, Verrucomicrobia, Firmicutes, and Fibrobacteres were driving factors for the regional differences.

Livestock gut microbiota may be affected by diet and practices in farms. Our results indicated significant regional differences in swine gut microbiota, suggesting that future livestock gut microbiota studies should be designed with the regional differences in mind.

Key words: growth promoter, gut microbiota, swine

The banning of the antibiotic growth promoters (AGPs) has caused increased death rates of livestock animals (Casewell *et al.*, 2003). Substantial AGP-alternatives have been reported to date. For example, the use of probiotic strains were found to be effective in increasing feed efficiency (Konstantinov *et al.*, 2008), and treating lysozyme to livestock was also showed effects similar to antibiotics (Oliver and Wells, 2013). On the other hand, rather than developing alternatives, substantial studies have reported that modulation of gut microbiota could be a new approach in regulating hosts health conditions (Million and Raoult, 2013; Shen, 2014; Tsabouri *et al.*, 2014; Xiao and Zhao, 2014).

Recent studies have indicated that gut microbiota is tightly related to the diet patterns of hosts. For example, gut microbiota between obese and lean people differ in ratio between abundances of two phyla, namely Firmicutes and Bacteroidetes (Ley *et al.*, 2006). In addition, antibiotics are also one of the factors that affect composition of gut microbiota (Looft *et al.*, 2014b). Therefore, it is possible that livestock gut microbiota could vary depending on type of feeds or veterinary practices in farms.

Fresh fecal materials were collected from 5-7 adult swine randomly selected from each pen and kept on ice during transportation. Fecal materials were frozen using liquid nitrogen, freeze-dried overnight, and then granulated using sterilized mortars and pestles. Fecal powders were stored at -20°C until

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processed. Number of feces obtained in Jeju, Haenam, and Gwangju are 40, 30, and 28 feces, respectively. DNA was extracted from the fecal materials using MOBIO Power Fecal DNA isolation kit (MOBIO Laboratories Inc.). Sequences of v4 region of 16S rRNA gene were obtained as previously described (Kozich *et al.*, 2013). Briefly, forward primers were designed to contain Illumina' adapter region, 8 bp tag, pad (TATGGTAATT), and V4 forward (GTGCCAGCMGCCGCGGTAA) and reverse primers contains Illumina's adapter region, 8 bp tag, pad (AGTCAGTCAG), and V4 reverse (GGACTACHVGGGTWTCTAAT). It should be noted that V4 primers used in this study were designed to amplify both bacterial and archaeal 16S rRNA genes. The equimolar amplicons were pooled and sequenced using Illumina MiSeq platform (Illumina) at Macrogen Inc. according to the manufacturer's instructions. Obtained sequences were processed according to MiSeq SOP (http://www.mothur.org/wiki/MiSeq_SOP) suggested by developers of Mothur.

A total of 98 samples were sequenced using MiSeq, resulting in 5,642,125 sequences. Nearly 65% (3,868,143) of the raw sequences remained after Mothur removed erroneous sequences based on alignment, chimera check, and taxonomy. Number of reads obtained for each sample varied from 9,949 to 127,179, thus normalization was done by randomly sampling 5,000 reads from each sample. All sequences used in this study are available at our swine microbiome project database (http://biotech.jejunu.ac.kr/~abl/swine_microbiome/DB). Species richness and evenness was evaluated based on Chao and Shannon indices, respectively. Results in Fig. 1 show that Jeju swine differ from Gwangju and Haenam swine in species evenness and richness, respectively.

In contrast, no significant difference in ecological indices was observed between Gwangju and Haenam swine.

Bacterial composition difference was examined at the phylum level, and heatmap was created based on read-abundance of each phylum (Fig. 2). Figure 2 shows relatively tight clusters according to the regions. The greatest portion of Firmicutes was

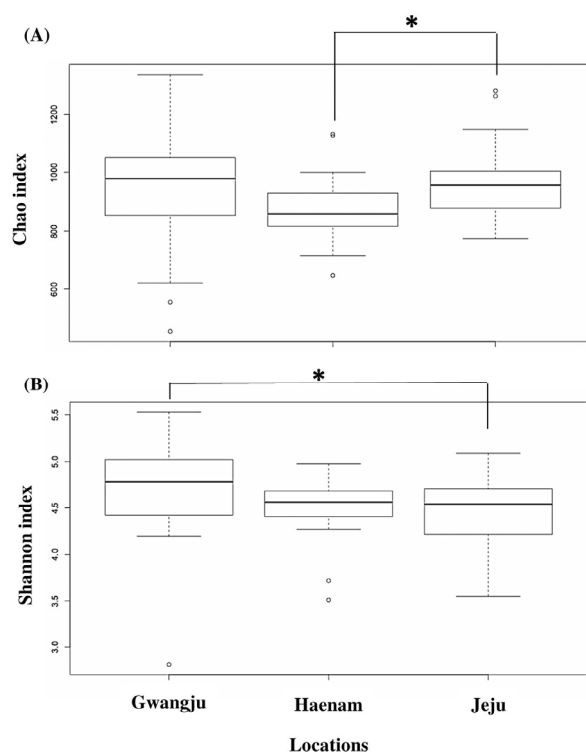


Fig. 1. Comparison of species richness and evenness based on Chao and Shannon indices, respectively. * indicates significant difference ($P < 0.05$) based on Tukey's HSD test.

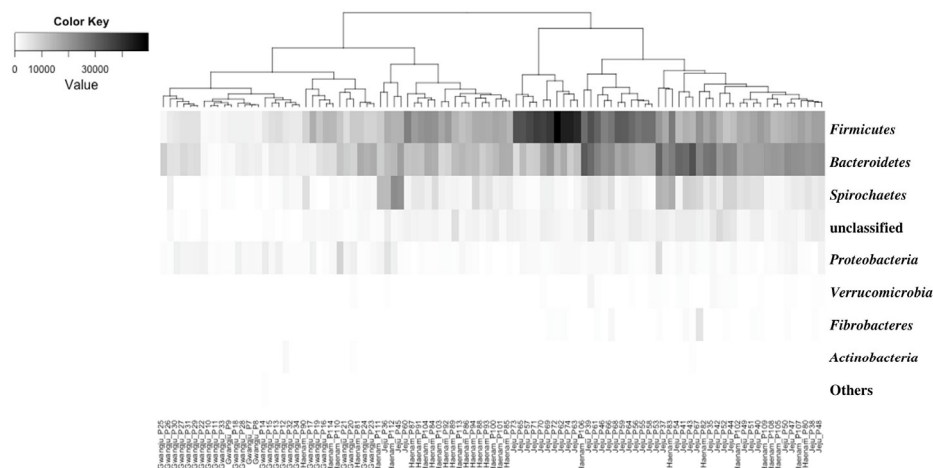


Fig. 2. Heatmap cluster analysis based on read abundance at the phylum level.

observed mostly for Jeju swine, while moderate portions of Firmicutes and Bacteroidetes were observed in Haenam swine and, to a lesser extent, Gwangju swine. Relatively greater percentage of Proteobacteria was observed for Gwangju swine. Verrucomicrobia and Fibrobacteres were found more prevalent in Jeju swine. These differential abundance grouped swine gut microbiota according to regions. Results from Fig. 2 suggest that regional differences in swine gut microbiota can be observed for bacterial composition at the phylum level. Previously, the ratio of the two major phyla in fecal microbiota, Firmicutes and Bacteroidetes, are significantly associated with obesity (Ley *et al.*, 2005, 2006), suggesting that difference at the phylum level may affect feed efficiency, fat content of meat, and host health. However, further studies should be done to clarify physiological differences among swine in different regions.

Yue-Clayton theta coefficient was calculated from the distribution of OTUs and clustered using Mothur (Fig. 3). Regional differences were observed between clusters in Fig. 3 with a few exceptions. While Gwangju and Haenam swine were tightly clustered, Jeju swine were divided into two clusters. Further studies should include information such as feed contents and veterinary practices to clarify what caused the difference between the two Jeju swine clusters. Nevertheless, our results showed the clear regional differences among swine gut microbiota.

To investigate the correlation between bacterial taxonomy and regional difference, we have conducted spearman correlation analysis between read abundance of each phylum and non-

metric multidimensional scaling (NMDS). Figure 4 shows NMDS plots with a 95% confidence interval on regional differences. Although ellipses overlap, analysis of molecular variances confirmed the significant differences between the

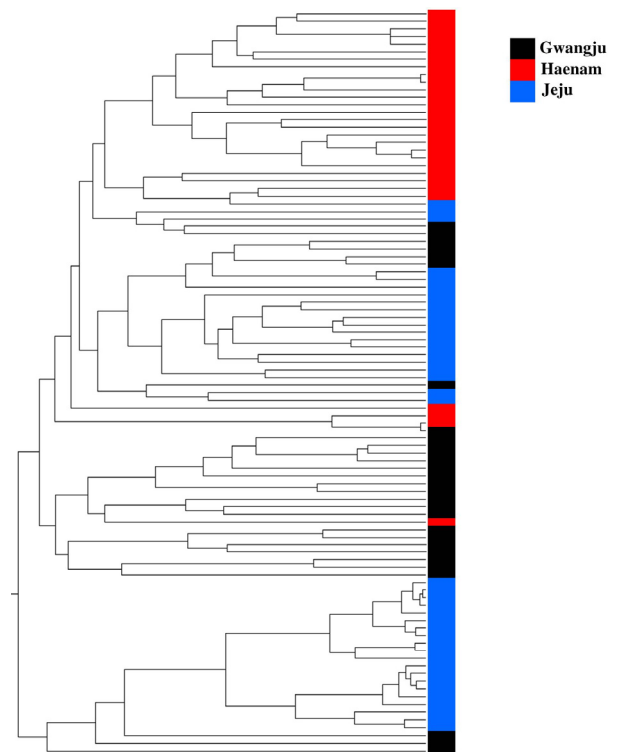


Fig. 3. Cluster analysis based on the distribution of operational taxonomic units calculated according to the theta coefficient.

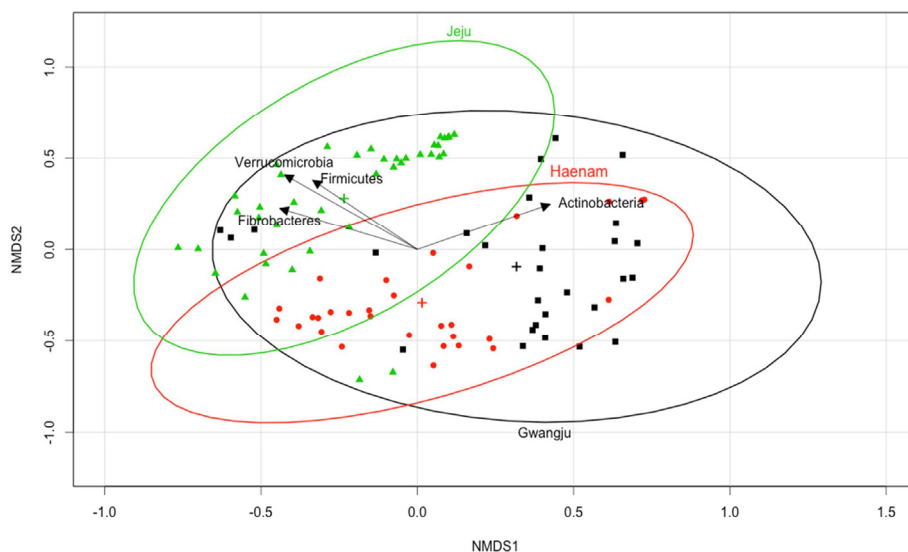


Fig. 4. Regional differences in swine gut microbiota based on non-metric multidimensional scaling.

three regions ($P < 0.001$) (Supplementary data Table S1). Biplots indicate the positive correlation between regional difference and phyla. Results from Fig. 4 suggest that prevalence of the phylum Actinobacteria is a positive determinant for Gwangju swine, while the phyla Firmicutes, Fibrobacteres, and Verrucomicrobia are for Jeju swine. Having less of these phyla would indicate Haenam swine.

It has been reported that swine gut microbiota were similar between housing-related than maternally related likely due to coprophagy (Thompson *et al.*, 2008). In addition, veterinary practice can significantly change swine gut microbiota depending on types and frequency of antibiotics use (Kim *et al.*, 2012; Looft *et al.*, 2014a). It has been reported that per-head antibiotics usage for pigs was as twice as high compared to UK and USA (Kim *et al.*, 2011). Although the use of AGPs has been banned in Korea in 2012, controlling the use of AGPs in a number of private farms in Korea may not be as easy as farms in western countries where farms are run by companies.

We conducted analysis of molecular variance (AMOVA) by amova mothur subroutine to investigate differences in swine gut microbiota between farms in each region. Results from AMOVA showed that microbial communities were also different depending on farms ($P < 0.001$), although similar swine gut microbial communities were observed between some farms (Supplementary data Table S2).

Previous studies have shown promises in improving host health through modulation of gut microbiota, although the mechanisms by which gut microbes change host's physiological functions are yet to be understood. Most of studies done for gut microbiota with respect to host health are focused on humans and fewer studies have been conducted for livestock animals, therefore fundamental information regarding to livestock gut microbiota should be helpful in designing future researches. Fecal microbiota analysis has been applied to many of human obesity studies to date (Sanz *et al.*, 2008). The composition of the bacterial microflora present in the feces was similar to that at the mucus layer of the terminal ileum and colon regions (van der Waaij *et al.*, 2005). Therefore, study of fecal microbiota may directly apply to elucidate functional roles of gut microbiota. The study presented here reports regional differences among swine gut microbiota, thus future livestock gut microbiota studies should keep regional variations in mind.

적 요

성장촉진제로 항생제 사용이 금지가 된 이후, 가축들의 사망률이 증가되어 항생제 대체제를 개발해야 하는 것이 시급하다. 그러한 대체제 개발에 새로운 접근 중 하나는 숙주의 신체적 기능에 영향을 준다고 알려진 장내미생물생태를 조절하는 것이다. 하지만 가축의 장내미생물에 대한 이해가 인간과 비교하여 볼 때 많이 부족한 실정이다. 본 연구에서는 돼지장내미생물생태가 지역적 차이가 있음에 대한 기본적인 정보를 제공한다.

돼지 분변샘플은 제주(n=40), 광주(n=28), 해남(n=30) 농가로부터 채취하였으며, MiSeq을 이용하여 16S rRNA V4 지역을 시퀀싱하였다. 또한 Mothur 파이프라인을 이용하여 MiSeq으로부터 얻은 데이터를 처리하였다. 총 5,642,125 reads를 얻었으며, 에러시퀀스들을 제거한 후 최종적으로 3,868,143 reads가 남았다. Phylum 수준의 taxonomic composition 분석에서는 제주 돼지들이 Firmicutes를 가장 많이 포함하고 있었으며, Operational Taxonomic Units 분포분석에서 또한 지역적 차이에 따라 돼지장내미생물생태가 다르다는 것을 확인하였다. Non-metric multidimensional scaling 과 Phyla의 풍부함 사이의 상관관계분석에서는 Actinobacter, Verrucomicrobia, Firmicutes, Fibrobacteres이 세 개의 지역에 있는 돼지들의 장내미생물생태 차이를 나타나게 하는 장내미생물 요소라는 것을 확인하였다.

그러한 가축의 장내미생물생태는 농장에서 사용하는 사료와 사양관리에 의해 많은 영향을 미치는 것으로 생각된다. 본 연구결과는 돼지장내미생물생태가 지역적으로 많은 차이가 있다는 것을 나타내며, 추후에 가축의 장내미생물생태에 관한 연구는 지역적 차이가 있다는 것을 고려하여 설계해야 될 것이다.

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