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The Metagenomic Analysis of the Pig Digestive System Microbiome as a Basis for Disease Control on Farming in Tangerang District, Indonesia

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ABSTRACT

Characteristics of microbiome of the pig's digestive tract play an important role in the animal's physiology, including metabolism, nutrient processing, the body's immune response, and disease resistance. This study aims to analyze the abundance and diversity of microorganisms (pathogenic and non-pathogenic) from the digestive system of pigs on farms in Tangerang District. The samples used in this research were pig feces from 43 pig farms in Tangerang District. Then DNA extraction was carried out using the Zymo Quick-DNA Fecal/Soil Microbe Kit according to manufacturing procedures. Sequencing was performed using an MGI DNBSEQ-G50 machine with a DNBSEQ-G50RS High-throughput sequencing set (FCL PE100) according to manufacturing procedures. The quality of fastq files was evaluated with FastQC. Taxonomic classification of the processed sequences was performed using Diamond, followed by MEGAN6. The microbiome of the pig's digestive system was dominated by bacteria (85%) with the taxonomic profile at the phylum level of abundance dominated by Firmicutes (84.46%) and Bacteroidetes (9.11%). Abundance at the genus level was dominated by *Enterococcus* **(20.44%) and** *Clostridium* **(10.31%), and at the species level it was dominated by** *Escherichia coli* **(4.92%) and** *Levilactobacillus brevis* **(4.84%). There were 10 species of pathogenic bacteria detected, with** *E. coli* **showing the highest relative abundance (4.92%). Changes in the gut microbiome play an important role in the physiology of animal health and disease. In addition, the pathogenic bacteria detected not only affect the health and productivity of pigs but also have the potential to threaten public health.**

Keywords: digestive system microbiome; metagenomic; microbial diversity; microbial pathogens; pigs

INTRODUCTION

According to Marchesi & Ravel (2015), the microbiome is the entire microhabitat, including the genomes of microorganisms and the surrounding environment. The microorganisms that make up the microbiome can include bacteria, archaea, fungi, parasites, and viruses, both non-pathogenic and pathogenic in certain environments, for example, the intestines, skin, soil, or certain parts of the ocean (Waite & Taylor, 2015). The composition of microbial communities in animal digestive systems can vary greatly at the taxonomic level. This can be influenced by various factors such as breed, age, feed, use of antibiotics, stress, and environment (Patil *et al.,* 2020).

The microbiome plays an important role in host function and health. The vital role of the digestive system microbiome includes metabolism, nutrition, physiology, immune response, and disease resistance (Waite & Taylor, 2015). The pig intestine is home to dynamic microbial populations, forming a complex ecosystem and having a symbiotic relationship with its host (Brestoff & Artis, 2013). The gut microbiome

provides many benefits in protection against colonization by pathogenic microbes, assisting the development and maturation of the immune system as well as the production of vitamins and metabolites (Patil *et al.,* 2020). However, this microbial population also includes disease-causing organisms such as *Escherichia coli*, *Salmonella* sp., and *Clostridium* sp., as well as other pathogenic microbes that can cause morbidity and mortality in pigs. Based on this, microbiome research has the potential to contribute solutions to overcoming health problems in animals, including infectious diseases, increasing feed efficiency, and increasing livestock productivity (Fouhse *et al.,* 2016). Detection of pathogenic microbes in the pig's digestive system can help prevent the early spread of disease in pigs and humans, which can result in economic losses.

Pigs are known to harbor many pathogenic microbes that can be transmitted to humans (Khan *et al.,* 2013). Pig feces are an important reservoir for spreading various kinds of pathogenic microbes to animals, humans, and the environment. Based on analysis of 57,000 publications over the last 50 years, we have identified the top 40 priority pathogens (including zoonoses) for

pig diseases, of which 40% originate from viruses, 37.5% from bacteria, 20% from worm parasites, and 2.5% from protozoa. The globalization of the pig industry has also contributed to the emergence and global spread of pigderived pathogens (VanderWaal & Deen, 2018).

High-throughput metagenomic sequencing is widely used for rapid and efficient characterization of the digestive system microbiome of many organisms and has provided many benefits regarding comprehensive information about the microbiome, including its role and function (Li *et al.,* 2020; Jiang *et al.,* 2021). In addition, it can detect the presence of known pathogenic microbes as well as rare and new pathogens so that it can reveal whether they have the potential to spread and cause a pandemic in the future (Miller *et al.,* 2013). This research uses metagenomic shotgun sequencing from pig feces on farms in Tangerang District, making it possible to understand the entire microbial community in the pig's digestive system and their role and function.

Tangerang District has a number of pig farms to meet the community's need for pork. According to the Central Statistics Agency of Banten Province, in 2021, the population of pigs in Tangerang District was 7,218 heads (BPS, 2023). Currently, comprehensive data regarding the abundance and diversity of microorganisms, especially pathogens, in the digestive systems of pigs on Tangerang District farms is still limited, and testing using shotgun metagenomic sequencing on pig farms is the first of its kind in Indonesia. The resulting data are needed as a basis for interventions to prevent and control diseases originating from pigs and as a selection of good microbial candidates that can later be used in efforts to improve livestock health and productivity. The aim of this study was to analyze the abundance and diversity of microorganisms (pathogenic and non-pathogenic) in the digestive systems of pigs on farms in Tangerang District.

MATERIALS AND METHODS

Sample Size and Sampling Technique

A total of 43 pig farms in Tangerang District were taken as samples. Fresh pig feces samples were taken from each pig farm. The pigs sampled in this study were pigs that did not show any symptoms of illness from the Yorkshire and Landrace breeds, aged 25–28 weeks. Samples were taken aseptically and placed in a sterile sample bag. Sample transportation uses a cool box equipped with an ice pack and maintained at a temperature of 4–8 °C during transit. The samples were placed in a freezer at -80 °C after arriving at the laboratory for storage before entering the next stage. This research does not require ethical approval because there is no treatment of animals.

Extraction and Quality Control of DNA

Sample DNA was extracted using the Zymo Quick-DNA Fecal/Soil Microbe Kit according to manufacturing procedures. DNA quality was evaluated via electrophoresis on a 1% agarose gel. DNA purity was evaluated using MaestroNano Micro-Volume Spectrophotometer analysis. The DNA purity value obtained from the MaestroNano Micro-Volume Spectrophotometer must comply with the predetermined value, namely 1.8–2.0 (A260/A280). DNA concentration was evaluated using the Qubit fluorometer dsDNA BR Assay with the condition that the DNA concentration value for sequencing was ≥15 ng/μL.

Sequencing

Sequencing was carried out using the MGI DNBSEQ-G50 sequencer machine. The initial stage starts with library preparation, followed by the sequencing process using the DNBSEQ-G50RS high-throughput sequencing set (FCL PE100) according to manufacturing procedures.

Bioinformatics Analysis

The quality of fastq files is evaluated with FastQC. Next, assembly was carried out using SPAdes software to sequence the cut DNA fragments into complete DNA sequences. After that, taxonomic analysis was carried out to see the abundance and diversity of microorganisms using MEGAN6 software (Bağcı *et al.,* 2021).

RESULTS

Sequencing of pig feces samples at pig farms in Tangerang District can describe the microbiome in the pig's digestive system, especially the lower digestive system. Sequencing results from pig feces samples showed that the most dominant abundance was bacteria, with a relative abundance percentage of 85%, followed by viruses (14%), archaea (1.03%), eukaryotes (0.1%), and unclassified organisms (0.02%).

The taxonomic profile of bacteria at the phylum level shows that Fermicutes was the most dominant, with a relative abundance percentage of 84.46%, followed by Bacteroidetes (9.12%) and Proteobacteria (2.73%) (Figure 1). The taxonomic profile of bacteria at the class level shows that Clostridia was the most dominant, with a relative abundance percentage of 45.46%, followed by Gammaproteobacteria (16.75%) and Bacilli (15.17%) (Figure 2). The most dominant order-level bacterial taxonomic profile was Eubacteriales, with a relative abundance percentage of 63.99%, followed by Bacteroidales (21%) and Lactobacillales (6.41%) (Figure 3).

Lactobacillaceae was the most dominant bacteria at the family level, with a relative abundance percentage of 31.8%, followed by Enterobacteriaceae at 14.9% and Clostridiaceae at 9.1% (Figure 4). *Enterococcus* was the dominant bacteria at the genus level with a relative percentage of 20.44%, followed by *Clostridium* 10.31% and *Pseudomonas* 10.19%. Details of bacteria at the genus level in the digestive system of pigs in Tangerang District pig farms can be seen in Figure 5. The most dominant bacteria at the species level was *Escherichia coli* with a relative abundance of 4.92%, followed by *Levilactobacillus brevis* (4.84%) and *Enterococcus asini* (3.25%) (Figure 6).

There were several pathogens from various bacterial species detected. *Escherichia coli* was the most dominant pathogenic bacteria detected, with a relative abundance

% Relative abundance

Figure 1. Taxonomic profile of bacteria at the phylum level in pig feces samples

Fusobacteriia	0.02
Candidatus Nanosyncoccalia	0.04
Sphingobacteriia	0.04
Epsilonproteobacteria	0.06
Flavobacteriia	0.08
Tissierellia	0.36
Negativicutes	0.49
Betaproteobacteria	\blacksquare 0.53
Coriobacteriia	0.55
Bacteria (Class) Spirochaetia	\blacksquare 0.57
Mollicutes	0.62
Anaerolineae	0.76
Deltaproteobacteria	1.51 TITUL
Alphaproteobacteria	1.59 Ш
Bacteroidia	THEFTER THE 6.48
Actinomycetia	MUNICIPALITY 8.95
Bacilli	15.17 CONTRACTOR
Gammaproteobacteria	16.75 <u> The Community of the Community of</u>
Clostridia	45.46
	0.00 10.00 20.00 30.00 40.00 50.00
	% Relative abundance

Figure 2. Taxonomic profile of bacteria at the class level in pig feces samples

percentage of 4.92%, followed by *Enterococcus fae* abundance of 100%. Taxonomy a *cium* (1.83%) and *Enterococcus faecalis* (0.21%). Details of pathogenic bacterial species in the digestive systems of pigs in Tangerang District pig farms can be seen in Figure 7.

The second-largest relative abundance after bacteria was viruses. Viruses were dominated by the order Caudovirales, with a relative abundance of 100%. Taxonomy at the family level was dominated by Myoviridae with a relative abundance of 47.81%, then Siphoviridae (40.98%), Podoviridae (8.20%), Herelleviridae (1.91%), Autographiviridae (0.82%), and Ackermann Viridae (0.27%) (Figure 8). The species level of the most dominant virus was *Siphoviridae* sp., with a relative abundance of 68.7%, followed by *Myoviridae* sp. (8.8%), *Bacteriophage* sp. (7.1%), *Podoviridae* sp. (6.9%), and *CrAss-like virus* sp. (3.5%) (Figure 9).

The taxonomic profile at order-level archaea was dominated by Methanobacteriales, with a relative

abundance of 100%. Taxonomy at the family level was dominated by Methanobacteriaceae with a relative abundance of 100%. Methanosphaera was the dominant archaea at the genus level, with a relative abundance of 87.46%, followed by *Methanobrevibacter* (12.54%) (Figure 26 10a). The species level is dominated by *Methanosphaera stadtmanae*, with a relative abundance of 34.86%, followed by *Methanosphaera* sp. DEW79 (19.11%) and *Methanosphaera* sp. SHI1033 (16.97%) (Figure 10b).

DISCUSSION

This research showed that Firmicutes was the most dominant phylum-level bacteria, followed by Bacteroidetes and Proteobacteria. The same thing was also reported in previous studies on the digestive tract of various pig breeds worldwide (Bergamaschi *et al.,* 2020; Giuffrè *et al.,* 2021). However, at the genus and species level, there were differences with previ-

% Relative abundance

Figure 3. Taxonomic profile of bacteria at the order level in pig feces samples

Figure 4. Taxonomic profile of bacteria at the family level in pig feces samples

ous research. The dominant genera in this study were Lactobacilli and Clostridia were the ϱϵϳ In contrast to previous research by Xiao *et al*. (2018), *Enterococcus*, *Clostridium*, and *Pseudomonas* (Figure 5).

Lactobacilli and *Clostridia* were the two most abundant genera in the ileum, cecum, and large intestine of Landrace pigs, while according to Quan *et al*. (2020), the

Figure 5. Taxonomic profile of bacteria at the genus level in pig feces samples

most abundant genera in the ileum were *Clostridium*, *Clostridioides*, and *Escherichia*, but *Prevotella*, *Bacteroides*, and *Treponema* were the most abundant genera in the cecum and large intestine. Several studies have proven that changes in the gut microbiome play an important role in animal physiology, including nutrient processing, health, and disease (Pascoe *et al.,* 2017; Patil *et al.,* 2020).

The highly variable composition of microbial communities at the taxonomic level may be influenced by various factors such as breed, age, feed, antibiotic use, stress, and environment (Patil *et al.,* 2020). The pigs sampled in this study were pigs that did not show any symptoms of illness from the Yorkshire and Landrace breeds, aged 25–28 weeks. In addition, gut location is a

major determinant of taxonomic and functional profiles that can be influenced by different ecological and physiological environments. For example, *Clostridium* sp. is abundant in the hindgut. This bacterium was associated with polysaccharide metabolism (Wu *et al.,* 2011), which was in accordance with the physiological capacity of the large intestine. *Clostridium* sp. in this study also showed a dominant abundance after *Enterococcus*.

The ratio of Firmicutes to Bacteroidetes changes as the animal ages and impacts polysaccharide breakdown, nutrient absorption, intestinal permeability, and inflammatory response. Bacteroidetes have been shown to participate in carbohydrate degradation; however, in pigs, the proportion of bacterial species belonging to this phylum group decreases with age

0.54 Bifidobacterium pseudolongum Þ 0.57 Clostridium sp. CAG:302 P 0.59 Clostridium sp. CAG:524 μ 0.61 h Lactococcus raffinolactis 0.61 Pseudoflavonifractor sp. MSJ-30 0.72 μ Enterococcus massiliensis 0.72 μ m Treponema succinifaciens 0.77 Corynebacterium xerosis μ 0.77 Enterococcus dongliensis P 0.83 Lapidilactobacillus concavus ш 0.85 Sodaliphilus pleomorphus m 0.85 Clostridium sp. CAG:1193 ш 0.86 Clostridium sp. CAG:1000 ш 0.90 Lacticaseibacillus paracasei W 0.91 Treponema bryantii Ш 0.91 Latilactobacillus curvatus ш 1.00 Streptococcus alactolyticus Ш 1.00	Clostridium sp. CAG:798 HIIII 1.82 Enterococcus faecium WUUT 1.83 Lentilactobacillus kribbianus HIIIII 1.96 Enterococcus hirae WINNE 2.03 Treponema porcinum HIIIII 2.04 Clostridium sp. CAG:221 2.09 WWW Clostridium sp. CAG:914 WWW 2.11 Alistipes sp. CAG:435 <i>MUNIT</i> 2.20 Alistipes sp. CAG:514 HILLIN 2.47 Lactiplantibacillus plantarum TUTTUTTI 2.64 Vescimonas fastidiosa 2.73 Clostridia bacterium ALLIN DINA 2.93 Enterococcus asini 3.25 Levilactobacillus brevis 4.84 Escherichia coli HILLING CONTRACT 4.92 Others 24.87

Figure 6. Taxonomic profile of bacteria at the species level in pig feces samples

and causes subsequent weight gain (Zhou *et al.,* 2015). abundance of the *Lactobacillus* genus The high relative abundance of Firmicutes in this study could influence the increase in body weight in pigs. In accordance with previous research showing that Firmicutes are important in producing energy, increasing Firmicutes in pig intestines can increase pig body weight gain (Yang *et al.,* 2018).

Lactobacillus is a member of the lactic acid bacteria (LAB). Many species, such as *Lactobacillus johnsonii*, can be used as probiotics, which are able to ferment and produce lactic acid (Pridmore *et al.,* 2004). The relative abundance of the *Lactobacillus* genus in the study was 3.67% and was ranked 6th highest (Figure 5). Pigs with high feed efficiency have a greater abundance of the genus *Lactobacillus*. In addition, many species of the genus 30 *Lactobacillus* are associated with weight gain in animals and humans (Million *et al.,* 2013). Increasing beneficial microbes, such as *Lactobacillus*, can prevent infection with pathogenic microbes by increasing mucin production, which improves the intestinal barrier (Che *et al.,* 2014). Limiting pathogenic microbes from attaching to the intestinal epithelium is an effort to defend against

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Figure 7. Pathogenic bacteria in pig feces samples

% Relative abundance

feces samples

Figure 9. Taxonomic profiles of viruses at the species level in pig feces samples

Figure 10. Taxonomic profiles of archaea in pig feces samples. (A) Archaea at the genus level; (B) Archaea at the species level.

disease development. The mucus produced by intestinal alsease development. The macas produced by intestinant goblet cells creates a dense mucus layer that is impermeable to pathogens and toxins (Jacobi & Odle, 2012). ϵ

The *Treponema* genus in this study showed a relative abundance of 3.02% (Figure 5) and usually plays a role in carbohydrate digestion in the pig intestine. *Treponema* is more abundant in the cecum microbiota of pigs with high feed efficiency (Quan *et al.,* 2020). High numbers of Bacterioidiales accompanied by low

restinal numbers of Clostridiales can reduce inflammation (Schwab *et al.,* 2014), whereas increasing Clostridiales and decreasing Bacteroidetes can be beneficial for high-energy diets (Magnusson *et al*., 2015). The relative abundance of Bacterioidiales in this study was 21% (Figure 3) and was the dominant order level of bacteria after Eubacteriales. Likewise, Bacteroidetes was the dominant phylum-level bacteria after Fermicutes, with a relative abundance of 9.12% (Figure 1).

A higher relative abundance of *Prevotella* was associated with high-carbohydrate diets. However, the role of *Prevotella* was also in contrast to that of other microbes, such as Bacteroides, which was associated with the protein breakdown process. The relative abundance of *Prevotella* in this study was 1.98% (Figure 5). IgA concentration is positively correlated with *Prevotella* abundance and increased animal growth (Mach *et al.,* 2015). Mucosal immunoglobulin, IgA, is stimulated by microbial fermentation and limits the entry of pathogens through intestinal epithelial cells (Che *et al.,* 2014). Similar to the impact on long-term microbial colonization, continued microbial exposure during piglet development is important for balancing immune cell populations (Inman *et al.,* 2010). The abundance of *Prevotella* plays an important role in pig feeding behavior and is a key bacterium for controlling pig appetite (Yang *et al.,* 2018).

Various pathogenic bacteria were also detected in this study, including important pathogenic bacteria for pigs and zoonoses that can endanger public health, including *Escherichia coli*, *Salmonella enterica*, *Clostridium perfringens*, *Clostridium botulinum*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Shigella sonnei*, and *Clostridium difficile* (Figure 7). *E. coli* was one of the pathogenic bacteria with the highest abundance in the digestive tract of pigs in this study. According to Kirk *et al*. (2015), all of these important pathogenic bacteria are mostly the main reservoirs of foodborne pathogens. These bacteria can contaminate food of animal origin at several stages in the production chain and can threaten public health. The composition of the pig microbiota is an important determinant of food safety for humans. Pigs act as a reservoir for potential foodborne pathogens and are a major cause of foodborne illnesses in humans. Pigs carry pathogenic bacteria, which are shed through their feces, thereby contaminating the pen, food consumed by humans, and the environment.

E. coli, *S. enterica*, *K. pneumoniae*, and *S. sonnei* are bacteria from the Enterobacteriaceae family group that often cause problems in the pig farming industry. These bacteria often cause colibacillosis in piglets, the general symptoms of which are diarrhea. Enterotoxigenic *E. coli* (ETEC) is a pathogenic *E. coli* that often causes colibacillosis in piglets, which causes diarrhea, especially after weaning. Infection of pigs by ETEC can reduce body weight and even cause high mortality in piglets, so it can cause significant economic losses for the pig farming industry (Luppi *et al.,* 2017; Fairbrother & Nadeau*,* 2019).

E. coli infection in humans can cause gastrointestinal diseases such as diarrhea by intestinal pathogenic *E. coli* (InPEC) strains and also cause extraintestinal diseases by extraintestinal pathogenic *E. coli* (ExPEC) strains such as uropathogenic *E. coli*, which is associated with neonatal meningitis, and *E. coli* cause sepsis (Dale & Woodford, 2015). *E. coli* O157:H7 is the most well-known serotype of EHEC, which has caused many outbreaks of waterborne and foodborne diseases in various countries worldwide. The incidence of non-O157 STEC has increased recently, including those caused by serotypes O26, O45, O103, O111, O121, and O145 (Farrokh *et al.,* 2013).

Salmonella sp. is the main digestive system patho-

genic bacteria in pigs and has a high potential for pork contamination, which negatively impacts public health. *Salmonella* sp. infection causes clinical disease in pigs, especially septicemic salmonellosis associated with *Salmonella enterica* serovar *S. typhimurium* and the monophasic variant *S. enterica* subsp. *enterica*, which causes significant economic losses due to increased mortality, stunted growth, and increased medical costs and is often associated with cases of salmonellosis in humans (Soliani *et al.,* 2023).

S. enterica infection in humans causes symptoms of fever, nausea, stomach cramps, vomiting, headaches, and diarrhea. These clinical symptoms generally last several days to a week. *S. enterica* serovar *S*. *typhimurium* causes a more severe type of salmonellosis called typhoid fever and causes a serious illness that can result in up to 10% mortality if left untreated. *Salmonella* is the second most common foodborne infection in humans after *Campylobacter* since 2005 in the European Union (European Food Safety Authority & European Centre for Disease Prevention and Control, 2022). The disease caused by *Salmonella* is estimated at around 1.35 million cases, with 26,500 hospitalizations and 420 deaths each year in the United States, with the source of infection coming from consuming contaminated food (CDC, 2024). Data regarding the incidence of salmonellosis in Indonesia is not yet available. This may be because of this self-limiting disease, and many cases are not reported. There are many cases of diarrhea in health center units and hospitals, but no further laboratory testing is carried out regarding the causative agent.

C. perfringens is frequently associated with significant systemic and enteric diseases in humans and pigs and is associated with food poisoning, nonfoodborne diarrhea, and enterocolitis (Sim *et al.,* 2015; Heida *et al.,* 2016). *C. perfringens* strains are known to secrete >20 identified toxins and enzymes. This could potentially be a principal virulence factor involved in the pathophysiology (Revitt-Mills *et al.,* 2015). *C. perfringens* type A is the most common strain and the most variable in terms of its toxigenic properties. Alpha-toxin production is associated with diarrhea in pigs. *C. perfringens* types B and C cause severe enteritis, dysentery, toxemia, and high mortality in pigs (Kiu & Hall, 2018). An estimated 90,000 cases of *C. perfringens* per year, with an incidence of 1.5/1000 people per year, are reported in the United Kingdom (UK) (Tam *et al.,* 2012). It is estimated that 8%–13% of gastrointestinal foodborne disease outbreaks associated with *C. perfringens* occur in the UK (Gormley *et al.,* 2011). Cases of food poisoning due to *C. perfringens* are rarely reported in Indonesia. This is because the symptoms often disappear by themselves within 24 hours (Dolan *et al.,* 2016).

An imbalance in the microbiome in the digestive system can increase the population of pathogenic microbes. Probiotics can be used to prevent and treat microbial imbalances by changing the intestinal population of the epithelial lining and lymphoid tissue associated with the gut. Probiotics, known as directfed microbials (DFM) in the livestock industry, have long been proposed as a sub-therapeutic alternative to antibiotics. (Hill *et al.,* 2014). *Bacillus spp., Lactobacillus,*

Bifidobacterium, and *Enterococcus* are lactic acidproducing bacteria that are commonly used in probiotic mixtures because of their characteristics (Pringsulaka *et al.,* 2015; Yang *et al.,* 2015; He *et al.,* 2020). All of these bacterial genera were detected in this study and can be used as probiotic candidates. In addition, recent research shows that the *Treponema* and *Prevotella* genera, which in this study showed quite high abundance, could potentially be probiotic candidates for pigs in the future (Yang *et al.,* 2018; Quan *et al.,* 2020), although further in vivo testing needs to be carried out in the future.

Feeding *Lactobacillus* originating from pig intestines as a probiotic can reduce the abundance of Enterobacteriaceae, including pathogenic *E. coli* and *Salmonella* sp. so it can reduce the incidence of diarrhea, improve the immune response during infection (Naqid *et al.,* 2015), and increasing body weight (Konstantinov *et al.,* 2008). *Lactobacillus reuteri* can protect by reducing intestinal pH through the production of lactic acid via *Bifidobacterium* spp., then reducing the abundance of *E. coli* (Hou *et al.,* 2015). Administration of *Bacillus* probiotics can increase mucosal abundance of *Clostridium*, *Lactobacillus*, and *Turicibacter*, thereby further increasing the expression of atonal BHLH transcription factor 1 (Atoh1), resulting in increased regulation of goblet cell proliferation in the ileum. The greater number of goblet cells can increase the preservation of mucin 2 (MUC2) production, which functions as an intestinal barrier (Zhang *et al.,* 2017). Piglets fed supplemental feed with *Bacillus subtilis* can result in an increase in *Lactobacillus* spp. and reduce *Clostridium perfringens* in the ileum (Baker *et al.,* 2013).

The second-largest relative abundance after bacteria in this study was viruses. Species-level viruses were dominated by *Siphoviridae* sp., *Myoviridae* sp., and *Bacteriophage* sp. (Figure 9). Viruses from pig feces samples in this study mostly consisted of viruses infecting bacteria and archaea, with several unclassified viruses.

The taxonomic profile of archaea at the genus level in this study was dominated by *Methanosphaera* (87.46%) and *Methanobrevibacter* (12.54%). Previous research also showed that *Methanobrevibacter* and *Methanosphaera* dominate the diversity of archaea in pigs, although the relative abundance of *Methanobrevibacter* is more dominant (0.23%) compared to *Methanosphaera* (0.023%) (Xiong *et al.,* 2022). Archaea can significantly influence the nutrition, metabolism, and growth performance of pigs, especially during weaning (Deng *et al.,* 2022). *Methanobrevibacter* dominance was consistently observed, with early colonization of methane-producing archaea in piglet intestines showing breed-specific variation and dramatic changes with age. The structure of the archaea community in the pig intestine is strongly influenced by diet (Feehan *et al.,* 2023). Pigs fed a variety of fiber diets showed significant changes in the archaeal structure of the intestine, especially in *Methanobrevibacter* (Cao *et al.,* 2016). Luo *et al*. (2012) also revealed that the diversity and abundance of archaea, especially *Methanobrevibacter*, were higher in feces samples from lean-type pigs compared to obese-type pigs, thus indicating the possible role of archaea in the intes-

tine in the formation of pork fat. Archaea can be a key component of the pig gut microbiome and are especially inhabitants of anaerobic environments, where they play a critical role in health and digestion. Archaea play a role in breaking down complex carbohydrates and producing important short-chain fatty acids (SCFAs). Increasing *Methanosphaera* in pig feed can improve feed efficiency (Deng *et al.,* 2021). There is a relationship between the abundance of certain archaea, especially *Methanobrevibacter A smithii* and *Methanobrevibacter A sp900769095*, feed efficiency, growth rate, and meat quality (Deng *et al.,* 2022; Zhao *et al.,* 2022). Therefore, in the future, certain beneficial archaea may function as "archaebiotics" and play an important role in the pig farming industry.

CONCLUSION

The microbiome of the pig's digestive system is dominated by bacteria with a taxonomic profile at the phylum level, whose abundance is dominated by Firmicutes, Bacteroidetes, and Proteobacteria. Abundance at the genus level was dominated by *Enterococcus*, *Clostridium*, and *Pseudomonas*, and at the species level, it was dominated by *Escherichia coli*, *Levilactobacillus brevis*, and *Enterococcus asini*. There were 10 species of pathogenic bacteria detected, with *E. coli* showing the highest relative abundance. The highly variable composition of microbial communities at the taxonomic level can be influenced by various factors, such as breed, age, feed, antibiotic use, stress, and environment. Changes in the gut microbiome play an important role in the physiology of animal health and disease. In addition, the pathogenic bacteria detected not only affect the health and productivity of pigs but also have the potential to threaten public health.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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