Structural Analysis of Intestinal Archaea in Mice Receiving Fecal Microbiota Transplantation from Jinhua and Landrace Pigs: Postprint

Authors: Yang Hua, Wang Junjun, Li Tiantian, Tang Biao, He Xiangxiang, Kaifeng Li, Xiao Yingping

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Abstract

This study aimed to compare the differences in gut archaeal community structure between obese-type Jinhua pigs and lean-type Landrace pigs, and to analyze the intestinal archaeal structure in fecal microbiota transplantation (FMT) mice. Fresh feces from Jinhua and Landrace pigs were collected and transplanted into the intestines of mice treated with broad-spectrum antibiotics via gavage. Total genomic DNA was extracted from the feces of the two pig breeds and the cecal contents of the transplanted mice. Universal archaeal primers were used for PCR amplification of the V4 region of the archaeal 16S rRNA gene. The amplicons were subjected to high-throughput sequencing using the Illumina HiSeq sequencing platform, and the resulting sequences were analyzed and statistically processed using QIIME and other software. The results showed that the archaea in the feces of Jinhua and Landrace pigs and the cecal contents of their transplanted mice mainly comprised three phyla: Thaumarchaeota, Euryarchaeota, and Crenarchaeota, with the abundance of Euryarchaeota being higher in Jinhua pig feces than in Landrace pig feces. At the genus level, five known genera were identified, with Cenarchaeum being the dominant genus. Regarding the archaeal community structure in the cecal contents of the transplanted mice, at the phylum level, Thaumarchaeota accounted for over 70% of the archaea; nearly all Thaumarchaeota archaea originated from the genus Cenarchaeum. No significant differences were observed in the cecal archaeal community structure between mice transplanted with Jinhua pig feces and those transplanted with Landrace pig feces at either the phylum or genus level ($P>0.05$); however, principal component analysis (PCA) based on operational taxonomic unit (OTU) levels showed good within-group clustering. Therefore, the genus Cenarchaeum within the phylum Thaumarchaeota is the dominant archaeal taxon in the feces of Jinhua and Landrace pigs and in the ceca of their transplanted mice. The abundance of Euryarchaeota was higher in Jinhua pig feces than in Landrace

pig feces, and there were certain differences in the archaeal community structure in the cecal contents between the two groups of transplanted mice at the OTU level.

Full Text

Intestinal Archaea Community Structure Analysis of Mice Transplanted with Jinhua and Landrace Pig Feces

YANG Hua[1], WANG Junjun[2,3], LI Tiantian[2,3], TANG Biao[1], HE Xiangxiang[1], LI Kaifeng[1], XIAO Yingping[1]*

[1]Institute of Quality and Standard for Agro-Products, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China
[2]Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing 100193, China
[3]College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

Abstract

This study aimed to compare the intestinal archaeal community structures between obese-type Jinhua pigs and lean-type Landrace pigs, and to analyze the archaeal composition in mice following fecal microbiota transplantation. Fresh feces were collected from both pig breeds and transplanted into the intestines of mice pretreated with broad-spectrum antibiotics via gavage. Total genomic DNA was extracted from the pig feces and the cecal contents of transplanted mice. The V4 region of the archaeal 16S rRNA gene was amplified using universal archaeal primers, and the amplicons were subjected to high-throughput sequencing on the Illumina HiSeq platform. Sequence analysis was performed using QIIME and other software packages. The results revealed that archaeal communities in both pig feces and transplanted mouse cecal contents were dominated by three phyla: Thaumarchaeota, Euryarchaeota, and Crenarchaeota, with higher abundance of Euryarchaeota observed in Jinhua pig feces compared to Landrace pigs. At the genus level, five known genera were identified, with *Cenarchaeum* as the predominant genus. In transplanted mice, Thaumarchaeota accounted for over 70% of the archaeal community at the phylum level, and was almost exclusively composed of *Cenarchaeum* at the genus level. No significant differences were detected between the two mouse groups at either phylum or genus levels ($P>0.05$). However, principal coordinate analysis based on operational taxonomic units (OTUs) demonstrated clear within-group clustering. These findings indicate that *Cenarchaeum* within Thaumarchaeota represents the dominant archaeal taxon in both pig feces and transplanted mouse cecal contents. While Euryarchaeota was more abundant in Jinhua pig feces than in Landrace pig feces, distinct differences in archaeal community structure between the two transplanted mouse groups were evident at the OTU level.

Keywords: archaea; Jinhua pig; Landrace pig; fecal microbiota transplantation; high-throughput sequencing
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Pork has long been the primary source of meat for Chinese consumers and holds significant importance in national economy and people' s livelihood. Jinhua pig, a renowned local breed in China known as the "Panda Pig," is characterized by black heads and tails (hence also called "two-end-black"), thin skin, fine bones, delicious meat, early sexual maturity, strong reproductive capacity, and remarkable heterosis. However, it exhibits slow growth rate and low lean meat percentage [1]. In contrast, Landrace pigs are an excellent exotic breed originating from Denmark, featuring long and lean bodies with white skin and hair, rapid growth rate, and high lean meat percentage, but they are relatively weak, have poor stress resistance, require higher feeding standards, and show low reproductive performance [2].

The gut microbiota constitutes the most complex and extensive microecosystem in mammals, profoundly influencing host health, evolution, and behavior [3-4]. Archaea represent an essential component of the gut microbial community. Initially discovered in extreme environments characterized by high temperature, salinity, and hypoxia (such as volcanic craters and salt lakes), archaea were later recognized as a distinct domain through 16S rRNA comparisons, forming the three-domain system alongside Bacteria and Eukarya [5-6]. Archaea are widely distributed across diverse natural habitats including oceans, hot springs, saline-alkaline lakes, soil, marshes, and animal digestive tracts. Currently, Euryarchaeota and Crenarchaeota are the most extensively studied archaeal phyla, particularly the former which encompasses a large group of methanogens prevalent in animal digestive systems and represents a focal point in animal nutrition research [7-9].

Intestinal archaea contribute to maintaining a healthy microecosystem in the digestive tract. Thaumarchaeota, a newly discovered archaeal group found widely in mesophilic environments, may play crucial roles in the biogeochemical cycling of essential elements such as nitrogen and carbon [10-12], yet their distribution and function in animal digestive tracts have been rarely reported [13-14].

Fecal microbiota transplantation (FMT), a historically established therapy for reconstructing gut microbiota, has regained clinical attention in recent years for treating both intestinal and extra-intestinal diseases [4,15]. As a gain-of-function experimental approach, FMT has been increasingly applied to functional studies of animal microbiota [16], with mice being the most commonly used animal model [17-18]. Previous studies have demonstrated that transplantation of gut microbiota from pigs and zebrafish into mice results in donor microbial communities being influenced and reshaped by the host to some extent [19-21]. However, most FMT studies have focused on bacterial communities, with limited investigation into archaeal dynamics during transplantation.

This study established mouse models simulating the gut microbial environments of Jinhua and Landrace pigs through FMT. Using high-throughput sequencing technology, we analyzed the archaeal community structures in feces from both pig breeds and in the cecal contents of transplanted mice, providing valuable data for understanding archaeal structural changes during inter-species fecal microbiota transplantation.

1. Materials and Methods

1.1 Experimental Animals Animal experiments were conducted at Beijing Sibifu Experimental Animal Technology Company. Twenty-four 28-day-old specific pathogen-free (SPF) C57BL/6J mice (12 males and 12 females) were housed in sterile isolators and fed sterile rodent chow. The environmental conditions were maintained at a constant temperature of 23°C, relative humidity of 63%, and a 12-hour light/dark cycle.

1.2 Mouse Antibiotic Treatment Mice were housed separately by sex (6 mice per cage). Following the protocol described by Wang et al. [22], we employed broad-spectrum antibiotics to establish a germ-free mouse model. The antibiotic cocktail consisted of 0.5 g/L vancomycin, 1 g/L neomycin sulfate, 1 g/L metronidazole, and 1 g/L ampicillin, administered ad libitum in drinking water for 28 days.

1.3 Pig Feces Processing Fresh feces were collected from one multiparous Jinhua sow and one multiparous Landrace sow (both in their second parity). Following the method of Pang et al. [23], we prepared the donor intestinal microbiota. Briefly, fresh feces were immediately suspended in pre-reduced sterile physiological saline (1 g feces in 4 mL saline, 5-fold dilution), vortexed for 1 minute, allowed to settle for 1 minute, and the supernatant was quickly collected. The supernatant was aliquoted into 1.5 mL sterile centrifuge tubes and stored at -20°C for FMT, with a portion reserved for high-throughput sequencing analysis of archaeal community structure.

1.4 Mouse Inoculation and Sampling Antibiotic-treated mice were divided into two groups (n=12 each, with 6 males and 6 females per group) and gavaged with 0.2 mL of either Jinhua or Landrace pig fecal microbiota suspension daily for 7 consecutive days. Mice were maintained for an additional 28 days post-inoculation (allowing for several cycles of intestinal epithelial and mucus layer renewal) before euthanasia. Cecal contents were collected for high-throughput sequencing analysis of archaeal community structure. The cecum was selected because its luminal contents can be easily and reliably obtained and harbor rich microbial populations [20].

1.5 DNA Extraction and Archaeal 16S rRNA Gene Amplification Total genomic DNA was extracted from pig fecal suspensions and

mouse cecal contents using the QIAamp DNA Stool Mini Kit (QIAGEN). The V4 region of the archaeal 16S rRNA gene was amplified using universal archaeal primers 519F (5 -CAGCCGCCGCGGTAA-3) and Arch915R (5 -GTGCTCCCCCGCCAATTCCT-3).

1.6 High-Throughput Sequencing Analysis Sequencing was performed by Beijing Novogene Bioinformatics Technology Co., Ltd. using the Illumina HiSeq platform to sequence the V4 region of archaeal 16S rRNA genes from pig fecal suspensions and mouse cecal contents.

1.7 Data Processing and Analysis Paired-end reads were assigned to samples based on barcode sequences, after which barcodes and primer sequences were trimmed. FLASH 1.2.7 software (http://ccb.jhu.edu/software/FLASH/) was used to merge paired-end reads into raw sequences. QIIME 1.7.0 software (http://qiime.org/scripts/split_libraries_fastq.html) was then employed for quality control and filtering to obtain high-quality sequences [24-25]. Chimeric sequences were identified and removed by comparing against the reference database (Gold database, http://drive5.com/uchime/uchime_download.html) using the UCHIME algorithm, yielding effective sequences [26-27].

Effective sequences passing quality control were clustered into operational taxonomic units (OTUs) at 97% similarity using Uparse 7.0.1001 software (http://drive5.com/uparse/) [28]. QIIME 1.7.0 default parameters were used to calculate alpha diversity indices and taxonomic distributions for each sample. To compare archaeal community structures between mice transplanted with feces from the two pig breeds, principal coordinate analysis (PCoA) based on weighted UniFrac distances was performed using the FactoMineR package in R 2.15.3, and scatter plots were generated using the ggplot2 package.

2. Results

2.1 Sequencing Data and OTU Information Statistics As shown in Table 1 , after quality control, filtering, assembly, and chimera removal, we obtained a total of 1,428,899 high-quality archaeal sequences. Specifically, the Jinhua pig fecal sample yielded 60,979 effective sequences, the Landrace pig fecal sample yielded 61,369 effective sequences, and the 24 transplanted mouse cecal samples yielded between 46,362 and 61,524 effective sequences each, showing minimal inter-individual variation. OTU clustering at 97% similarity resulted in 307 OTUs for the Jinhua pig fecal sample, 202 OTUs for the Landrace pig fecal sample, and 104-133 OTUs for the transplanted mouse cecal samples. Coverage indices for all samples ranged from 0.999 to 1.000, indicating extremely low probability of undetected sequences and confirming that the sequencing results accurately represented the true diversity of each sample.

2.2 Alpha Diversity Analysis Based on OTU information at 97% similarity, we assessed archaeal community richness and diversity using Chao I index, ACE

index, Shannon index, and Simpson index (Table 1). OTU numbers (123±2 vs. 117±2, P=0.0405), Chao I index (146.846±18.969 vs. 139.330±17.755, P=0.7752), and ACE index (147.606±18.852 vs. 140.350±17.995, P=0.7834) consistently indicated slightly higher archaeal community richness in mice transplanted with Jinhua pig feces (JM1-12) compared to those transplanted with Landrace pig feces (LM1-12), though differences were not statistically significant for Chao I and ACE indices (P>0.05). Similarly, Shannon index (3.811±0.065 vs. 3.898±0.063, P=0.3454) and Simpson index (0.882±0.007 vs. 0.889±0.006, P=0.5076) revealed marginally higher archaeal diversity in Jinhua-transplanted mice, but these differences were also not significant (P>0.05).

2.3 Archaeal Structure of Transplant Donors OTUs obtained from donor fecal samples were taxonomically annotated at phylum and genus levels (Table 2 and Figure 1 [Figure 1: see original paper]). Approximately half of the donor OTUs belonged to Thaumarchaeota, while Euryarchaeota and Crenarchaeota exhibited relatively low abundances. Notably, Euryarchaeota was more abundant in Jinhua pig feces than in Landrace pig feces. At the genus level, Thaumarchaeota was almost exclusively represented by *Cenarchaeum*, with Landrace pig feces also containing minor proportions of *Nitrososphaera*. Within Euryarchaeota, *Methanobrevibacter* showed relatively higher abundance in Jinhua pig feces, along with trace amounts of the candidate genus *Candidatus* Methanomethylophilus, whereas Landrace pig feces exhibited higher abundance of *Methanosphaera*.

2.4 Archaeal Structure in Transplanted Mouse Cecal Contents Following gavage with fecal microbiota from Jinhua and Landrace pigs, archaeal communities in antibiotic-treated germ-free mice were analyzed 28 days post-inoculation. Taxonomic annotation at phylum and genus levels revealed that Thaumarchaeota increased to over 70% of the archaeal community in transplanted mice, while Euryarchaeota and Crenarchaeota were barely detectable (Table 3 and Figure 2 [Figure 2: see original paper]). At the genus level, Thaumarchaeota was almost exclusively composed of *Cenarchaeum*. No significant differences in archaeal community structure were observed between the two mouse groups at either phylum or genus levels, though substantial differences were noted compared to the donor profiles.

2.5 Beta Diversity Analysis of Transplanted Mouse Samples Weighted UniFrac distances were calculated based on OTU composition and abundance across samples, followed by principal coordinate analysis (Figure 3 [Figure 3: see original paper]). Except for samples JM12 and LM12, which showed greater dispersion, all other samples within each group exhibited high clustering. The two groups displayed distinct within-group aggregation along the PC2 axis according to their respective donor sources.

3. Discussion

3.1 Similarities and Differences in Archaeal Structure Between Jinhua and Landrace Pig Feces Jinhua pigs, a famous local Chinese breed with slow growth rate and high fat deposition, contrast sharply with Landrace pigs, an excellent exotic breed with rapid growth and high lean meat percentage [1]. Cross-species transplantation of gut microbiota between different pig breeds provides important insights for functional analysis [19], yet the role of intestinal archaea in this process remains understudied. This study established mouse models simulating the gut microbial environments of both pig breeds through FMT and employed high-throughput sequencing to analyze archaeal community differences, laying a foundation for further functional and mechanistic investigations.

Previous research on intestinal archaea in animals and humans has primarily focused on methanogens. Studies indicate that methanogen diversity in animal guts is closely associated with environmental conditions, developmental stages, and dietary factors, leading to structural variations across different breeds [7-9]. Regarding fat metabolism, one report suggested that lean Landrace pigs harbor higher density and diversity of fecal methanogenic archaea compared to obese Erhualian pigs, as methane production affects energy harvest and adipose formation [7]. Conversely, Zhang et al. [29] found that obese individuals had significantly higher levels of hydrogen-utilizing methanogenic archaea compared to lean individuals or those who underwent gastric bypass surgery, with inter-species hydrogen transfer between bacteria and archaea proposed as a key mechanism for increased energy uptake in the large intestine of obese subjects. These contrasting results highlight discrepancies arising from different study subjects and methodologies. Our study revealed that although Euryarchaeota was present at low abundance in both pig breeds, its abundance was significantly higher in Jinhua pig feces than in Landrace pig feces. The implications of this difference for growth and metabolism in the two breeds warrant further investigation.

Thaumarchaeota have been reported in only a few animal digestive systems, including certain termites and silkworms [13-14], and typically at very low abundance in humans and other animals [30]. Surprisingly, our study identified Thaumarchaeota as the dominant archaeal phylum in both Jinhua and Landrace pig feces, accounting for nearly half of all archaea, with *Cenarchaeum* as the predominant genus. This finding contradicts previous reports, and its underlying causes and significance require further experimental validation and discussion.

In 2006, Rawls et al. transplanted zebrafish gut microbiota into germ-free mice and observed significant compositional changes post-transplantation, and vice versa, demonstrating that donor microbial communities are influenced and reshaped by the host during inter-species FMT [20]. Our results confirm that archaeal communities are similarly affected by the host during pig-to-mouse

FMT. Although Euryarchaeota abundance was markedly higher in Jinhua pig feces than in Landrace pig feces, this difference disappeared after transplantation into mice. Substantial structural changes occurred from phylum to genus levels, with *Cenarchaeum* within Thaumarchaeota becoming even more dominant. While no significant differences were observed between the two mouse groups at phylum and genus levels, principal coordinate analysis at the OTU level revealed distinct clustering patterns, indicating differences in archaeal community structure at finer taxonomic resolution.

Conclusions

This study demonstrates that: (1) Thaumarchaeota, particularly the genus *Cenarchaeum*, represents the dominant archaeal taxon in both Jinhua and Landrace pig feces and in the cecal contents of transplanted mice; (2) Euryarchaeota abundance is significantly higher in Jinhua pig feces than in Landrace pig feces; and (3) Following transplantation into broad-spectrum antibiotic-treated mice, the archaeal community structure in mouse cecal contents differs markedly from that of the donors, with distinct differences observed between the two transplanted groups at the OTU level.

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