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Original Research Article

Comparative analysis of fecal microbiota between diarrhea and non-diarrhea piglets reveals biomarkers of gut microbiota associated with diarrhea

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ABSTRACT

Diarrhea poses a significant threat to the health and well-being of weaned piglets, leading to substantial morbidity and mortality and economic loss in the pig industry. However, the structural characteristics of the gut microbiota and the key genera associated with early diarrhea in piglets within large-scale production systems are poorly understood. This study aimed to investigate the differences in the microbial community structure and the specific genera alteration between the healthy piglets and diarrhea piglets, and to identify the biomarkers of gut microbiota associated with diarrhea in piglets. A total of 250 fecal samples, including 130 healthy piglets (Duroc × Landrace × Large Yorkshire) in the Control group and 120 from diarrhea piglets in Diarrhea group, were collected from three large-scale farms as discovery cohorts and were used for 16S rRNA gene sequencing. Additionally, 150 fecal samples from another large-scale pig farm were collected for the validation trail. The Chao1 and ACE indices were obviously lower ($P < 0.01$) in the diarrhea piglets compared to the healthy ones. Principal coordinate analysis showed significant differences in the distance matrix of gut microbiota between the healthy and diarrhea piglets (Bray-Curtis: $P = 0.001$, Jaccard: $P = 0.001$). Eighty-five genera were differentially enriched ($P < 0.001$) between healthy and diarrhea piglets. Notably, *Treponema*, *Sphaerochaeta*, *Escherichia-Shigella*, *Slackia*, and *Staphylococcus* were identified as potential biomarkers of diarrhea susceptibility; *Clostridium sensu stricto 1*, *Prevotella_9*, *Olsenella*, *Dorea*, and *Lachnospiraceae NK4A136 group* were found to be beneficial for maintaining intestinal homeostasis. These differentially enriched genera of healthy and diarrhea piglets were further confirmed in the validation cohort. In conclusion, this study identified the diarrhea-associated and beneficial genera in the faces of piglet, providing a theoretical basis for the diagnosis and intervention of diarrhea in weaned piglets.

1. Introduction

Diarrhea is an important cause of morbidity and mortality in piglets, especially during weaning (Xiao et al., 2012). In modern pig production, early weaning is generally performed at 3 to 4 weeks of age to improve economic efficiency (Sutherland et al., 2014). The piglets at early weaning stage are subjected to nutritional, physiological, and psychological stressors, leading to diarrhea and gut injury (Wijtten et al., 2011). Diarrhea caused by early weaning stress is one of the most serious health problems faced by the pig

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industry, resulting in significant economic losses (Gresse et al., 2017). Weaning stress can damage the intestinal barrier and immune function of pigs, leading to diarrhea (Wang et al., 2023). It has been reported that diarrhea changes the microbial community in the gut, such as decreasing the proportion of probiotic bacteria and increasing the proportion of pathogenic bacteria (Liu et al., 2015).

Diarrhea in weaned piglets is associated with gut microbiota dysbiosis. The proportion of members of the phyla Fusobacteria and Verrucomicrobia, and most of the pathogenic genera such as *Enterococcus*, *Lactococcus* and *Escherichia-Shigella* were higher in diarrhea pigs than in healthy pigs (Bin et al., 2018; Koh et al., 2015; Song et al., 2017; Yan et al., 2017). *Escherichia coli* can adhere to the intestinal epithelial cells via surface structures, secrete enterotoxins and interact with the intestinal epithelial cells through various plasmid-encoded colonization factor genes encoding fimbrial, non-fimbrial and fibrillar structures, resulting in severe diarrhea in piglets (Luise et al., 2019; Pakbin et al., 2021). An oral challenge with an *E. coli* reduced the fecal microbiota diversity and decreased the beneficial genera such as *Prevotella* in the gut of diarrhea piglets (Rhouma et al., 2021). In addition to *E. coli*, *Treponema* was found to exert pathogenic synergism with two anaerobes (*Bacteroides vulgatus* and *Fusobacterium necrophorum*) to cause diarrhea in pigs (Whipp et al., 1979). Moreover, alterations were observed in the microbial diversity and composition in the gut causing diarrhea in *Salmonella Typhimurium*-challenge piglets (Li et al., 2024). Although specific pathogens are commonly used to establish infection models to explore changes in gut microbiota in diarrhea piglets (Breyer et al., 2024; Navez et al., 2023), few studies have systematically analyzed the relationship between gut microbiota and diarrhea in large-scale pig farm production. Therefore, a comprehensive review of the genera associated with healthy and diarrhea piglets in the context of pig production is needed.

In this study, the gut microbiota profiles of healthy and diarrhea piglets from 3 different farms were compared, and diarrhea-associated and beneficial genera were identified in the discovery cohort. Subsequently, a validation cohort study was performed to confirm the identified genera. Our findings contribute to identifying key biomarkers of diarrhea and providing new insights of early diagnosis of piglet diarrhea.

2. Methods and materials

2.1. Animal ethics statement

All animal experiments were approved by the Institutional Animal Care of the Zhejiang Academy of Agricultural Sciences in accordance with the relevant rules and regulations (2022ZAASLA014).

2.2. Experimental design and diets

Trial 1 (discovery cohort): To explore the biomarker microbiota associated with piglet diarrhea.

A total of 1500 weaned piglets (Duroc × Landrace × Large Yorkshire) at 21 d old with similar body weight (BW; 5.93 ± 0.61 kg) were selected and fed with corn-soybean meal (Table 1) in 3 typical large-scale pig farms (Farm 1–3). The crude protein, calcium (Ca), total phosphorus (P), and amino acids content in diet were measured according to China National Standards (GB/T 6432-2018, GB/T 6436-2018, GB/T 6437-2018, and GB/T 18246-2019, respectively). The diets were formulated to meet the requirements for pigs as recommended by NRC (2012). Fecal scores were recorded daily for 7 d as previously described: 0, normal; 1, pasty; 2, semi-liquid; and 3, liquid (Liu et al., 2010). After average fecal score was calculated, piglets with average fecal score ≥1 were set as the diarrhea group (Group D) and piglets with average fecal score <1 were set as the control group (Group H) (Wang et al., 2022). When 120 fecal samples were collected from piglets in Group D, 130 fecal samples were collected from piglets in Group H, which came from the same litters as those in Group D. Fecal samples were stored at −80 °C until DNA extraction. The piglets were weighed and feed intake was recorded on d 21 and 28 (Table S1).

Table 1
Ingredients and nutrient composition of basal diet.

Item	Content
Ingredients, g/kg, as-fed basis	
Corn	551
Extruded corn	100
Soybean meal	150
Extruded soybean	120
Fish meal	30
Soybean oil	18
Limestone	5
Dicalcium phosphate	10
Sodium chloride	3
L-Lysine HCl	2
DL-Methionine	1
Vitamin-mineral premix[1]	10
Calculated nutrient composition, g/kg	
Digestible energy[2], MJ/kg	14.1
Crude protein	180.5
Lysine	13.1
Methionine	3.9
Calcium	7.4
Total phosphorus	6.4
Analyzed nutrient composition, g/kg, DM basis	
Crude protein	181.3
Lysine	13.2
Methionine	4.0
Calcium	8.1
Total phosphorus	6.8

[1] Supplied per kilogram of diet: vitamin A, 17,500 IU; vitamin D_3, 5000 IU; vitamin E, 50 IU; vitamin K_3, 5.0 mg; riboflavin, 12.5 mg; thiamine, 5.0 mg; pyridoxine, 5.0 mg; pantothenic acid, 25 mg; folic acid, 2.5 mg; biotin, 0.2 mg; vitamin B12, 0.05 mg; Zn, 80 mg; Cu, 6 mg; I, 0.14 mg; Fe, 100 mg; Mn, 4 mg; Se, 0.3 mg.
[2] Digestible energy was calculated from data provide by Table of China Feed Composition and Nutrimental Values (Institute of Animal Science of CAAS and other institutes, 2022).

Trial 2 (validation cohort): To validate the identified biomarker microbiota in diarrhea piglets.

A total of 506 piglets at 21 d old originating from another typical large-scale pig farm (Farm 4) and 150 fecal samples were collected for the validation trial. From d 1 to 7 after weaning, 1 sample of diarrheal feces (Group D) and 2 samples of healthy piglet feces from the same litter were collected as controls (Group H). The methods of sample collection were the same as in trial 1.

2.3. DNA extraction and purification

Bacterial DNA was extracted from the fecal samples using the QIAamp DNA Stool Mini Kit (Mo Bio Laboratories, San Diego, United States) according to the manufacturer's instructions. The quantity and quality of DNA were determined by a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, United States). The V4–V5 hypervariable region of the 16S rRNA gene was then amplified by polymerase chain reaction (PCR) using two universal eubacterial primer pairs 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′), which were synthesized by Invitrogen Life Technologies (Shanghai, China). The PCR amplification was conducted using TransGen AP221-02: TransStart FastPfu DNA polymerase (TransGen Biotech, Beijing, China) and performed in a GeneAmp 9700 thermal cycler (Applied Biosystems,

Foster City, United States). The PCR reaction conditions were as follows: 95 °C for 3 min followed by 27 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, with a final extension at 72 °C for 10 min. The PCR reactions were executed in triplicate using a 20-μL mixture, comprising 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 μmol/L dNTPs, 0.8 μL of each primer (5 μmol/L), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. The PCR products were excised from a 2% agarose gel after electrophoresis, and purified using the AxyPrep DNA Gel Extraction Kit (Axygen, Union City, United States) and then quantified using QuantiFluor-ST (Promega, Madison, United States). The unprocessed sequencing reads have been duly deposited in the NCBI Sequence Read Archive (SRA) database for archival purposes (https://www.ncbi.nlm.nih.gov/sra/PRJNA984003).

2.4. 16S rRNA gene sequencing and data processing

The V4–V5 region of the 16S rRNA gene was sequenced on the Illumina MiSeq PE250 sequencing platform according to the manufacturer protocols. The 16S rRNA-derived sequence inventories were processed using the Quantitative Insights into Microbial Ecology (QIIME) platform. The Illumina raw data was filtered to remove low quality reads. The sequences with a mean quality score of no less than 20 and a length longer than 250 bp were retained. The UPARSE software (http://drive5.com/uparse/) was used for read clustering and the cutoff (based on 97% similar identity) for operational taxonomic Units (OTUs). More detailed information of 16S rRNA gene sequencing was performed and analyzed according to a previous study (Ma et al., 2024).

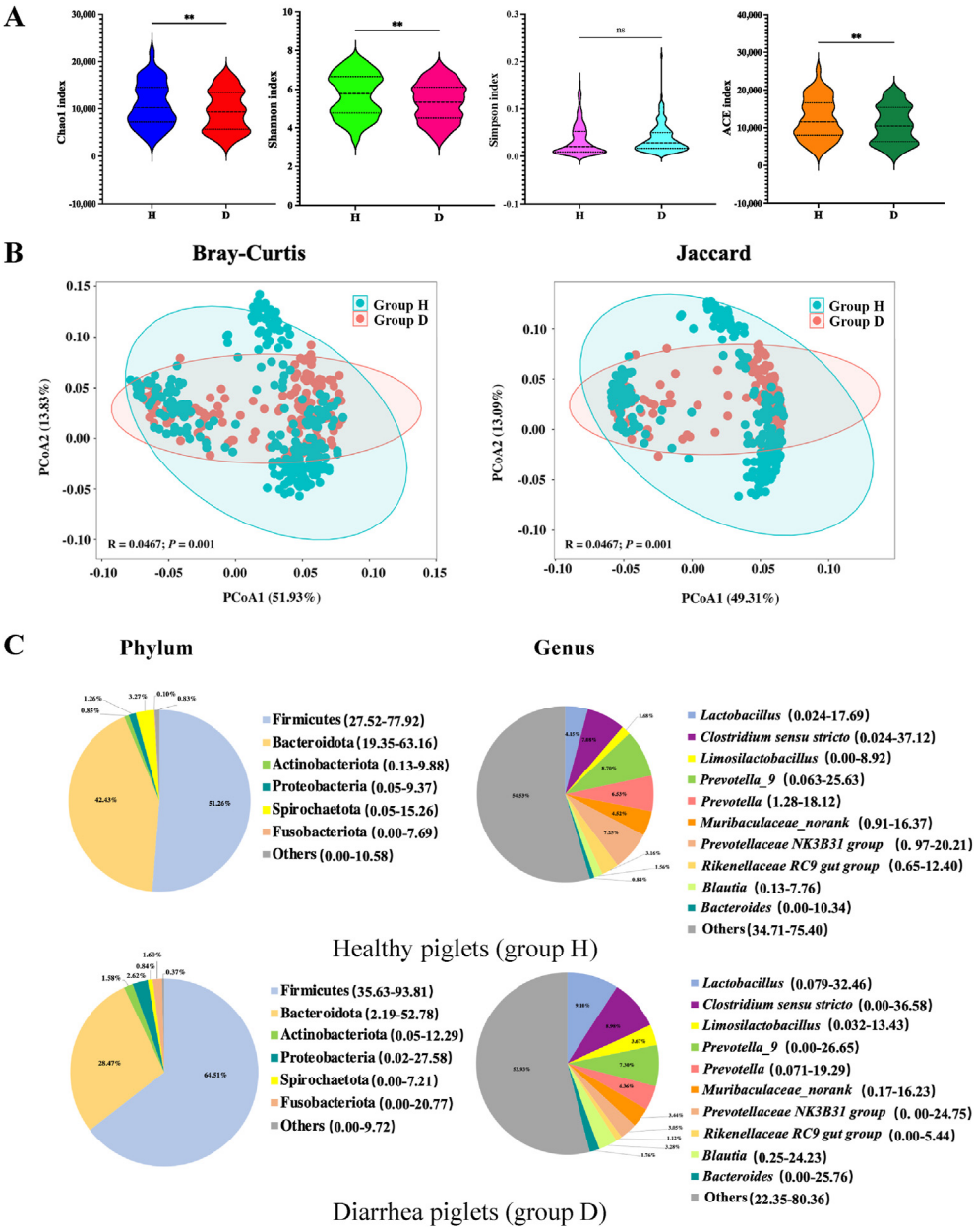

Fig. 1. Microbial diversity and community structure in the feces of healthy (group H) and diarrhea (group D) piglets. (A) The Chao1 index, Shannon index, Simpson index and ACE index (**$P < 0.01$). (B) PCoA based on Bray-Curtis and Jaccard distances ($P = 0.001$). (C) Microbial community structure in the feces of healthy piglets (group H) and diarrhea piglets (group D) at phylum and genus levels. ns = no significance.

2.5. Statistical analysis

Alpha diversity indices (Chao1 and Shannon indices) and beta diversity (Bray-Curtis and Jaccard) matrices were calculated using Mothur software (http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity). Linear discriminant analysis effect size (LEfSe) (http://huttenhower.sph.harvard.edu/lefse/) was used to identify differences in the taxa between healthy and diarrhea piglets. Correlation analysis was performed using Spearman's rank correlation test. The results were visualized in Graphpad (San Diego, CA, USA). Differences were considered significant when $P < 0.05$.

3. Results

3.1. Microbial diversity and community structure between diarrhea and non-diarrhea piglets

The Chao1, Shannon and ACE indices were used to determine the bacterial community richness, which was significantly decreased in diarrhea piglets compared to healthy piglets (Fig. 1). Principal co-ordinates analysis (PCoA) plots showed that the clusters of diarrhea and healthy groups were completely separated (Fig. 1 and Fig. S1).

Firmicutes, Bacterioidetes, and Proteobacteria were the dominant phyla in the piglet feces, which accounted for more than 90% of the total sequences in most samples (Fig. 1C). The Firmicutes to Bacteroidetes ratios were 2.27 and 1.21 in the diarrhea piglets and the healthy piglets, respectively. The most abundant genera included *Lactobacillus*, *Clostridium sensu stricto 1*, *Limosilactobacillus*, *Prevotella_9*, *Prevotella*, *Muribaculaceae*, *Prevotella NK3B31 group*, *Rikenellaceae RC9 gut group*, *Blautia*, and *Bacteroidetes*, which contained nearly 50% or more of the total sequences in most samples.

3.2. Identification of the differentially enriched genera between the diarrhea and non-diarrhea piglets

A total of 85 different genera were identified based on LEfSe analysis with LDA >2.0, which included 37 diarrhea-associated genera and 48 non-diarrhea-associated genera (Table S2). The diarrhea-associated genera mainly consisted of *Escherichia-Shigella*, *Slackia*, *Staphylococcus*, *Treponema*, *Sphaerochaeta*, *Lactobacillus*, *Limosilactobacillus*, *Collinsella*, and *Mogibacterium* ($P < 0.001$). The non-diarrhea-associated genera mainly consisted of *C. sensu stricto* 1, *Prevotella*, *Prevotellaceae NK3B31 group*, *Rikenellaceae RC9 gut group*, *Prevotella_7*, *Prevotella_9*, *Olsenella*, *Dorea*, *Lachnospiraceae NK4A136 group*, and *NK4A214 group* ($P < 0.001$). Differentially enriched genera are shown in a heatmap based on their relative abundance (Fig. 2A). Correlation heatmaps are used to show the relationship among these 85 different genera (Fig. 2B). The identified genera associated with diarrhea are colored in the co-occurrence patterns (Fig. 3 and Fig. S2).

3.3. Correlation analysis of fecal scores and identified genera

Correlation analysis was conducted to investigate the relationship between fecal score and relative abundance of genera, suggesting a strong relationship with each other. The higher the relative abundance of *Treponema*, *Sphaerochaeta*, *Escherichia-Shigella*, *Slackia* and *Staphylococcus*, the higher fecal scores were observed. Trends of *C. sensu stricto 1*, *Prevotella _ 9*, *Olsenella*, *Dorea* and *Lachnospiraceae NK4A136 group* were inverse (Fig. 4, Fig. S3).

3.4. Microbial diversity and community structure of piglets in validation trial

The Chao1, Shannon and ACE indices in the validation trial followed the same trend in discovery trial, being significantly decreased in diarrhea piglets (Fig. 5). The clusters of diarrhea and healthy groups were completely separated in PCoA plots. The microbial community structure was observed to be similar to the discovery trial (Fig. S4).

3.5. The validation of identified bacterial genera associated with diarrhea in weaned piglets

Based on the 85 different genera identified in the discovery cohort (Trial 1), the differences in relative abundance can be clearly visualised through the heatmap in the validation cohort (Trial 2) (Fig. S5). *Terrisporobacter*, *Limosilactobacillus*, *Treponema*, *Sphaerochaeta*, *Escherichia-Shigella*, *Slackia*, *Staphylococcus* and *Alloprevotella* were found to be in higher abundance in group D. Conversely, *Clostridium_sensu_stricto_1*, *Prevotella*, *Prevotella_9*, *Prevotellaceae NK3B31 group*, *Olsenella*, *Dorea* and *Lachnospiraceae NK4A136 group* were significantly enriched in group H

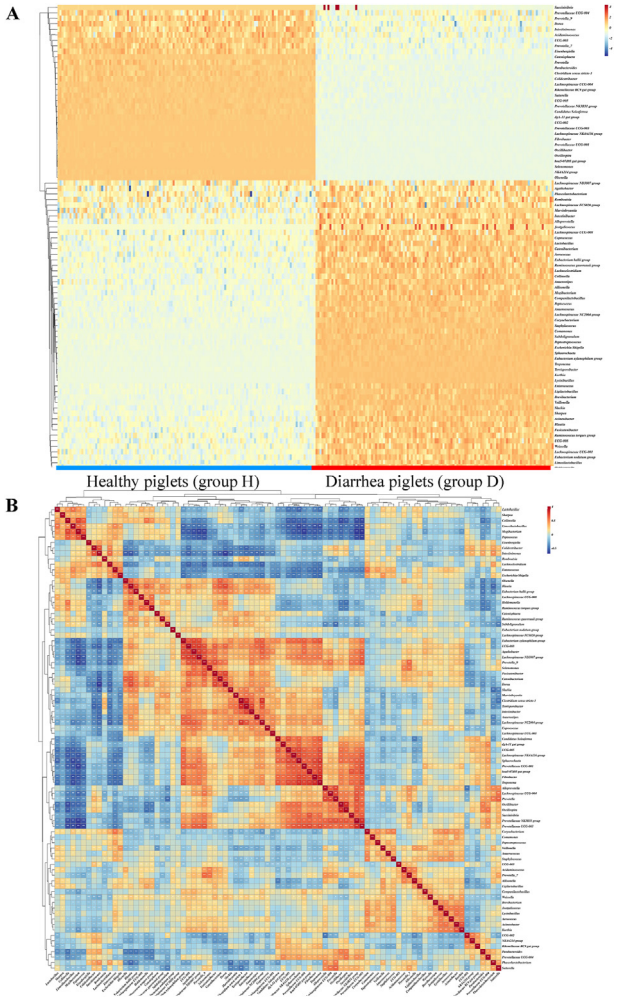

Fig. 2. Different genera in the feces of healthy (group H) and diarrhea (group D) piglets. (A) The heatmap of 85 differentially enriched bacterial genera between group H (blue bar) and group D (red bar). (B) The correlations between the different genera in healthy and diarrhea piglets (*$P < 0.05$, **$P < 0.01$).

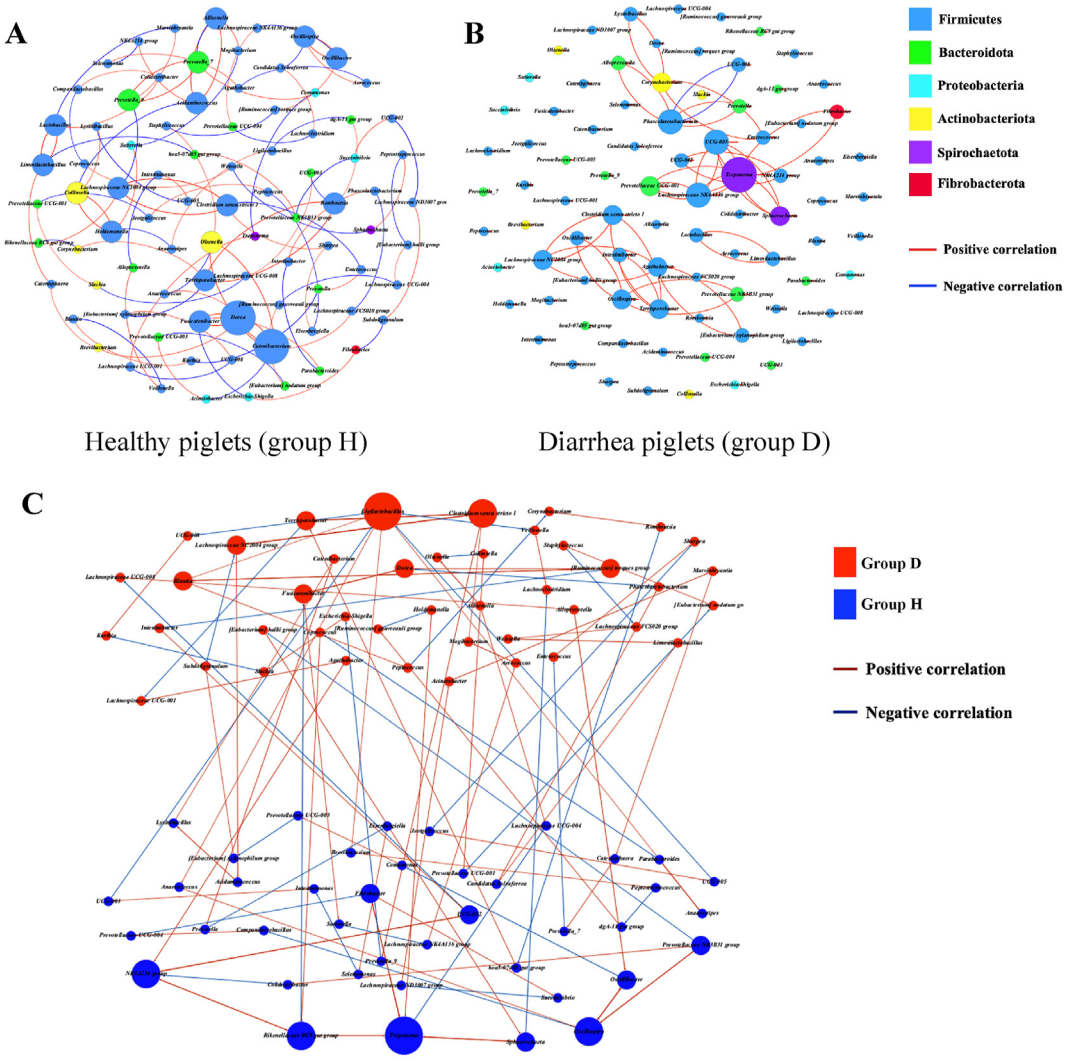

Fig. 3. Correlation analysis of the bacterial community in the feces of piglets. (A) Correlation network of genera across healthy samples of piglet feces. Colorful nodes belong to different phyla. (B) Correlation network of genera across diarrhea samples of piglet feces. (C) Correlation network based on genera between diarrhea piglets (group D) and healthy piglets (group H).

(Fig. S5A). Correlation results indicated that *Lachnospiraceae FCS020* and *Acinetobacter* were highly correlated with other genera (Fig. S5B). The co-occurrence pattern of group H had 84 nodes and 140 edges and the diarrhea group had 81 nodes and 153 edges (Fig. S6A). Furthermore, within group D, notable prominence was attributed to *Marvinbryantia*, *Romboutsia*, *Escherichia-Shigella*, *Treponema*, *Sphaerochaeta*, and *Staphylococcus*. The nodal properties of *C. sensu stricto 1*, *UCG-008*, *Prevotella 9*, *Olsenella*, *Dorea*, and the *Lachnospiraceae NK4A136* assemblage demonstrated greater magnitude in group H. Conversely, the distinctive presence of *Slackia*, *Staphylococcus*, and *Escherichia-Shigella* was discernible within group D, as depicted in Fig. S6B.

3.6. Validation of the correlation between identified specific bacterial genera and fecal score

A correlation analysis was diligently performed to further elucidate the intricate interrelation between the assigned fecal scores and the relative prevalence of identified bacterial genera. Notably, an the abundance of *Treponema*, *Sphaerochaeta*, *Escherichia-Shigella*, *Slackia*, and *Staphylococcus* directly corresponded to higher fecal scores, indicating a heightened severity of diarrhea (Fig. 6A). Conversely, an inverse trend was perceptible in the case of *C. sensu stricto 1*, *Prevotella_9*, *Olsenella*, *Dorea*, and the *Lachnospiraceae NK4A136 group*, as illustrated in Fig. 6A–B and Fig. S7A-B.

4. Discussion

Gut microbiota plays an important role in regulating the digestion, nutrition, metabolism and gut-associated mucosal immunity in pigs (Ji et al., 2023; Ma et al., 2024; Xiao et al., 2018). Gut microbiota dysbiosis may lead to the development of various diseases such as chronic diarrhea and irritable bowel syndrome (Khoo et al., 2023; Kong et al., 2021). Weaning stress commonly induces gut barrier injury in pigs leading to gut microbiota dysbiosis, inflammatory bowel disease and even severe diarrhea

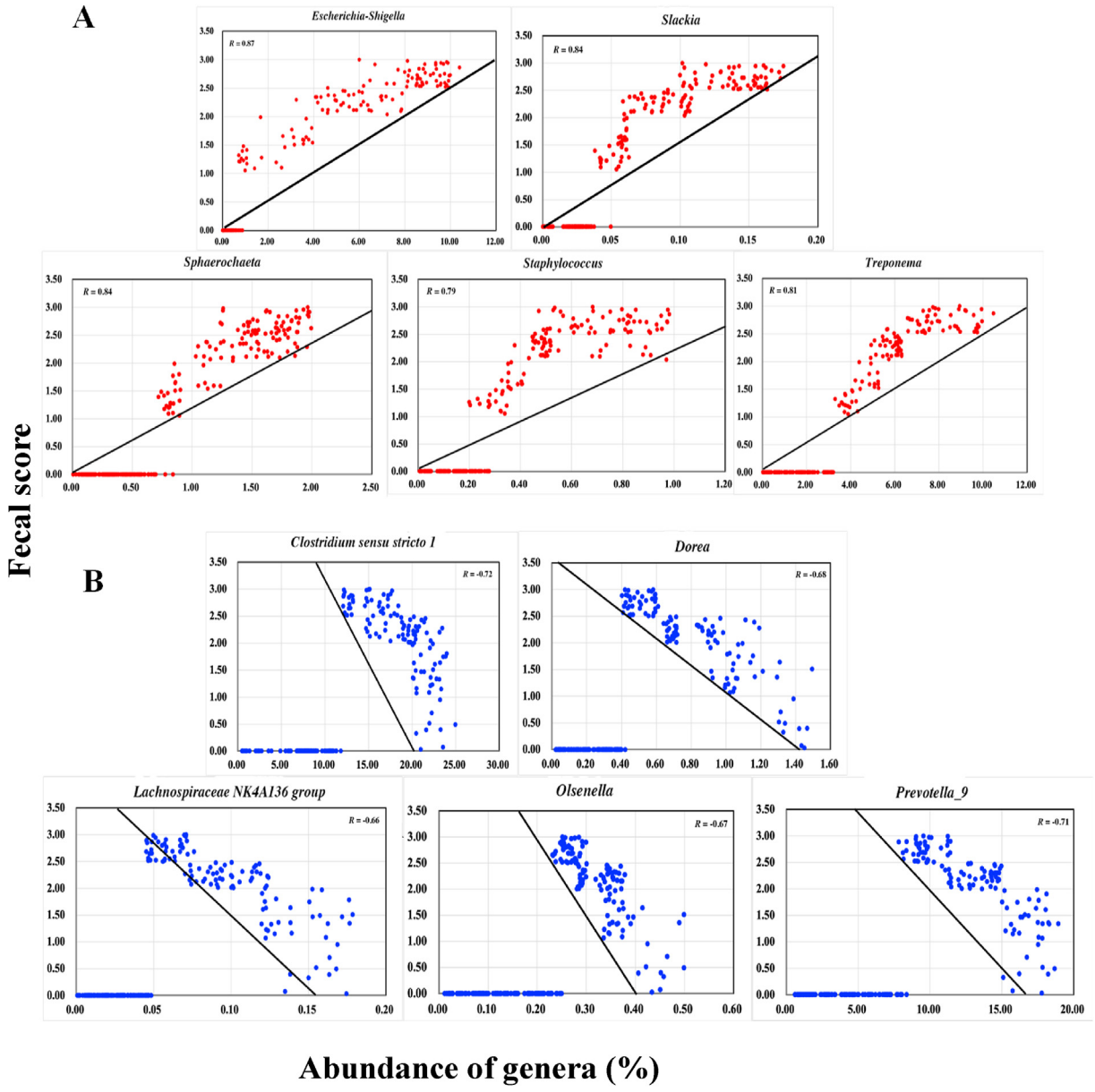

Fig. 4. Scatter plots which indicate the correlations between fecal score and the abundance of genera in the feces of piglets from validation cohort. (A) The positive correlation of fecal score with bacterial abundances of *Treponema*, *Sphaerochaeta*, *Escherichia-Shigella*, *Slackia*, *Staphylococcus* in group D. (B) The negative correlation of fecal score with bacterial abundances of *Clostridium sensu stricto 1*, *Prevotella_9*, *Olsenella*, *Dorea* and *Lachnospiraceae NK4A136* group in group H.

(Xiao et al., 2012; Zhang et al., 2022). However, research on the characteristics of the gut microbiota and the key genera associated with early diarrhea in weaned piglets within large-scale production systems is limited. In this study, the bacterial richness and diversity as measured by Chao1 and ACE indices was lower in diarrhea piglets than those in healthy piglets, which was in accordance with the previously reported ranges for the α-diversity of fecal bacterial communities in pigs (Kim et al., 2011; Pajarillo et al., 2014). Our results showed the Firmicutes to Bacteroidetes ratio in the diarrhea piglets was significantly higher than that in the healthy piglets. The greater the ratio of Gram-positive Firmicutes to Gram-negative Bacteroidetes, the more diarrheal and unhealthy the animal (An J et al., 2023; Chen et al., 2016; Riva et al., 2017). It has been suggested that *E. coli*-induced diarrhea may result in a higher Firmicutes:Bacteroidetes ratio because the diarrhea may create a more suitable environment for the survival and growth of Firmicutes compared with Bacteroidetes (Chaban et al., 2012; Costa et al., 2014; Swidsinski et al., 2008). One of the possible reasons is the oxygen level in the gut, which can be diffused from the host tissues into the intestinal lumen. After pathogenic bacterial infection, there is an abnormal increase in the intestinal level of oxygen, which inhibits the growth of anaerobic organisms belonging to Bacteroidetes, as well as leading to the accumulation of facultative anaerobes such as Firmicutes (Albenberg et al., 2014; David et al., 2015; Espey, 2013).

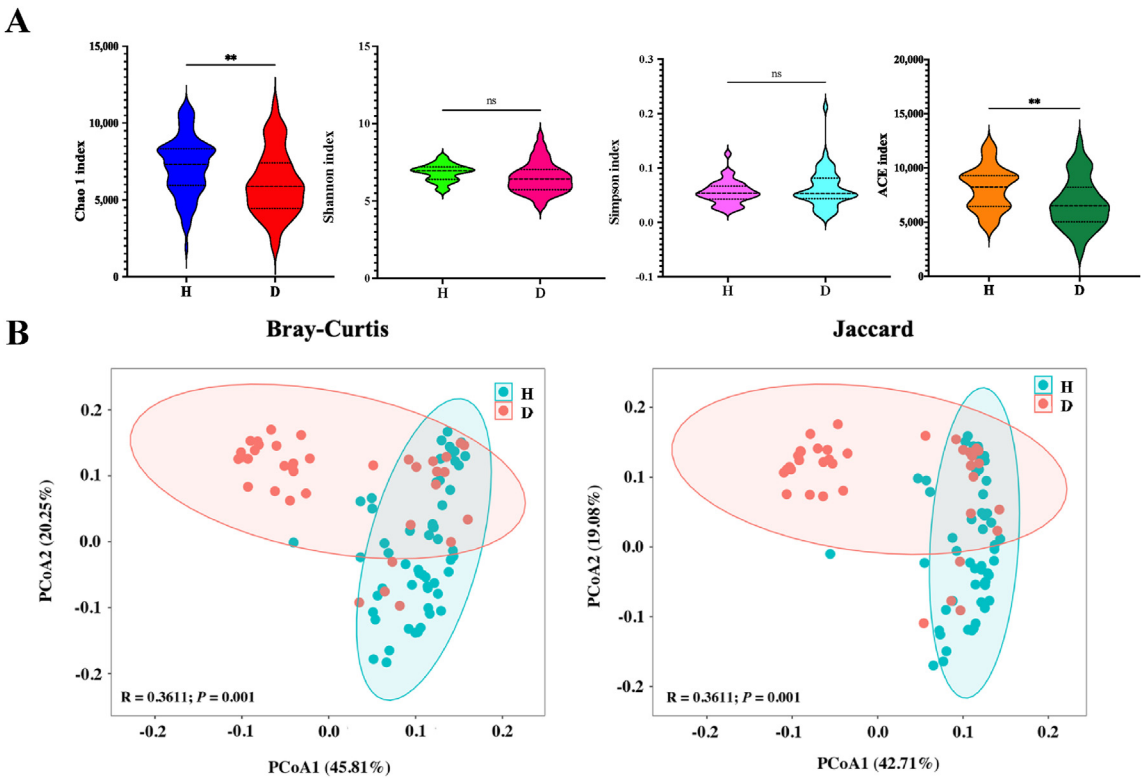

Fig. 5. Microbial diversity and community structure in the feces of healthy (group H) and diarrhea (group D) piglets of validation cohort. (A) The Chao1 index, Shannon index, Simpson index and ACE-index (**$P < 0.01$). (B) PCoA based on Bray-Curtis and Jaccard distances of fecal samples at genus level ($P = 0.001$). ns = no significance.

Changes in the genera of gut microbiota reflect pathogenic infection and even imbalance in the gut of pigs (Wang et al., 2023). *Enterococcus, Escherichia-Shigella,* and *Helicobacter* have been associated with diarrhea in piglets (Gryaznova et al., 2022; Panah et al., 2023). In this study, a total of 85 significant different genera were identified between healthy and diarrhea piglets. The abundance of *Treponema*, *Sphaerochaeta*, *Escherichia-Shigella*, *Slackia*, and *Staphylococcus* were found to be positively associated with the fecal score of piglets, which was consistent with previous studies (Bin et al., 2018; Jacques et al., 1989; Kohrman et al., 1989). *Treponema* and *Staphylococcus* affect microbiota functions with modulations in carbohydrate-active enzymes (CAZymes) mainly including GH25, GH10, GH9, and cellulase (EC 3.2.1.4), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database pathways including cholic acid, allocholic acid, and 1,3-butadiene, then leading to diarrhea in weaned piglets through inducing metabolic disorders in the gut (Chen et al., 2022). *Treponema* represents a taxonomically diverse genus which is pathogenic to animals in some conditions, such as through pathogenic synergism with other select anaerobes in gnotobiotic pigs (Buyuktimkin et al.,, 2019). Clinical cases also have linked methicillin-resistant *Staphylococcus* to enterotoxin expression, leading to diarrhea (Shrestha et al., 2009). Several *Escherichia-Shigella* species are believed to play critical roles in the development of diarrhea in piglets and have serious effects on the gut barrier function of the animal (Chen et al., 2017). Importantly, as one of the hallmarks of diarrhea, *Escherichia-Shigella* mainly colonizes the gut through adhesins and produces Shiga-like toxin (Stx2e, also known as Vero cytotoxin). Some members of *Escherichia-Shigella* can produce several enterotoxins at the same time, such as heat stable enterotoxin and heat labile enterotoxin, which cause severe diarrhea in piglets (Janke et al., 1989).

As for *C. sensu stricto 1, Prevotella, Dorea,* and *Lachnospiraceae NK4A136,* which maintain a complex and symbiotic relationship with the host, they are increasingly known to a play critical role in the maintenance of intestinal homeostasis (Chi et al., 2022; Park et al., 2023; Tremaroli and Bäckhed, 2012; Yu et al., 2024). *Clostridium, Prevotella,* and *Lachnospiraceae NK4A136* could regulate gut health through improving nutrition for the organism, enhancing metabolic abilities, producing short chain fatty acids (SCFAs) and restoring colonic barrier damage such as tight junction protein dysfunction (Wen et al., 2023; Zhao et al., 2021). Our data revealed that several SCFA-producing genera, such as *C. sensu strico 1* and *Prevotellaceae NK3B31 group* were found to have a higher abundance in healthy piglets than in diarrhea piglets and showed a strong positive correlation with each other. These genera can breakdown resistant starch and dietary fiber and convert them to SCFAs, which play key roles in energy homeostasis (Ma et al., 2022a, 2022b). Furthermore, the anti-inflammatory role of *Clostridium butyricum* in ulcerative colitis suggests that the genus *Clostridium* could promote gut health (Ma et al., 2023). *Prevotella* has been shown to improve glucose metabolism in piglets, and is often considered to be a beneficial bacteria for gut health (Song et al., 2021). In addition, *Prevotella* in the hindgut are known to possess extensive repertoires of polysaccharide utilization loci and carbohydrate-active enzymes for the metabolism of various plant polysaccharides (Accetto and Avguštin, 2018). Random forest machine learning algorithm revealed that *Dorea* may be key microbial

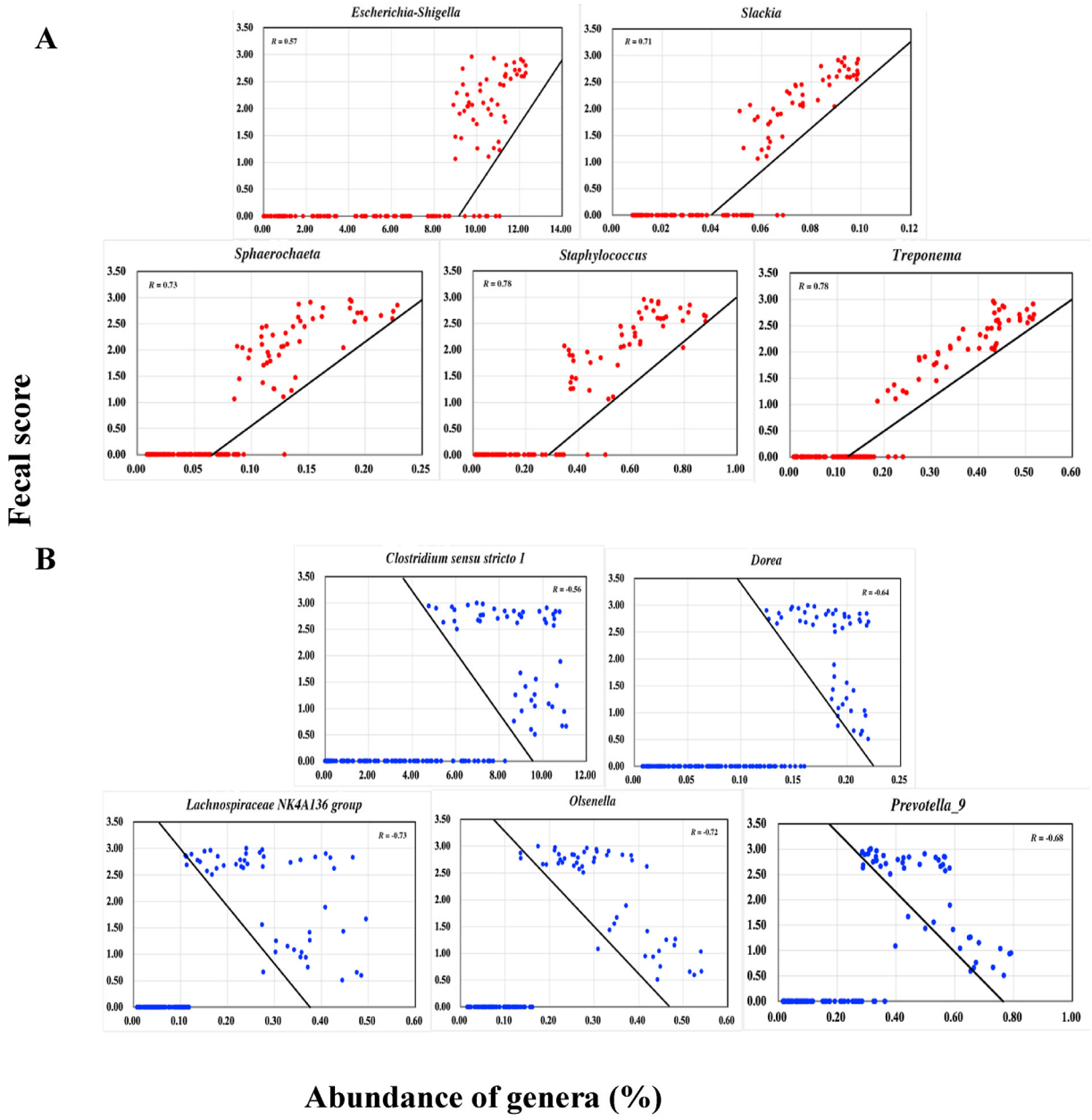

Fig. 6. Scatter plots with regression line showing correlations of fecal score with bacterial abundances (abundance of genera) in piglet feces of the validation cohort. (A) The positive correlation of fecal score with bacterial abundances of *Treponema*, *Sphaerochaeta*, *Escherichia-Shigella*, *Slackia*, *Staphylococcus* in group D. (B) The negative correlation of fecal score with bacterial abundances of *Clostridium sensu stricto 1*, *Prevotella_9*, *Olsenella*, *Dorea* and *Lachnospiraceae NK4A136* group in group H.

markers that can differentiate "healthy" and "unhealthy" (diarrhea) gut microbiota, as they predicted early life diarrhea with an accuracy of 84.3% (Ma et al., 2020).

5. Conclusion

In summary, 85 differentially enriched genera were identified between healthy and diarrhea piglets through the discovery cohort in 3 large-scale pig farms. *Treponema, Sphaerochaeta, Escherichia-Shigella, Slackia,* and *Staphylococcus* had a strongly positive relationship with diarrhea and identified as diarrhea-associated genera, whereas *C. sensu stricto 1, Prevotella_9, Olsenella, Dorea,* and *Lachnospiraceae NK4A136* were enriched in the healthy piglets. These bacteria might be essential diarrhea biomarkers and provide a foundation in early diagnosis of piglet diarrhea.

Credit author statement

Jiang Zhu: Conceptualization, Methodology, Investigation, Writing—Original draft. **Yue Sun:** Methodology, Investigation, Validation. **Lingyan Ma:** Investigation, Validation. **Qu Chen:** Formal analysis, Validation. **Caihong Hu:** Supervision. **Hua Yang:** Investigation, Validation data curation. **Qihua Hong:** Conceptualizations, Fund acquisition. **Yingping Xiao:** Conceptualization, Fund acquisition, Writing—Review & editing, Supervision.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or

company that could be construed as influencing the content of this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.05.013.

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