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Characterization of vaginal microbiota of endometritis and healthy sows using high-throughput pyrosequencing of 16S rRNA gene

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ABSTRACT

Endometritis is one of major challenges in reproduction infections caused by bacteria in sows. Understanding of the vaginal bacterial community between endometritis and healthy sows serves as a critical step to develop more effective ways to improve reproduction ability in pig industry. The aim of the present study is to evaluate and compare the vaginal microbiota of endometritis and healthy sows using high-throughput pyrosequencing of 16S rRNA gene. The main bacterium found at the phylum level were *Firmicutes* (60.88% vs. 45.86%), *Proteobacteria* (20.45% vs. 32.19%) and *Bacteroidetes* (9.19% vs. 12.99%) for healthy and endometritis sows, respectively. Most notable difference at the phylum level was the *Proteobacteria* which occupied high abundance in the endometritis sows but less abundance in the healthy sows. At the genus level, the highest abundant were *Bacillus* (27.13% vs. 16.15%), *Paenibacillus* (14.78% vs. 8.92%), *Alkaliphilus* (3.99% vs. 2.87%) and *Cronobacter* (4.04% vs. 2.37%), in healthy and endometritis sows, respectively. Notable differences were *Escherichia-Shigella*, *Bacteroides*, *Fusobacterium* and *Clostridium_sensu_stricto_1* which were more abundant in the endometritis than the healthy sows respectively. The present results for the first time demonstrate vaginal microbial community of sows and indicate that endometritis affected the vaginal microbiota of sow.

1. Introduction

Endometritis is one of the common microbial diseases in female domestic animal which causes a huge economic loss [1,2]. Although antibiotics have been widely used to treat endometritis in female domestic animals, problems such as microbial resistance and antibiotic residues in milk are becoming more and more prominent [3]. Hence the critical need to develop new methods for treating endometritis. Previous studies have showed that the microbial community of vagina has a crucial role in sustaining the health of reproductive tracts through a variety of mechanisms such as biological barrier, producing lactic acid, bacteriocin and hydrogen peroxide [4,5]. Defining vaginal microbial diversity is of importance for understanding the role of the microbiota in reproductive tracts health and developing new ways to prevent and treat reproductive tracts infections by modulating the microbial community. Previous study has shown that vaginal microbial community of endometritis cows was more complicated and without dominant bacteria as compared to the healthy cows [6]. In recent years, high-throughput pyrosequencing of 16S rRNA gene have been proved to be powerful tool to investigate the phylogenetic diversity of the microorganisms in different ecological niches [7,8]. Endometritis is one of the common diseases in pig industry [2]. However, information on vaginal microbial community of healthy and endometritis sows and their differences is few. In the present study, high-throughput pyrosequencing of 16S rRNA gene was used to profile the vaginal microbiota of healthy and endometritis sows.

Abbreviations: QIME, Quantitative Insights into Microbial Ecology; OTUs, operational taxonomic units; PCA, Principal Coordinate Analysis; UPGMA, Unweighted Pair Group Method with Arithmetic mean.

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Table 1
Descriptive of pyrosequencing data and diversity indices at species level.

Sample name	OTU	Chao	Ace	Shannon	Coverage
H1	598	632	629	3.67	0.996805
H2	643	671	667	4.14	0.997083
H3	600	649	651	3.66	0.995061
H4	599	626	624	3.85	0.997129
M1	620	674	666	3.93	0.995740
M2	555	670	676	4.15	0.993471
M3	417	545	545	2.08	0.993702
M4	631	665	669	4.17	0.996434

OTU, operational taxonomic units; Ace, Abundance-based coverage estimator.
OTU definition at >97% identity cutoff.

2. Material and methods

2.1. Animals and samples

Vaginal samples were collected from 4 healthy sows (H1, H2, H3 and H4) and 4 endometritis sows (M1, M2, M3 and M4) at 5d postpartum in a pig farm located in Jilin province, China. In order to avoid contamination of the vaginal samples during sampling, special swab equipment was used to collect the samples. The structure of swab equipment was the same as that described by Thiago et al. [9]. Briefly, the swab was placed inside a metal tube, and then the sterile cotton swab was rotated to exposed to the sampling sites of vagina, the sterile cotton swab was then rotated to be covered by the metal tube during the metal tube being pulled out from the vagina of the sows. Third parity Landrace sows were used in the present study. Endometritis sows were diagnosed by the presence of purulent or mucopurulent uterine exudate in the vagina. All the sows had not received antibiotics treatment at least 1 month before sampling. The samples were collected with sterile swabs and stored at -20 °C until use.

2.2. DNA extraction

The swabs were individually subjected to vigorous agitation in 1 mL of RNAase free water and then the samples were centrifuged

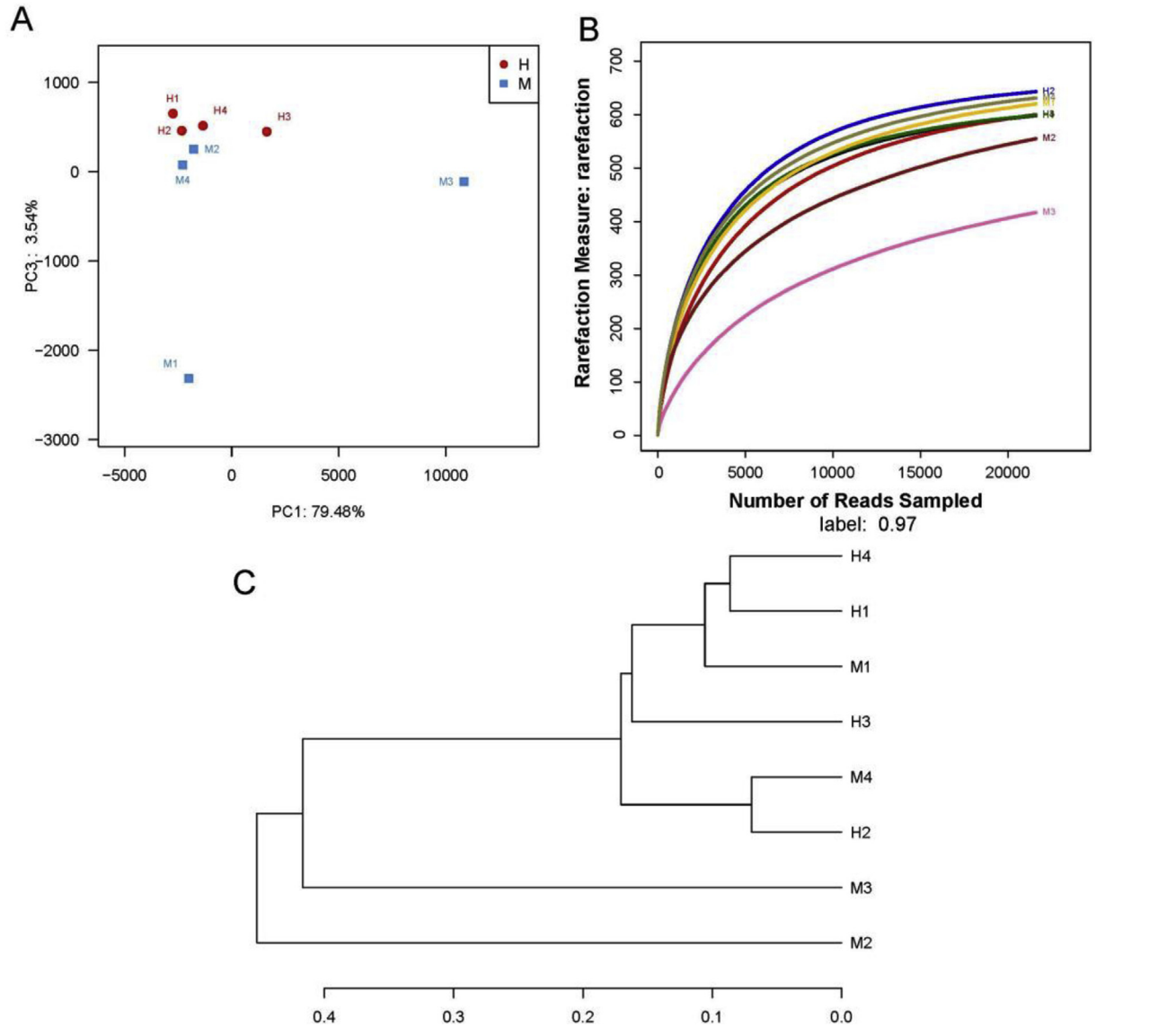

Fig. 1. Comparison of the vaginal microbiomes of the healthy and endometritis sows. The analyses were performed on 16S rRNA gene sequences. The coding names are coded as follows: Group H refers to the healthy sows group and Group M refers to the endometritis group. H1 to H4 refers to the samples per individual from the healthy group and M1 to M4 refers to the samples per individual from the endometritis group. (**A**) Principal Coordinate Analysis (PCA) by bacterial microflora. (**B**) Rarefaction analysis per sample. (**C**) The UPGMA clustering tree from samples per individual.