The Distribution Characteristic of Microbial Aerosols Inside a Nursery Pig House and the Respiratory Tract of Piglets

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Research

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Abstract

Background: The particulate matter (PM) is a carrier of many substances. Microorganisms are vital constituents contained in PM and the kinds and concentration are closely connected to human health and animal production. This study was aimed to investigate the distribution characteristics of microbial aerosols inside the pig house and as well as in the respiratory tract of pigs. The environment inside a nursery pig house was monitored in winter, including temperature, relative humidity, TSP, PM_{10}, $PM_{2.5}$, NH_3, CO_2, CO and NO. The concentrations of airborne culturable bacteria, fungi and *Escherichia coli* were detected. Then 16 S rRNA sequencing technology was applied to identify bacteria contained in different sized PM and the bacteria in the respiratory tract of piglets.

Results: The results showed that the concentration of airborne culturable bacteria inside the pig house was significantly higher than that outside. The concentration of airborne culturable bacteria was reduced with the decrease of the size of PM; the concentration of airborne culturable fungi was mostly distributed in the size of 2.1-3.3 μm and 1.1-2.1 μm PM; most airborne culturable *Escherichia coli* were distributed in the size of >7.0 μm and 2.1-3.3 μm. Besides, these three types of microbial aerosols did not exhibit significant change during different time points. The 16 S rRNA results showed that the bacteria contained in PM had high similarity with that in the respiratory tract of pigs. The bacteria assemblage in the size of 1.1-3.3 μm PM had high similarity with that in the lower respiratory tract (bronchus and lung) of pigs. In addition, four potential pathogenic bacteria genera (*Escherichia-Shigella*, *Streptococcus*, *Acinetobacter*, *Pseudomonas*) were identified in the PM samples and the respiratory tract.

Conclusions: These results will provide a significant scientific basis in exploring the potential risk of aerosol from animal houses for human and animal health.

Introduction

Modern pig production with high animal densities in confinement buildings can cause a series of environmental problems. Particulate matter (PM), as an important indicator of air pollution, has aroused widespread concern. As reported, it has correlated with many respiratory diseases[1-3]. The animal farmers, they are at a higher prevalence of chronic bronchitis and chronic obstructive pulmonary disease (COPD) than the no-farming people[4-6]. When compared to the farmers working in chicken, cattle or sheep houses, pig house farmers have higher risk of developing work-related symptoms, such as shortness of breath and a cough with phlegm, thus, they have higher incidence of respiratory disease than others[7, 8]. PM is not a single pollution and it also can carry some toxic and harmful substance to enter the body, such as heavy metals, gas, polycyclic aromatic hydrocarbons, and microorganisms[9]. Obviously different from PM in atmosphere, the biological sources of PM from pig houses are abundant, including feed, feces, urine, dander, bedding, skin, and hair, hence the microorganisms contained are very rich[10, 11]. A large proportion of the microbial aerosols in livestock production system are bacteria aerosols[12]. Some pothogenic bacteria can be a great challenge for the health of animals and farmers. Hong et al.[13] identified 12 kinds of potential pathogenic bacterial genera and White et al.[14] totally

classified 28 different species of bacterial and fungal pothogens in pig farms. Usually, the deposition depth of aerosol particle in the respiratory tract was shaped by its size[15]. It remained to be determined that whether these bacteria can colonize the respiratory tract of animals. Although the lung environment were classically believed to be sterile, more and more published studies have confirmed the existence of microbial communities in the lung of humans and animals[16, 17]. Nowadays, the effect of the lung microbes on animal health has attracted more and more attention. Prior to study these implications, it is necessary to figure out the dynamics of lung microbial composition as well as the relationship with the environment[16].

In order to investigate the distribution of different sized microbial aerosols inside the pig house, six-stage microbial sampler was used to collect the culturable bacteria, fungi and *Escherichia coli*. Furthermore, 16 S rRNA sequencing technology was applied to identify the different sized bacterial aerosols and the bacteria in the respiratory tract of piglets, then aimed to compare the similarity between the both. This study provides a better understanding of the microbial aerosol distribution inside the pig house, and also reveals the relationship between the bacteria aerosols with the bacteria in the respiratory tract of pigs. The study will help us better evaluate the potential risk of microbial aerosols inside the animal houses.

Materials And Methods

Description of the nursery pig house

The nursery pig house was located in the city of Huaian in Jiangsu Province, China (30°45'-35°20' northern latitude, 116°18'-121°57' east longitude). The house was 24.0 m long, 9.0 m wide and 2.5 m high. There was a 1.5 m-deep basement manure pit under the slatted floor. During the experiment, all fans were closed and the heat lamps were turned on all the time. The population of the nursery pigs was 396, aged approximately 4-weeks old. The nursery pigs were fed manually at 7:00, 11:00 and 17:00.

Measurement of environmental indexes inside and outside the nursery pig house

The measurement of environmental indexes was conducted from 18^{th} to 20^{th} in January, 2018. The monitoring was conducted inside (in the middle of the house 0.5 m above the floor, the breathing height of the nursery pigs) and outside the nursery pig house (5 m from the house). DustTrak II model 8533 aerosol monitor (TSI Inc., Shoreview, USA) was used to measure the concentrations of TSP, PM_{10} and $PM_{2.5}$, based on the principle of dynamic light-scattering laser. The PM concentration was detected per second, then the mean value was stored in the device per minute. Model 1412 photoacoustic multigas monitor (Lumasense Technologies, Inc., USA) was used to determine the concentrations of NH_3, CO_2, CO and NO, and the data were recorded every 30 min. RC-4HC miniature temperature and humidity recorder (Jingchuang Electric Co., Ltd., China) was used to detect the temperature and relative humidity every 30 min.

Determination of airborne culturable microorganisms inside and outside the nursery pig house

Airborne culturable bacteria, fungi, and *Escherichia coli* were collected by a PSW-6 air microorganism sampler (Changzhou Pun Sen Electronic Instrument Factory, China) at the airflow rate of 28.3 L/min [18]. The sample was set inside (in the middle of the house 0.5 m above the floor) and outside (5 m from the house). Luria-Bertani agar medium, rose bengal chloramphenicol agar medium and eosin-methylene blue medium (Solarbio Science & Technology Co., Ltd, Beijing, China) was used to collect airborne culturable bacteria, fungi and *Escherichia coli*, respectively. The sampling time was set at 3 min for each time. Then the samples were taken to the laboratory and cultured at different conditions: 37 °C for 24 h (bacteria, *Escherichia coli*), 28 °C for three days (fungi). The airborne microorganisms were collected at different time points (3:00, 9:00, 15:00, 21:00). The airborne particles were divided into six stages according to the aerodynamic diameters, the first stage (7.0 μm), the second stage (4.7–7.0 μm), the third stage (3.3–4.7 μm), the fourth stage (2.1–3.3 μm), the fifth stage (1.1–2.1 μm), and the sixth stage (0.65–1.1 μm). The formula for calculating unites of colonies in per cubic meter of air is: CFU/m^3= [colony total collected from air microorganism sampler/28.3 L/m^3 × sampling time (min)] × 1000.

16S rRNA gene sequencing

Mouth and nose swab were sampled from three piglets located in the middle of the nursery pig house, then the piglets were slaughtered for sampling bronchus and lung. Porcine samples were collected for three consecutive days, corresponding to the collection time of aerosol. Then the same site of respiratory samples were mixed together, thus four kinds of porcine samples were obtained (mouth swab, nose swab, bronchus and lung). The teflon fiber filter (90 mm diameter, Whatman Inc., Clifton, NJ, USA) were set inside the PSW-6 air microorganism sampler in the middle of the nursery pig house 0.5 m above the floor. Then the PM samples with the desired size fraction were collected. The sample time was 12 h for consecutive three days from 9:00 to 19:00 during daytime. A quarter of the filter at each level was cut and then mixed together, thus six kinds of airborne PM samples were obtained (> 7.0, 4.7–7.0, 3.3–4.7, 2.1–3.3, 1.1–2.1 and 0.65–1.1 μm).

As described above, a total of 10 samples were performed for 16S rRNA MiSeq sequencing. The total genomic DNA was extracted as described previously[39]. The concentration and quality (OD260/OD280) of extracted DNA were detected using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Next, the primers 338 F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806 R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the V3-V4 hyper variable region of the bacterial 16S rRNA gene [19]. The PCR reaction was performed on a Master cycler Gradient (Eppendorf, Germany) using 25 μL reaction volumes, containing 12.5 μL 2 × Taq PCR Master Mix, 1 μL each Primer (5 uM), 2 μL template DNA (10 ng) and 8.5 μL ddH_2O. The PCR process included 95 °C for 5 min, followed by 32 cycles of 95 °C for 45 s, 55 °C for 30 s and 72 °C for 45 s with a final extension at 72 °C for 10 min. Then the PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN, Germany) and paired-end sequenced (2×300) on Illumina Miseq platform. These raw sequences were discarded if they were shorter than 200 bp, had a low quality score (≤ 20), contained ambiguous bases or did not exactly match to primer sequences and barcode tags. The normalized reads were separated using the sample-specific barcode sequences and trimmed with Illumina Analysis Pipeline Version 2.6. Operational taxonomic units

(OTUs) were counted at a similarity level of 97% using UCLUST function in QIIME [20] to generate rarefaction curves and to calculate the richness and diversity indices. The taxonomy of each 16S rRNA gene sequence was analyzed by Ribosomal Database Project (RDP) Classifier algorithm against the Silva database [21]. The alpha-diversity indices (observed species, ACE, Simpson, Shannon and Chao 1) were calculated for each sample, and beta-diversity was analyzed by nonmetric multidimensional scaling (NMDS) based on the unweighted-UniFrac distance matrix, which was conducted to show differences of microbial community structure between groups. We failed to detect bacteria in the size of 0.65-1.1 μm, finally nine samples were analyzed.

Statistical Analysis

All statistical analyses were conducted by GraphPad Prism version 7.01 (GraphPad Software, Inc. CA).The data were analyzed by unpaired t test if they were in Gaussian distribution or by nonparametric test (Mann-Whitney U test) if they were not normally distributed. The data are presented as the mean ± SEM. *p* values less than 0.05 were considered as significantly different.

Results

Measurement of microclimate variables and microorganism concentration inside and outside the nursery pig house

As shown in Table 1, the temperature, concentrations of different-sized particulate matter (TSP, PM_{10}, $PM_{2.5}$), and gas pollutants (NH_3, CO_2, CO, NO) inside the nursery pig house were significantly higher than those outside the house ($p < 0.05$). Meanwhile, lower relative humidity was detected inside the house ($p < 0.05$).

Size and time distribution of airborne culturable bacteria, fungi and *Escherichia coli*

As shown in Figure 1, the concentrations of airborne culturable bacteria, fungi and *Escherichia coli* were compared inside and outside the nursery pig house. The concentration of airborne culturable bacteria inside the nursery pig house was significantly higher than that outside the house ($p < 0.05$). No significant difference of the concentrations of airborne culturable fungi and *Escherichia coli* was observed between the inside and the outside ($p > 0.05$).

The proportions of different-sized airborne culturable bacteria, fungi and *Escherichia coli* were shown (Figure 2 A, B, C). For bacteria, the ratio was reduced with the decrease of aerodynamic diameter of aerosols. Most airborne culturable bacteria were distributed at the first (>7.0 μm) and second stage (4.7-7.0 μm), and the total percentage reached to 57.7%. The least was observed in the sixth stage (0.65-1.1 μm), only accounting for 4.2% (Figure 2 A). For airborne culturable fungi, most were distributed at the fourth stage (2.1-3.3 μm) and the fifth stage (1.1-2.1 μm), and the least were observed at the sixth stage (0.65-1.1 μm) (Figure 2 B). For airborne culturable *Escherichia coli*, they displayed an irregular change

trend; the most were distributed at the first (>7.0 μm) and fourth stage (2.1-3.3 μm), while the least were observed at the fifth stage (1.1-2.1 μm) (Figure 2 C).

As shown in Figure 2 D, E, F, the concentrations of airborne culturable bacteria, fungi and *Escherichia coli* peaked at 15:00, 9:00 and 22:00, respectively. However, no significant difference was found among different time points ($p > 0.05$).

The bacterial taxonomic diversity of PM and respiratory tract of piglets

The bacteria contained in the samples of PM and respiratory tract of piglets were analyzed by sequencing 16S rRNA V3-V4 region. It is noted that the bacteria contained in the particles sized 0.65-1.1 μm failed to be detected. As shown in Figure 3A, no significant difference of bacterial diversity (Shannon and Simpson) and richness (observed species, ACE, Chao 1) indices were observed between the bacterial composition of PM and respiratory tract of pigs. The PCoA analysis also revealed that the samples of PM and respiratory tract of pig were clustered together (Figure 3B). From the hierarchical clustering tree on OTU level (Figure 3C), the samples of the first (>7.0 μm) and the second stage (4.7-7.0 μm) clustered together; the samples of the nose swab, the third stage (3.3-4.7 μm) and the mouth swab clustered together; the samples of the fourth stage (2.1-3.3 μm) and the bronchus clustered together; the samples of the fifth stage (1.1-2.1 μm) and the lung clustered together.

The Bacterial composition of PM and respiratory tract of pigs

A total of 19 phyla, 30 classes, 73 orders, 136 families, and 300 genera were identified in the samples of PM and 17 phyla, 25 classes, 61 orders, 114 families, and 268 genera in the samples of the respiratory tract of pigs. At the phylum level, Bacteroidetes was the most dominant across the samples of the first (>7.0 μm) and second (4.7-7.0 μm) stage, Firmicutes was the most dominant across the samples of the third stage (3.3-4.7 μm) and the mouth swab; Proteobacteria was the most dominant across the samples of nose swap, lung, the fourth and the fifth stage (1.1-2.1 μm) (Figure 4A). No significant difference of any phyla was observed between these two group ($p > 0.05$) (Figure 4B).

At the genus level, the dominant bacteria varied from samples (Figure 5A). Compared with the respiratory tract of pigs, the relative abundance of *Bacillus* was higher in the samples of PM ($p < 0.05$) (Figure 5B). There were four potential pathogenic bacterial genera were identified from all the samples, including *Escherichia-Shigella*, *Streptococcus*, *Acinetobacter* and *Pseudomonas* (Table 2). The genus *Escherichia-Shigella* accounted for very large proportion of the fourth stage (2.1-3.3 μm), the fifth stage (1.1-2.1 μm), the bronchus and lung. The genus *Streptococcus* was the most abundant in the third stage (1.1-2.1 μm) and the mouth swab. The genus *Acinetobacter* was the most abundant in the second stage (4.7-7.0 μm). The genus *Pseudomonas* was the most abundant in the fourth stage (2.1-3.3 μm) and the bronchus.

Discussion

The environmental indicators were monitored inside and outside the nursery pig house in winter. To keep warm for the nursery pigs, the heating method of warm air furnace was adopted inside the house. Hence, the temperature inside the house was higher than the outside, accompanying with lower relative humidity. The average concentrations of TSP, PM_{10} and $PM_{2.5}$ were 1.28, 0.57 and 0.25 mg/m^3, respectively. The occupational exposure limit for TSP was 2.5 mg/m^3 for pig farmers and 3.7 mg/m^3 for animals, respectively [22-23]. In the present study, the TSP concentration was within the range. Compared with the World Health Organization air quality guideline, 50 μg/m^3 for PM_{10} and 25 μg/m^3 for $PM_{2.5}$, our results markedly exceeded this threshold. The concentrations of PM in nursery pig houses varied in some studies. Shen et al. [24] reported that the concentrations of TSP, PM_{10} and $PM_{2.5}$ inside were 0.635, 0.388 and 0.210 mg/m^3, respectively. Kwon et al. [25] reported that the concentration of TSP and PM_{10} was 1.5 mg/m^3 and 1 mg/m^3, respectively. Our results were in the range of these values.

The current total concentrations of airborne culturable bacteria, fungi and *Escherichia coli* was 3.46×10^3, 648 and 88 CFU/m^3, respectively. Jo WK et al.[26] reported the concentration of airborne culturable bacteria and fungi inside the swine houses in Korea was 1.34×10^5 and 454 CFU/m^3, respecively. Duchaine et al.[27] reported that the airborne culturable fungi concentration inside swine buildings in Canada ranged from 547 to 2.9×10^3 CFU/m^3. Kim et al.[28] measured the airborne culturable bacteria concentration inside a nursery pig house in winter, ranging from 4.6×10^3 to 7.6×10^3 CFU/m^3. Compared with the above studies, the bacterial concentration in our research was lower, and the fungal concentration was similar. This difference of concentrations of particulate matter and airborne microorganisms can be attributed to many factors, including animal, housing system, management and season[11].

According to our results, the airborne culturable bacteria with aerodynamic diameter larger than 3.3 μm (stage 1 to 3) accounted for 74.6%, and the size ranging from 0.6 to 3.3 μm (stage 4 to 6) accounted for 25.4%. For airborne culturable fungi with aerodynamic diameter larger than 3.3 μm (stage 1 to 3) accounted for 45.4%, and the size ranging from 0.6 to 3.3 μm (stage 4 to 6) accounted for 54.6%. In the present study, the aerosols smaller than 3.3 μm (stage 4-6) were defined as fine particles, because the air microorganism sampler does not have a cut-off point sized 2.5 μm . Hence, the aerosols larger than 3.3 μm (stage 1-3) were defined as coarse particles. The above results implied that the airborne culturable bacteria were dominant in coarse particles rather than in fine fraction. However, for airborne culturable fungi, the proportion in fine particles was slightly higher than that in coarse particles. Other studies also used six-stage cascade impactor to identify the culturable microorganisms. Kim et al.[28] reported that the airborne bacteria sizes smaller than 3.3 μm (stage 4 to 6) accounted for 40% of the total inside the nursery pig house, which was a little higher than our result. As reported, in Beijing city, the percentages of airborne culturable bacteria and fungi at fine particle sizes (stage 4 to 6) ranged from 15.34% to 45.95% and from 32.0% to 63.81% in winter, respectively, which was similar with our results[29].

It is not known from where we obtain our putative bacterial lung microbiota however it is most likely to be in a flux state with the environment. The airborne microorganisms contained in PM can be breathed into

the respiratory tract. The ability of aerosols to enter the respiratory tract depends on their dynamic diameters. In the current study, this is the first time to use high-throughput sequencing technology to identify the bacterial composition of different-sized particles and respiratory tract of pigs. The bacterial composition between the third stage and mouth and nose swab, between the fourth stage and bronchus, between the fifth stage and lung exhibited great similarity. Few studies performed this analysis, hence it is hard to compare with other results. However, some research studied the deposition of different sized PM in human respiratory tract. Liu et al. found that 2.5-10 μm PM had the highest deposition mass in nasopharyngeal, and 1-2.5 μm PM had the highest deposition mass in lung [30]. When a grown man did light exercise outside the environment, 80-90% aerosols could deposite in the respiratory tract, including about 70% in the upper respiratory tract, 5-7% in the alveoli, and 3% in the bronchial couple with bronchiolar regions[29]. To some extent, these results also confirmed the relationship between the bacteria contained in PM and the respiratory tract. In addition, in the present study, although the specific relative abundance of the upper respiratory bacteria was different from those of the lower respiratory tract, they have similar composition. The upper and lower respiratory bacteria shared the common bacteria at the phylum and genus level. There are several factors affecting the composition and diversity of pulmonary microbiota, the type and number of microorganisms immigrating into the lungs; the elimination race of microorganisms from the lung, and the reproduction rates of the microorganism itself in the lungs[31, 32].

The dominant bacterial phyla in bronchus and lung were Proteobacteria, Bacteroidetes, Firmicutes and unclassified_k_norank_d_Bacteria. In mouse lung, Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes and Cyanobacteria were the most abundant bacteria phyla[33]. When analyzing the bacteria in bronchoalveolar lavage from healthy adults, Beck et al. found that Bacteroidetes, Firmicutes, and Proteobacteria were the most common bacterial phyla[17]. From the results, we can conclude that the pulmonary bacterial composition of animals and human have a great similarity at the phylum. While the dominant genera (*Escherichia-Shigella*, *Empedobacter*, and *unclassified_k_norank_d_Bacteria*) in the lung of pigs were very different from those of the human beings (*Prevotella*, *Veillonella*, *Pseudomonas*, *Fusobacteria*, and *Streptococcus*)[17]. This difference can be attributed to the geography, including the climate, environmental mictobiota [34, 35].

In addition, four potential bacterial genera (*Escherichia-Shigella*, *Streptococcus*, *Acinetobacter*, *Pseudomonas*) were found in the samples of aerosol and respiratory tract of pigs. The relative abundance of the genus *Escherichia-Shigella* accounted for a large proportion in the size of 1.1-3.3 μm bacterial aerosols and the lower respiratory tract of piglets; Among the genus *Escherichia-Shigella*, the Shigella can cause severe diarrhea disease, particularly in children and infants[36, 37]. *Escherichia coli*, as a common species of *Escherichia-Shigella*, is a conditional pathogenic bacteria. Pathogenic strains of *Escherichia coli* can cause diseases both in people and animals. For people, they can cause diarrhea, neonatal meningitis, hemolytic uremic syndrome, urinary tract infections, and hemorrhagic colitis. For pig, they can cause diarrhea and edema disease during post-weaning[38]. In the current study, the genus *Streptococcus* was dominant in the nose and mouth swab. *Streptococcus suis*, as the common pathogenic bacteria among the genus *Streptococcus*, naturally inhabited the upper respiratory tract of

pigs, particularly the tonsils and nasal cavities[39].*Streptococcus suis* can cause wide manifestations of diseases, including septicemia, meningitis, endocarditis, pneumonia and arthritis. However, in peracute cases of infection, pigs are usually found dead with no premonitory signs of disease[40]. Hence, isolation and characterization of the pathogenic bacteria is necessary [39]. The genus *Pseudomonas*, important gram-negative bacteria, are widely distributed in the environment. The *Pseudomonas* infections are primarily due to *Pseudomonas aeruginosa*. It can infect many tissues, such as the lung, throat, urinary tract, blood stream, bone, skin, ear, eye, and central nervous system[41]. *Acinetobacter* are widely distributed in nature. *Acinetobacter baumannii*, as a typical opportunistic gram-negative pathogen, can cause pneumonia, urinary tract, bloodstream and burn wound infections[42].

In conclusion, the concentration of airborne culturable bacteria inside the nursery pig house was high than that outside. And the 16 S rRNA results showed that the bacteria aerosol inside the pig house had high similarity with the bacterial composition in the respiratory tract of pigs. And we also identified several potential pathogenic bacteria genera both in the aerosol and respiratory tract. Together, the airborne microorganisms are an important factor to evaluate the potential risk of air quality inside animal houses.

Abbreviations

PM: particulate matter; COPD: chronic obstructive pulmonary disease; OTUs: Operational taxonomic units; RDP: Ribosomal Database Project; NMDS: nonmetric multidimensional scaling.

Declarations

Acknowledgments

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Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Authors' contributions

Qian Tang and Chunmei Li designed the experiments. Qian Tang and Kai Huang performed the experiments and analyzed the experimental data. Tang Qian wrote this paper. Kai Huang and Junze Liu assisted with sampling and laboratory analyses. Xiaoming Jin and Chunmei Li participated in editing the paper. All authors read and approved the final manuscript.

Ethics approval

All procedures performed in studies involving animals were conducted according to the Guidelines for the Care and Use of Animals of Nanjing Agricultural University, 1999. The study was approved by the Ethical Committee of Nanjing Agricultural University, Nanjing, China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1: The microclimate variables and different sized-PM concentrations measured inside and outside the nursery pig house

	Outside the nursery house	Inside the nursery house
Temperature (°C)	3.74±0.24	20.82±0.40*
Relative Humidity (%)	82.74±0.46	51.34±0.30*
TSP (mg/m^3)	0.39±0.01	1.28±0.06*
PM_{10} (mg/m^3)	0.17±0.01	0.57±0.02*
$PM_{2.5}$ (mg/m^3)	0.15±0.01	0.25±0.01*
NH_3 (mg/m^3)	7.91±0.01	8.22±0.01*
CO_2 (mg/m^3)	1622.50±9.63	2053.33±8.24*
CO (mg/m^3)	9.06±0.09	9.68±0.05*
NO (mg/m^3)	2.51±0.02	2.74±0.01*

Note: The values are expressed as the mean ± SEM of the group based on 4 measurements per day for 3 days (n=12), *$p < 0.05$. TSP: total suspended particulate; PM: particulate matter.

Table 2: The relative abundance of potential bacterial pathogen genera (%)

Samples	*Escherichia-Shigella*	*Streptococcus*	*Acinetobacter*	*Pseudomonas*
> 7.0	0.01	0	8.27	0.25
4.7-7.0	0.40	0.01	19.81	1.15
3.3-4.7	0.08	24.07	0.38	0.01
2.1-3.3	48.74	0.63	2.04	6.76
1.1-2.1	32.81	0.26	3.10	1.07
Nose swab	0.10	11.27	0.36	0.02
Mouth swab	0.02	24.62	0.35	0.01
Bronchus	48.05	0.67	1.95	6.92
Lung	33.02	0.29	3.01	1.15

Figures

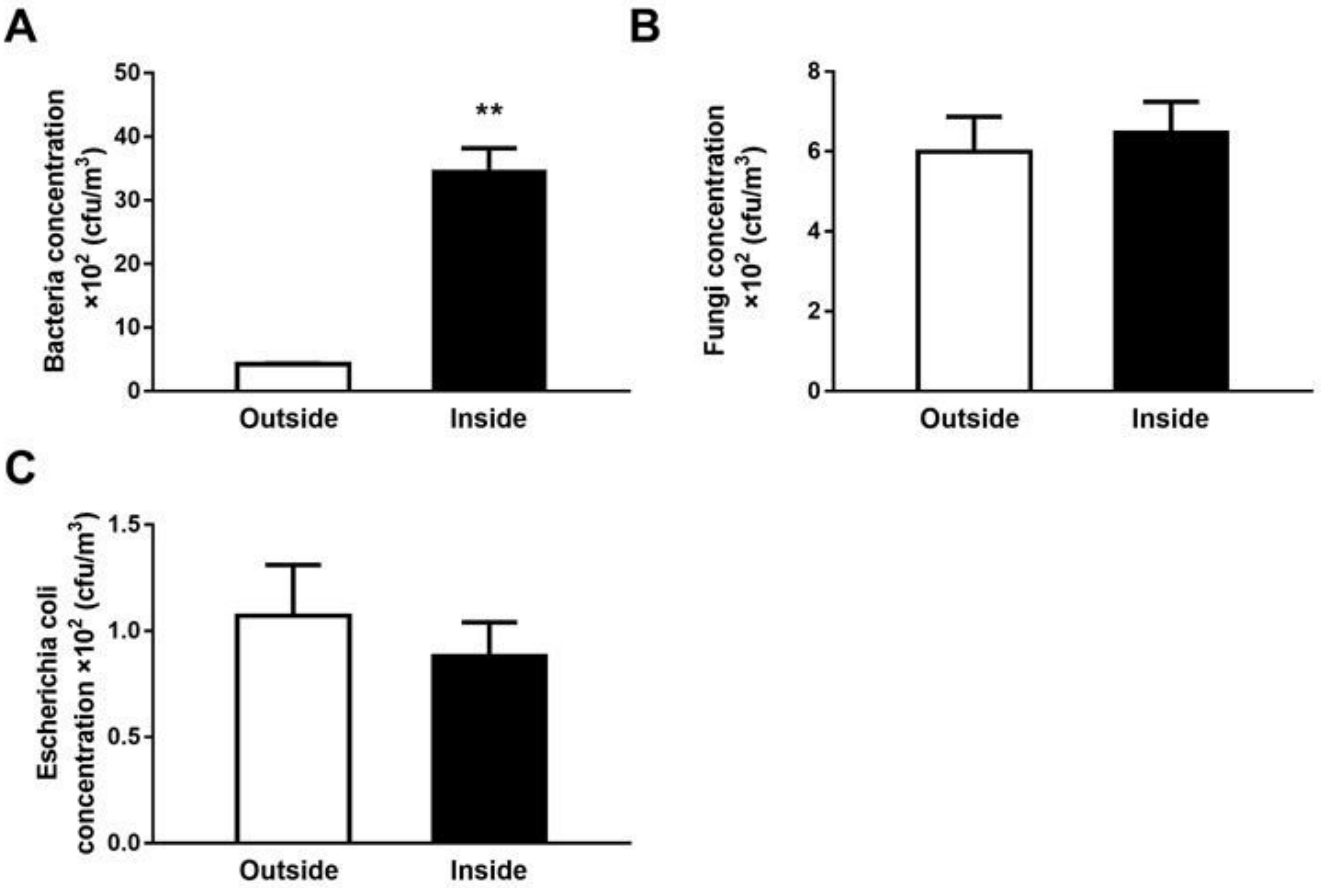

Figure 1

Comparison of concentrations of airborne bacteria (A), fungi (B) and Escherichia coli (C) inside and outside the nursery pig house. Values are expressed as the mean ± SEM (n=3). ** p < 0.01.

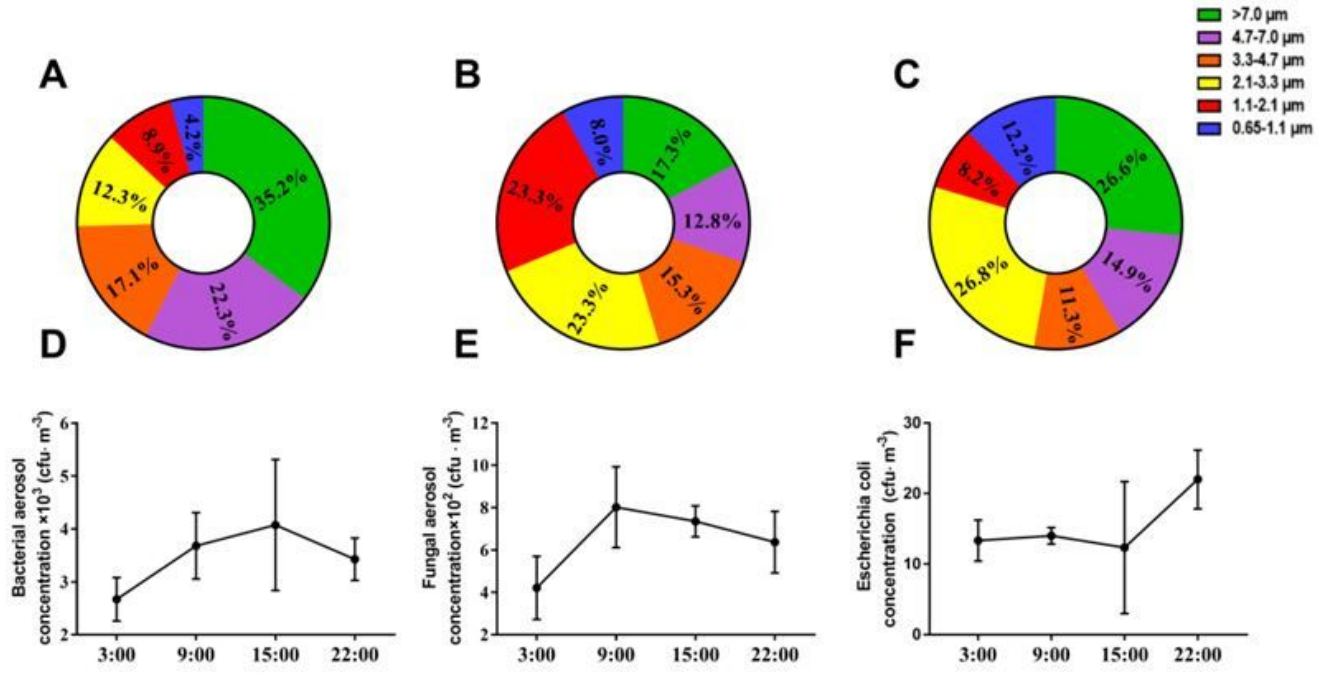

Figure 2

The size and time distribution of the airborne microorganisms inside the nursery pig house. The size distribution of airborne bacteria (A), fungi (B) and Escherichia coli (C), and the values are shown as the mean (n=3). The time distribution of airborne bacteria (A), fungi (B) and Escherichia coli (C), and the values are shown as the mean ± SEM (n=3).

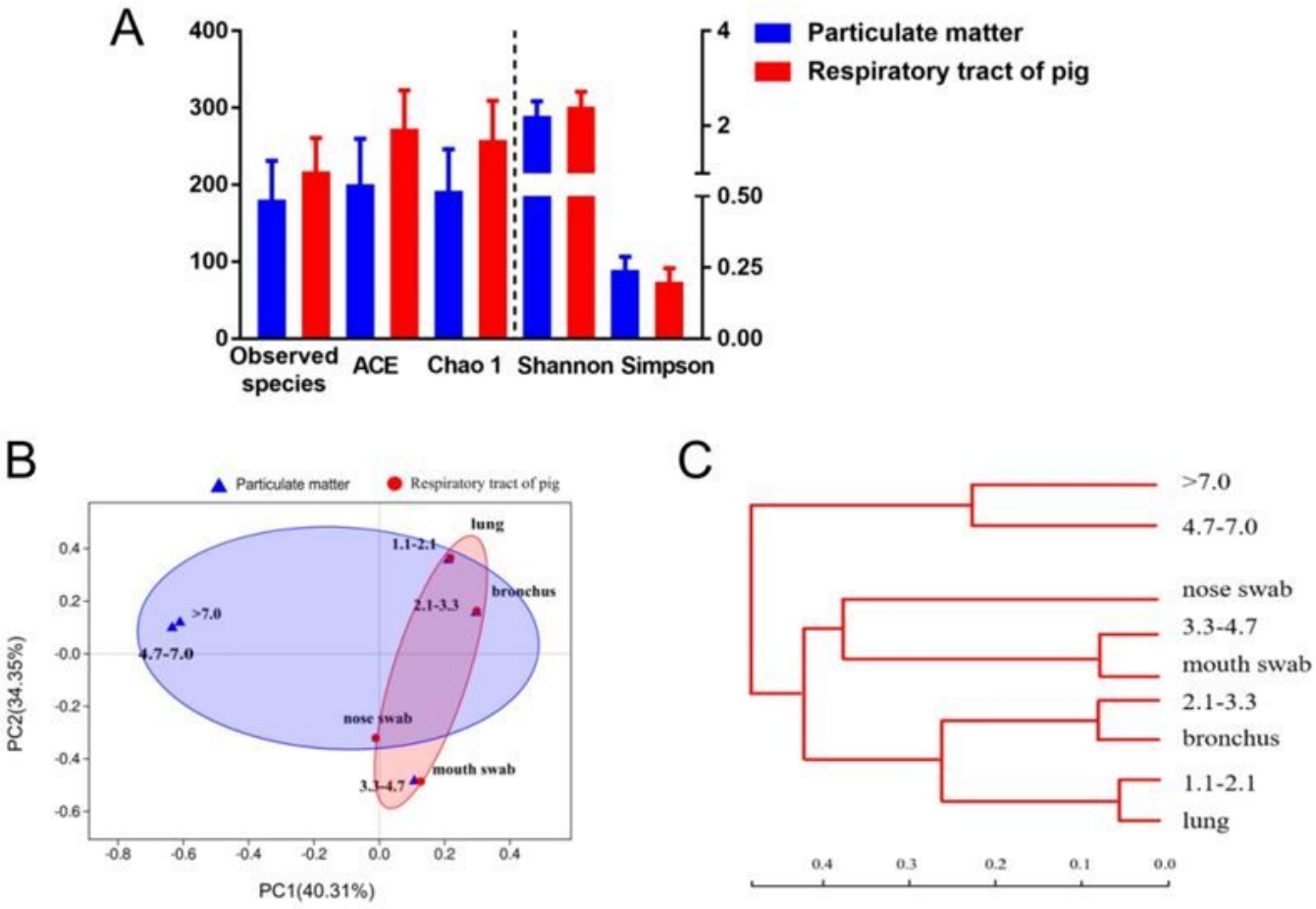

Figure 3

The alph- and beta-diversity of the bacteria contained in the particulate matter and the respiratory tract of piglets. Bacterial diversity (Shannon and Simpson) and richness (observed species, ACE, Chao 1) indices were determined and the data are shown as the mean ± SEM (n=4-5) (A). Nonmetric multidimensional scaling (NMDS) based on unweighted-UniFrac distance matrix of OTUs (B). The hierarchical clustering tree on OTU level (C).

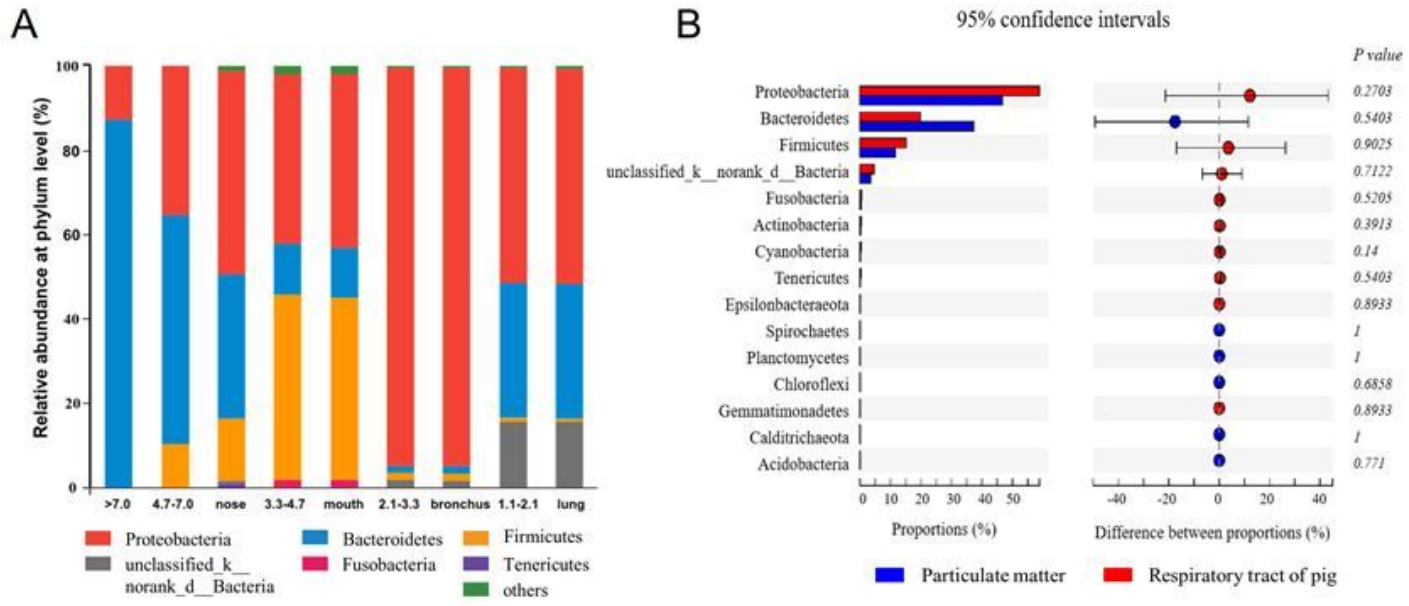

Figure 4

Phylogenetic classification of the bacteria contained in the particulate matter and the respiratory tract of piglets at the phylum level. The relative abundance of the dominant phyla are shown in each sample (A). The statistical significance was compared among the top 15 phyla between these two groups (n=4-5) (B).

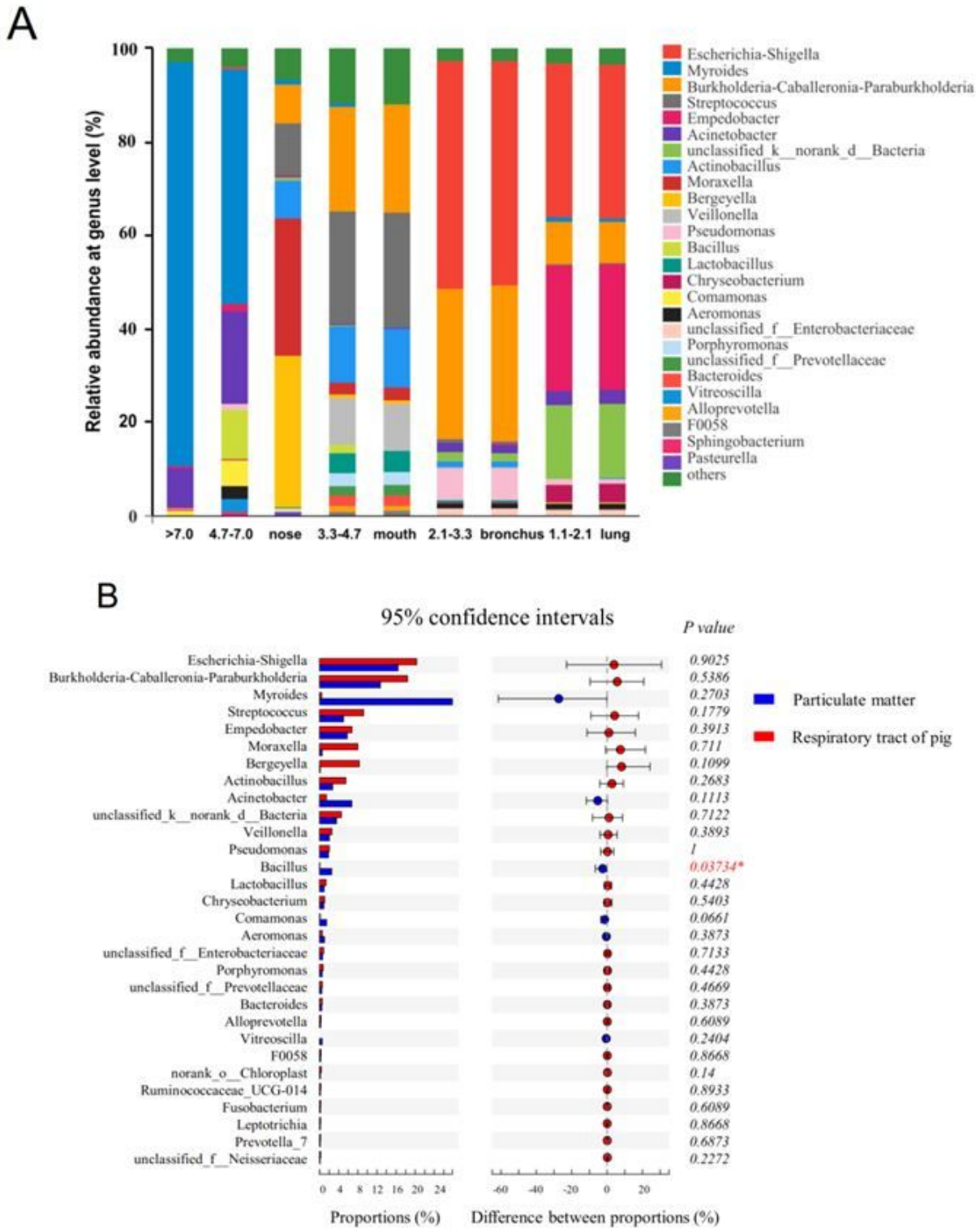

Figure 5

Phylogenetic classification of the bacteria contained in the particulate matter and the respiratory tract of piglets at the genus level. The relative abundance of the dominant genera were shown in each sample (A). The statistical significance was compared among the top 30 genera between these two groups (n=4-5) (B), * $p < 0.05$.