Yeasts of Burden: Exploring the Mycobiome–Bacteriome of the Piglet GI Tract

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Interactions between the bacteria and fungi in the gut microbiome can result in altered nutrition, pathogenicity of infection, and host development, making them a crucial component in host health. Associations between the mycobiome and bacteriome in the piglet gut, in the context of weaning, remain unknown. Weaning is a time of significant stress, dietary changes, microbial alterations, and a predisposition to infection. The loss of animal health and growth makes potential microbial interventions of interest to the swine industry. Recent studies have demonstrated the diversity and development of the microbiome in the gastrointestinal (GI) tract of piglets during weaning, resulting from the dietary and physiological changes. Despite these advances, the role of the mycobiota in piglet health and its contribution to overall microbiome development remains mostly unknown. In this study we investigated the bacteriome and the mycobiome after weaning in the GI tract organs and feces from 35-day old piglets. Following weaning, the $\alpha$-diversity and amplicon sequence variants (ASVs) counts of the bacteriome increased, proximally to distally, from the stomach to the feces along the GI tract, while the mycobiome $\alpha$-diversity and ASV counts were highest in the porcine stomach. $\beta$-diversity analyses show distinct clusters based on organ type in the bacteriome and mycobiome, but dispersion remained relatively constant in the mycobiome between organ/fecal sites. Bacteroidetes, Firmicutes, and Epsilonbacteraeota were the most abundant bacterial phyla present in the GI tract and feces based on mean taxonomic composition with high variation of composition found in the stomach. In the mycobiome, the dominant phyla were Ascomycota and Basidiomycota, and the stomach mycobiome did not demonstrate the same high level of variation observed in the bacteriome. Potential interactions between genera were found in the lower piglet GI bacteriome and mycobiome with positive correlations found between the fungus, Kazachstania, and several bacterial species, including Lactobacillus. Aspergillus demonstrated negative correlations with the short chain fatty acid-producing bacteria Butyricoccus, Subdoligranulum, and Fusicatenibacter. This study demonstrates the distinct colonization dynamics between fungi and bacteria in the GI tract and feces of piglets directly following weaning and the potential interactions of these microbes in the porcine gut ecosystem.

Keywords: mycobiome, bacteriome, microbiome, piglet, weaning, swine
INTRODUCTION

The microbiome plays a critical role in animal health through its ability to alter nutrition, physiology, immune system development and function, and through bacterial–fungal–host interactions. Fungi in the GI tract of piglets are ubiquitous members of the rare biosphere (Huffnagle and Noverr, 2013; Summers et al., 2019) and disruption of the mycobiome may result in disease, as it does in other species (Ott et al., 2008; Iliev et al., 2012; Mason et al., 2012a; Erb Downward et al., 2013; Li et al., 2014; Li Z. et al., 2019). Additionally, fungi affect gut community structure and function through genetic exchange, interactions with bacterial species, biofilm formation, secondary metabolite secretion, and potential antibiotic creation (Frey-Klett et al., 2011; Suhr and Hallen-Adams, 2015).

Recent studies have demonstrated that commensal fungi can alter host immunity during normal health, as well as modify the severity of some diseases (Mukherjee et al., 2015; Kureljusic et al., 2016; Weissenbacher-Lang et al., 2016; Iliev and Leonard, 2017; Limon et al., 2017; Richard and Sokol, 2019). Fungi can alter host immune responses through direct and indirect actions in the GI tract via pattern recognition receptors (PRRs), the production of metabolites such as prostaglandin E2 (PGE2), and multiple virulence factors that assist in host tissue invasion and nutrient acquisition. PGE2 is an immunomodulator typically produced by immune cells that can also be secreted by some fungi, such as Candida, leading to extensive immune changes in humans (Kim et al., 2014). Further, studies suggest that commensal fungi may promote immune tolerance to commensal bacteria (Li X.V. et al., 2019). In the context of pigs, studies have documented the effect of mycotoxins, fungal secondary metabolites known for contaminating agricultural feed, on the immune response. Different mycotoxins have the ability to up- or down-regulate the immune response in pigs, and immunosuppressive mycotoxins may increase piglet susceptibility to infectious diseases (Pierron et al., 2016). Due to the known sensitivity of piglets to these fungal metabolites, future studies are vital to understand the role of commensal fungi in porcine health.

The weaning transition is a stressful time in a pig's life and associated changes in the piglet gut microbiome can result in poor health and reduced growth performance, making it of critical interest to the swine industry (Campbell et al., 2013; Guevarra et al., 2018, 2019). Post-weaning diarrhea and susceptibility to opportunistic pathogens are common consequences of changes to the piglet gastrointestinal (GI) microbiome. Recently, studies have begun to elucidate the normal members of the microbiota in piglets, but details remain unknown as to interactions among members alter immune responses and promote growth performance. This information is necessary to identify potential alternative growth promotants as the use of antibiotics for growth promotion is banned in the United States. While studies have begun to show the importance of weaning and diet changes in the development of the GI microbiome (Bian et al., 2016; Han et al., 2017), the mycobiota remains a poorly understood, yet integral part of the gut ecosystem.

Members of the microbiota interact with each other within the host environment through a variety of means, including physical or chemical interactions, competition for resources or space, production of biofilms, or modulation of the surrounding environment (Krüger et al., 2019). For example, studies have demonstrated the ability of bacterial metabolites to directly inhibit Candida growth and colonization in the gut (Nguyen et al., 2011; Bulgasem et al., 2016) as well as the production of mycotoxins by bacteria residing within the fungal cytosol (Partida-Martinez and Hertweck, 2005). Bacteria can also indirectly inhibit fungal growth by activating different components of the immune system. One such example is the ability of lactobacilli to promote host resistance to gut colonization with Candida spp. through the activation of AhR, a transcription factor that stimulates the release of IL-22 (Kiss et al., 2011; Zelante et al., 2013; Lamas et al., 2016). In humans, Candida albicans can prevent the gut colonization of other fungal and bacterial pathogens (Tso et al., 2018) and Aspergillus fumigatus can inhibit Pseudomonas aeruginosa and alter the pro-inflammatory immune response in co-cultures (Reece et al., 2018). The microbial interplay in the piglet gut may significantly alter the growth and health of pigs long-term due to the numerous potential interactions between fungi and bacteria. Recent studies have demonstrated a link between certain fungal species and weight gain in other mammalian species (Mar Rodriguez et al., 2015), and while currently unknown, potential dietary intervention strategies for piglet weight gain is of great interest to industry (Sam et al., 2017). Previous work from our laboratory has shown that the dominant, post-weaning fungal species is Kazachstania slooffiae, but its role in animal health and development remains to be elucidated (Summers et al., 2019). We hypothesize that the bacteriome and mycobiome will significantly differ between organ sites. The current study investigated the microbiome and mycobiome in piglets 2 weeks post-weaning to evaluate the diversity, populations, and potential interactions between the bacterial and fungal members of the piglet GI tract and feces.

MATERIALS AND METHODS

Animal Procedures

A 23 Large White × Landrace piglets from 3 litters (L.119 = 8 piglets, L.120 = 8 piglets, and L.126 = 7 piglets) were assessed from birth through day 35 of age and were weaned at day 21. Piglets were not provided with creep feed or milk replacer at any point throughout the experiment. The diet was formulated to meet the National Research Council estimate of nutrient requirements (Supplementary Table S1). From days 21–28, piglets received Nursery Diet 1 followed by Nursery Diet 2 from days 29–35. Piglets were evaluated daily for health and were given free access to feed and water; all piglets used in this study were observed to be healthy. No antibiotics, antifungals, or supplementary additives were administered to the piglets at any time during the experiment. On day 35 of age, piglets were humanely euthanized, and the GI tract was removed from the abdominal cavity and immediately dissected. Sections from the stomach, proximal duodenum, jejunum, distal ileum, cecum, distal colon, and feces were collected under sterile conditions and
DNA Extraction and Sequencing
DNA was isolated from 0.25 g feces or organ sections using the MagAttract Power Microbiome Kit (Qiagen, Hilden, Germany) by the Microbial Systems Molecular Biology Laboratory at the University of Michigan. Cells were lysed to isolate DNA using mechanical bead beating for 20 total minutes with 20 frequency/second and extracted using magnetic bead technology according to the Qiagen protocol. The V4 region of the 16S rRNA-encoding gene was amplified from extracted DNA using the barcoded dual-index primers developed previously (Kozich et al., 2013). The ITS region was sequenced utilizing primers ITS3 (5′-GATCCTGATTTGATATGC-3′) and ITS4 (5′-TCTTCCGCTTATTGATATGC-3′) with the Illumina adaptor sequence added to the 5′ end (5′-TCGTCGGCAGCGTCAGATGTG TATAAGAGACAG—ITS3-3′) and (5′-GCTTCGTGGGCTCGGAGATGTGTATAAGAGACAG—ITS4-3′). Both 16S and ITS regions were sequenced with the Illumina MiSeq Sequencing platform.

Bacteriome (16S) and Mycobiome (ITS) Sequence Processing

Bacteria (16S)
Quality filtering, pairing, denoising, amplicon sequence variants (ASVs) determination, and chimera removal was conducted with the DADA2 plugin (Callahan et al., 2016) in QIIME2 v. 2019.4 (Caporaso et al., 2010). For quality trimming, paired-end sequences were truncated to 240 and 160 bp for forward and reverse reads, respectively, with an average median quality score of 34.8. Taxonomic classification of the ASVs was performed using the pretrained 16S 515F/806R from the Silva 132 database (Yilmaz et al., 2014). ASVs identified as Archaea, chloroplast, mitochondria, or unassigned were removed from further analysis.

Fungi (ITS)
Forward and reverse primers were removed from paired-end reads with cutadapt v 1.18 (Martin, 2011). QIIME2 plugin DADA2 was used to perform similar quality filtering and ASV identification described above for bacterial sequences. Because of the variable nature of fungal ITS sequencing length, however, no quality trimming was conducted on fungal sequences. Average median quality score was 35.9 and 32.3 for forward and reverse reads, respectively. Taxonomic classification was trained and conducted on fungal sequences using the UNITE (Koljalg et al., 2013) developer's full-length ITS reference sequences in QIIME2. Fungal ASVs without a phylum or higher classification or those identified as unassigned were removed. Additional classification using BLAST1 was performed on removed sequences to confirm non-fungal origin.

Characterization of the Bacteriome and Mycobiome
Calculations of α-diversity were performed on rarefied (n = 5,000 sequences) bacterial and fungal samples using the phyloseq package (McMurdie and Holmes, 2013). Shannon diversity indices and observed ASVs were normalized using box cox and square root transformations, respectively. Satisfaction of normality was tested using the Shapiro–Wilk test. Differences between bacterial and fungal Shannon diversity and observed ASVs were determined using a linear mixed model with organ as the fixed effect and pig as the random effect using the lmer4 and lmerTest. Non-metric multidimensional scaling (NMDS) was conducted using the vegan package on log-transformed bacterial and fungal sequences using Bray–Curtis dissimilarity distances. To reduce potential ASV artifacts, ASVs with <1 sequence in ≤5.0% of samples were removed prior to analysis. NMDs plots were visualized using the ggplot2 package (Wickham, 2016). Pairwise comparisons of mean Bray–Curtis distances to group centroids was calculated using the permutational analysis of multivariate dispersion (PERMDISP) function in vegan and plotted in R. Due to similarities between organ bacteriomes and mycobiomes, samples were recategorized by GI region: duodenum, jejunum, and ileum samples were recategorized as “Upper GI,” cecum and colon were recategorized as “Lower GI,” and stomach and feces remained categorized as “Stomach” and “Feces,” respectively. For visualization purposes, relative abundances of taxa are presented as mean% value by litter for each GI tract region and feces.

Correlation and Network Analyses of the Lower GI
For correlation analysis, samples were rarefied to their corresponding bacterial or fungal sample pair to account for sequencing depth differences between pairs while retaining similar community composition structure. Bacterial (n = 46) and fungal sample pairs (n = 46) were combined and ASVs were merged at the genus level. Genera found <30% of samples were removed to prevent degradation of correlation detection, which increases with increased numbers of 0 counts (Weiss et al., 2017). Correlations between fungus and bacteria were detected using the sparse correlations for composition (SparCC) python module (Friedman and Alm, 2012). Correlation values

1https://blast.ncbi.nlm.nih.gov
2https://www.R-project.org
were visualized using the corrplot package in R. P-values were corrected for multiple comparisons using FDR. A corresponding network analysis of SparCC correlation coefficients was created using the igraph (Csardi and Nepusz, 2005) and the Sparse and Computationally Robust Inference of Microbial Ecological Networks (Kurtz et al., 2015) R-packages. Only correlations with an absolute value $\geq 0.4$ were plotted. Unless otherwise stated, all statistical tests were performed in R, $p$-values of $<0.05$ were considered significant, and errors are given as $\pm$SE. All figures were created with GraphPad Prism 7, unless otherwise indicated.

RESULTS

Composition and Diversity of the Bacteriome and Mycobiome in the Piglet GI Tract

To analyze the microbiota communities in the piglet GI tract, the V4 and ITS2 regions of the bacterial 16S rRNA and fungal ITS genes, respectively, were amplified and sequenced from feces and six organ sections (stomach, duodenum, jejunum, ileum, cecum, and colon) collected from 23 piglets, aged 35 days. A total of 8,363,058 bacterial and 6,670,225 fungal high quality sequences were obtained following the QIIME processing and filtering pipeline. Rarefaction curves showed that a minimum sampling depth of 5,000 sequences was sufficient to capture both bacterial and fungal diversity in organs and feces (Supplementary Figures S2A,B). After removal of samples with $<5000$ sequences, the number of bacterial and fungal samples was reduced to 130 and 149, respectively (Supplementary Tables S2A,B). A mean sequencing depth of 24,909 $\pm$ 1,448 and a total of 2383 ASVs were detected in bacterial samples, and a mean sequencing depth of 35,187 $\pm$ 1,585 and a total of 592 ASVs were detected in fungal samples.

Indices for Shannon and observed ASVs were calculated to measure the $\alpha$-diversity in the bacteriome and mycobiome (Figures 1A,B and Supplementary Tables S3A,B). In the bacteriome, the overall trend showed an increase in diversity and observed ASVs from the stomach to the feces along the GI tract. The mycobiome showed a different trend, with the stomach showing higher diversity and observed ASVs, followed by a decrease in diversity and observed ASVs in the duodenum, jejunum, and ileum and an increase in diversity and observed ASVs in the colon. Compared to the mycobiome, diversity and observed ASVs were significantly higher in the bacteriome ($p < 0.001$, Supplementary Tables S2A,B).

Non-metric multidimensional scaling plot were used to visualize $\beta$-diversity between the different regions and organs of the piglet GI (Figures 2A,B). In both the bacteriome and mycobiome, stomach and feces showed distinct clusters from the other organs. The duodenum, jejunum, and ileum organs in the upper GI, and the cecum and colon in the lower GI had a high degree of overlap among their centroids within their respective GI tract region indicating similarities between the microbiota communities. Mean distances between group centroids (dispersion) for each organ were calculated using PERMDISP on Bray–Curtis dissimilarities (Supplementary Tables S4A,B). In the bacteriome, there was a significant decrease in dispersion from the stomach and upper GI tract to the lower GI tract and feces, signifying a larger amount of individual variation in the upper GI tract vs. the lower GI tract and feces ($p < 0.05$, Figure 3A and Supplementary Table S2A). This trend in dispersion directly contrasted with $\alpha$-diversity, which showed an increase in observed ASVs and Shannon diversity from the stomach to the lower GI as shown previously (Figure 1A). The mycobiome showed no significant trends in dispersion and remained relatively similarly dispersed throughout the piglet GI tract and feces ($p \geq 0.05$, Figure 3B, and Supplementary Table S2B).

Mean taxonomic composition by litter of bacterial and fungal families present in the piglet GI tract were compared across GI tract and feces (Figures 4A,B). In the bacteriome, the most abundant phyla (Supplementary Figure S2A) present in the GI tract and feces were Bacteroidetes (40.8 $\pm$ 1.9%), Firmicutes (37.2 $\pm$ 1.9%), and Epsilonbacteriota (19.5 $\pm$ 2.5%) comprising >97% of the bacteriome. Genera Prevotella 9, Prevotella 1, Prevotellaceae NK3B31 group, and Alloprevotella from family Helicobacteraceae (17.4 $\pm$ 2.4%), Lactobacillus from family Lactobacillaceae (10.3 $\pm$ 1.4%), Blautia from family Lachnospiraceae (7.2 $\pm$ 0.4%), and Veillonella from family Veillonellaceae (5.2 $\pm$ 0.6%) were among the most abundant and prevalent genera and families in the bacteriome (Supplementary Figure S2C). In general, bacterial families Helicobacteraceae and Lactobacillaceae decreased from the stomach and upper GI to the lower GI and feces, while families Prevotellaceae, Lachnospiraceae, and Ruminococcaceae increased along the GI tract and feces. Relative abundances of Helicobacteraceae in the feces were <1.0%. Of the different GI tract regions, the stomach showed high variation in taxonomic composition among litters, while the lower GI and feces showed relatively consistent taxa across litters. In the mycobiome, Ascomycota (90.7 $\pm$ 1.3%) and Basidiomycota (9.0 $\pm$ 2.3%), and Wallemia from family Wallemiaceae (6.3 $\pm$ 1.0%) were the dominant genera and families across all GI tract regions and feces (Figure 4B and Supplementary Figure S2D). Symbiotaphrina from family Symbiotaphrinaceae was dominant in the piglet GI tract organs (9.8 $\pm$ 2.0%) but was only found in 3 piglet feces samples at $<0.1\%$ abundance. In contrast to the stomach and feces bacteriome, the stomach mycobiome had relatively consistent taxa among the litters, while the feces mycobiome demonstrated a high degree of variation.

Interactions Between the Bacteriome and Mycobiome in the Piglet Lower GI

Potential interactions between genera found in the lower piglet GI bacteriome and mycobiome were determined with SparCC correlations and a corresponding network
Figure 1 | Alpha-diversity of the bacteriome and mycobiome in piglet GI organs. (A) Shannon diversity index values and (B) observed ASV counts for bacterial 16S rRNA and fungal ITS gene sequencing data by sample type. Linear mixed-models were performed to determine differences between bacterial and fungal indices by organ. Only samples with both bacterial 16S rRNA and fungal ITS gene sequencing data were plotted and analyzed. Significance indicated by *p < 0.001.
FIGURE 2 | Beta-diversity of gastrointestinal tract organs and litters. Non-metric multidimensional scaling (NMDS) plot of β-diversity based on Bray–Curtis dissimilarities in the (A) bacteriome and (B) mycobiome of the piglet GI tract. Ellipses indicate 1 standard deviation from organ centroid and spiders are drawn to GI tract region centroid. Colors indicate GI tract region, symbols indicate litter, and ellipses line types indicate specific organs of the upper and lower GI.
FIGURE 3 | Box plot of pairwise distances between piglet organ centroids. Plots represent the median and interquartile range in the (A) bacteriome and (B) mycobiome. Colors indicate piglet GI tract region: red = stomach, green = upper GI, blue = lower GI, purple = feces. Differences between organ centroids were analyzed using permutational analysis of multivariate dispersion on Bray–Curtis dissimilarities with significance indicated by letters ($p < 0.05$).
analysis (Figures 5A,B and Supplementary Tables S5A,B). Fungi genus Kazachstania showed significant positive correlations with bacteria genera Alloprevotella, Lactobacillus, Prevotella 9, and Subdoligranulum. Fungi genera Aspergillus, Cladosporium, Hyphopichia, and Wallemia showed mostly negative correlations with other bacteria genera. Aspergillus, in particular, showed predominantly negative correlations with short chain fatty acid-producing bacteria such as Butyricicoccus, Subdoligranulum and Fusicatenibacter. Fungi genera Diopadascus, Symbiotaphrina, and Trichosporon did not show correlations with other fungi or bacteria. Additionally, there were no strong correlations among any of the other fungi genera identified within the piglet gut mycobiome.
FIGURE 5 | Inferred interactions between the bacteriome and mycobiome in the piglet lower GI tract. (A) SparCC correlation plot showing significant individual correlations between bacterial and fungal genera in the lower GI organs of the post-weaning piglet. Red circles indicate negative correlations and blue circles indicate positive correlations ($p < 0.05$ after FDR adjustments). The size of circles represents correlation strength while non-significant correlations are not shown. (B) SparCC correlation network between bacterial and fungal genera with plotted correlations with an absolute value $\geq 0.4$. The edge color indicates sign of correlation: negative (red), positive (blue); node color indicates kingdom: bacteria (purple), fungus (green). The size of node is proportional to the mean centered-log ratio abundance for each genus.

**DISCUSSION**

Fungi, in addition to bacteria, are important members and contributors of the microbiome, and recognition of their role is an essential step forward in elucidating the dynamics of the GI environment. Of the limited gut studies examining bacteria and fungi together, none to our knowledge have explored the interaction of the bacteriome–mycobiome of the piglet GI tract. Our study characterized and compared the mucosal-associated bacteriome and mycobiome of 23 healthy piglets, aged 35 days, from 3 litters along different organs of the GI tract and feces. The lower GI tract, which included the cecum and colon, was further evaluated to assess differences in predicted interactions between the bacteriome and mycobiome and determine associations between bacterial and fungal genera. This research is a critical first step in revealing the complex interactions, including health and growth, promoted by the fungi in the piglet gut during weaning.

While studies investigating the microbiota have become common, extensive studies of the mycobiota have been limited due to a lack of technologies, databases, and consensus in techniques (Li X.V. et al., 2019; Richard and Sokol, 2019). Recently, Li X.V. et al. (2019) reviewed studies characterizing the mycobiome of different human sites and disease states. Despite significant recent advances, methodologies continue to
lack consensus for DNA isolation, primer design, sequencing, databases, and analysis of fungal species. Previous work in our laboratory aimed to determine which techniques and fungal primers were effective in studying the piglet mycobiome (Summers et al., 2019). Lower estimated diversity in the mycobiome compared to the bacteriome has been observed in human stool (Nash et al., 2017), piglet feces (Summers et al., 2019), and settling dust in pig farms (White et al., 2019). While there is no established consensus on what constitutes a healthy gut mycobiome, it is widely accepted that fungi are less abundant and demonstrate less diversity than bacteria in the human gut, comprising roughly 0.1% of the microbiome based on shotgun metagenome sequencing (Qin et al., 2010; Huffnagle and Noverr, 2013). In this study, compared to the bacteriome, estimated overall diversity and observed ASVs for all 6 GI organs (stomach, duodenum, jejunum, ileum, cecum, colon) and feces were significantly lower in the mycobiome (Figures 1A,B and Supplementary Figures S1A,B).

Within the bacteriome, there was an increasing trend in α-diversity along the GI tract from the stomach to the colon, with highest α-diversity found in the feces, coupled with a decreasing trend in dispersion (β-diversity) among the organs (Figure 1A and Supplementary Tables S3, S4). These trends are consistent with those seen by Crespo-Piazuelo et al. (2018), which characterized the bacteriomes of 120 day old pigs along the GI tract gradient from the duodenum to the distal colon. In general, the stomach and upper GI tract (duodenum, jejunum, and ileum) host fewer microorganisms than the lower GI tract (colon and cecum) due to shorter retention times for adherence to tissue or mucus (Donaldson et al., 2016) lower pH, and higher concentrations of bile acids (Mackie et al., 1999; Walter and Ley, 2011). The harsher environment of the stomach and upper GI may subsequently select for a smaller number of colonizing bacterial species resulting in reduced diversity. The stomach and organs associated with the upper GI bacteriome also demonstrated higher levels of dispersion than the lower GI and feces, indicating a greater level of individual variation among piglets. Unlike the lower GI, the stomach and upper GI are exposed to new and exogenous bacteria ingested with food particles (Donaldson et al., 2016). The stomach, in particular, serves to block ingested microbes from passing to the intestine (Martinsen et al., 2005). Despite identical piglet diets, individual variation was seen in the stomach and upper GI, potentially due to the amount and timing of the piglet’s meal. Other potential factors, including host immunity or fungal interactions, may influence bacterial variation in the upper GI of the piglet as the small intestine plays a critical role in the development of mucosal and systemic tolerance toward microbes (Villmone, 2018).

Much less is known about diversity trends in the gut mycobiome. Unlike the bacteriome, the mycobiome did not follow the same general linear increase in α-diversity along the GI tract (Figure 1B). Instead, the stomach mycobiome had the highest mean diversity, followed by the colon. The average gastric pH of 6-month old pigs fed ad libitum is 4.4, although this level can vary among individuals or timing of meals (Merchant et al., 2011). Compared to many bacteria, fungi are more acid tolerant. Many fungi have adaptive strategies to respond to low pH environments, and some fungi like Aspergillus sp. actively lower the surrounding pH of their environment (Vylkova, 2017). This suggests that the high diversity of the stomach mycobiome may be due to the greater survivability of fungi in highly acidic environments, as well as potentially less competition from bacteria for resources compared to the rest of the GI tract. Individual variation in the mycobiome remained relatively similar along the GI tract based on dispersion estimates (Figure 2B). Unlike the bacteriome, however, there was no reduction in dispersion in the lower GI or feces, and individual variation remained comparable to the upper GI bacteriome. Some studies have suggested that most fungi found in the GI tract are transient via environmental or dietary sources, and are unable to colonize or inhabit the gut long-term (Suhr and Hallen-Adams, 2015; Raimondi et al., 2019). The suspected temporary nature of some fungi in the piglet GI tract, as well as the genetic and immunity factors that affect the bacteriome, may all play a role in the relatively high level of individual variation in the mycobiome. Despite high individual variation within each organ mycobiome, distinct clusters were found for each GI tract region depicted in the NMDS (Figure 2B), indicating that fungal distribution along the GI tract is not random and may indicate different GI environmental niche effects on the mycobiome.

In both the bacteriome and mycobiome, there were dominant taxa throughout most of the piglet GI tract and feces (Figures 3A,B). In the bacteriome, Bacteroidetes, Firmicutes, and Epsilonbactereota were the dominant phyla, consistent with previous studies investigating the pig GI tract (Zhao et al., 2015; Kelly et al., 2017; Crespo-Piazuelo et al., 2018; Zhang et al., 2018). In previous studies, Proteobacteria was considered a dominant phylum, but recently Epsilonbactereota was reclassified as a separate phylum from Proteobacteria (Waite et al., 2017) and Epsilonbactereota was a more dominant phylum in our dataset. In general, there were increases in Prevotellaceae, Lachnospiraceae, and Ruminococcaceae from the stomach to the lower GI tract, corresponding with a decrease in Lactobacillaceae and Helicobacteraceae. Many of these taxa shifts may be attributed to changing environmental conditions that occur along the GI tract. Measured dissolved oxygen levels undergo a dramatic reduction from the duodenum in the upper GI to the cecum of the lower GI (Hillman et al., 1993), as well as a reduction in pH, an increase in resistant starches (Flint et al., 2012), and slower peristalsis times (Walter and Ley, 2011). Members of Helicobacteraceae and Lactobacillaceae are tolerant of bile acids and oxygen (De Boever and Verstraete, 1999; Okoli et al., 2007; Yasuda et al., 2015), and are able to adhere firmly to the surface of the small intestine (Donaldson et al., 2016) making them suitable for colonizing the upper GI tract. In comparison, Prevotellaceae, Lachnospiraceae, and Ruminococcaceae are oxygen-sensitive and are likely more competitive in the lower GI due to their ability to degrade complex carbohydrates (Arumugam et al., 2011; Flint et al., 2012; Liu et al., 2012).

The dominant phyla in the piglet GI tract and feces mycobiome were Ascomycota and Basidiomycota, which are similar to those found in human mycobiome gut studies (Hoffmann et al., 2013; Nash et al., 2017; Raimondi et al., 2019). Unlike human studies, however, commonly found yeasts from
genera *Candida* and *Saccharomyces* (Hallen-Adams and Suhr, 2017; Sam et al., 2017), were either absent or found at <1% relative abundance in our study samples. Instead, the dominant yeast throughout the piglet GI tract and feces was identified as *Kazachstania* (sp. *sloofiae*). *K. sloofiae* has been previously identified from different parts of the healthy pig GI tract (Uden and Carmo-Sousa, 1962; Urubschurov and Janczyk, 2011) and feces (Summers et al., 2019), and has been shown to establish quickly in the gut of piglets based on fecal analysis (Urubschurov et al., 2015; Urubschurov et al., 2017). Urubschurov et al. (2017) determined *K. sloofiae* may be responsible for maintaining piglet health by producing peptides, vitamin C, and formic acid in the piglet GI tract. Other dominant fungi genera found throughout the piglet GI tract and feces included *Hyphopichia* (sp. *burtonii*) and *Wallemia*. In contrast to *K. sloofiae*, both of these fungi are likely non-colonizing, transient fungi of the piglet GI tract. *Hyphopichia burtonii*, a commonly isolated yeast from corn, wheat, and rice (Kurtzman, 2011), has an estimated maximum growth temperature of 37°C (Burgain et al., 2015), and is unlikely to thrive at the internal temperature of a pig at around 38.7–40°C. *Wallemia*, commonly isolated from food sources as well as agricultural dust, is also unlikely to reside in the piglet GI environment due to its extremophilic and xerophilic nature (Zajc and Gunde-Cimerman, 2018).

Complex interactions between bacteria and fungi also occur within the gut. Several significant correlations were found between bacterial and fungal genera in the lower piglet GI tract, suggesting potential bacteriome–mycobiome relationships (Figures 5A,B). The dominant fungal genus in the GI tract of humans is *Candida* and several species have been found to interact directly with bacterial species like *Lactobacillus* (Mason et al., 2012b; Allonsius et al., 2017; Hallen-Adams and Suhr, 2017; Rossoni et al., 2018). *Kazachstania* was strongly and positively correlated with *Lactobacillus* in the lower GI of our piglets and as *Kazachstania* is genetically similar to *Candida* (Kurtzman et al., 2005), it may be a potential porcine analog to *Candida* in the guts of humans. Future studies will be needed to clarify its role in pig gut health and homeostasis, as well as its potential to act as an opportunistic pathogen. A positive correlation between *Kazachstania* and *Lactobacillus* was also found in piglet feces in Urubschurov et al. (2011) using culture methods and PCR-DGGE techniques. In co-cultures of *Lactobacillus* and some yeasts, *Lactobacillus* released organic acids that lower the surrounding pH and promoted yeast growth; these yeasts are then stimulated by *Lactobacillus* to release essential nutrients and vitamins utilized by *Lactobacillus* (Stadie et al., 2013). A similar mutualistic relationship may exist between the dominant yeast *Kazachstania* in the piglet GI tract and *Lactobacillus*.

A strong positive correlation was also observed between *Kazachstania* and *Prevotella* 2 and *Prevotella* 9 genera. It has been hypothesized from observed positive associations between *Candida* yeasts and *Prevotella*, that *Candida* and *Prevotella* are involved in a mutualistic relationship regarding the degradation and fermentation of complex carbohydrates in the human gut (Hoffmann et al., 2013). In the piglet gut, *Kazachstania* may fulfill the role of *Candida* in the potential Candida-*Prevotella* link to starch metabolism. The corresponding network plot of the bacteriome–mycobiome community correlations showed no interactions between *Cladosporium*, *Symbiotaphrina*, and *Trichosporon* and other microbiota of the bacteriome–mycobiome. Unlike the *Kazachstania*–bacterial potential relationship, these fungi may be truly transient in the lower piglet GI and pass through without interacting with the gut microbial community. Interestingly, *Hyphopichia* and *Wallemia* showed mostly negative correlations with several gut bacteria. While these fungi are also thought to be non-colonizing microbiota of the gut as mentioned previously, they may still have an impact on the gut bacteriome during passage or may be capable of establishing themselves in the lower gut environment. One interesting finding in the lower gut of piglets was the negative association between short chain fatty acid-producing bacteria and *Aspergillus*. In humans, *Aspergillus* can exacerbate allergic responses in farm workers and is a well-documented pathogen. Aspergillosis is less common in pigs but has been documented as a rare cause of porcine abortions (Eustis et al., 1981; Todd et al., 1985; Sabino et al., 2012; Li Z. et al., 2019). *Aspergillus* has been documented to directly interact with bacterial species such as *Stenotrophomonas* and *Pseudomonas* and may play a critical role in disease severity (Schroechk et al., 2009; Melloul et al., 2018; Briard et al., 2019). The negative association of *Aspergillus* with SCFA-producing bacteria, typically associated with beneficial gut health (Baxter et al., 2019; Peirce and Alvina, 2019) could be explained by the change in *Aspergillus* spp. behavior following sodium butyrate exposure (Philip et al., 1963).

In this study, we provided a comprehensive overview of the bacteria and fungi present along the piglet GI tract and feces in healthy piglets post-weaning, as well as new insight into potential interactions between the microbiome and mycobiome. The taxonomy and diversity of the mycobiome, in addition to the microbiome, demonstrated distinct differences in diversity between the bacterial and fungal members of the gut. In addition, fungal commensals, such as *Candida* spp., from the human gut were lacking in the pig. Potential interactions in the porcine gut show that bacteria may be acting in a beneficial way with thefungus, *Kazachstania*, and through negative interactions with *Aspergillus*. Further exploration of these significant correlations in the piglet gut will provide a greater understanding of the relationships that exist between the bacteriome and mycobiome that may potentially alter piglet growth and health.

**DATA AVAILABILITY STATEMENT**

The datasets generated for this study can be found using the Accession number PRJNA558038: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA558038.

**ETHICS STATEMENT**

The animal study was reviewed and approved by the USDA–ARS Institutional Animal Care and Use Committee of the Beltsville Agricultural Research Center.
AUTHOR CONTRIBUTIONS

AA and KS contributed to the conception and design of the study, and wrote the first draft of the manuscript. AA performed the statistical analysis. All authors performed the animal research and handling, contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.02286/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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