

Life-long dynamics of the swine gut microbiome and their implications in probiotics development and food safety

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ABSTRACT

The swine gut microbiome has received remarkable attention in recent years given that pigs serve not only as important sources for animal-derived food but also as excellent biomedical models for human health. However, despite recent advances in the understanding of the swine gut microbiome, many important biological and ecological questions are still largely unanswered. In a recent study, we characterized the life-long dynamics of the swine gut microbiome from birth to market. We showed distinct shifts in gut microbiome structure along different growth stages mainly driven by diet. Here, we summarize these discoveries and provide additional data related to the core swine gut microbiome, probiotics development in the swine industry, and foodborne pathogens in the pork supply chain.

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Introduction





The human microbiome has been extensively studied in the last decade and substantial progress has been made in that field regarding their membership, structure, dynamics, and correlations with different diseases.^{1–5} Pigs serve as important protein sources for human beings. With the increase in global population and preference for animal protein sources, it is critical to maintain sustainable and efficient swine production. Given the importance of pigs as both livestock species and as medical models for human diseases, the swine gut microbiome has been characterized by many groups throughout the world. It has been reported that the swine gut microbiome correlated with feed efficiency, fat deposition, and growth performance.^{6–10} Despite this progress toward understanding the swine gut microbiome, many important ecological questions are yet to be answered.

The swine industry faces enormous challenges. The emergence and spread of antimicrobial resistance (AMR) from the swine industry due to antibiotic application for disease treatment, prevention, and growth promotion have drawn pressing public health concerns,¹¹ necessitating the use of alternatives to

antibiotics, such as probiotics, in the swine industry. Furthermore, live pigs on farm serve as one of the major sources of foodborne pathogens in the pork supply chain and the environment. Given the critical roles that the gut microbiome plays in swine health and production, it is crucial to better understand the mechanisms underlying the assembly and succession of the microbial communities in pigs to identify beneficial bacteria that might serve as probiotics. It is also important to track the dynamics of the pathogens to establish an effective management strategy to reduce, if not prevent product contamination. Recently, we investigated the life-long dynamics of the swine gut microbiome in a longitudinal study at pre-harvest and addressed many important ecological questions.¹² Here, we build on the discoveries from that study, particularly on the core microbiome and stage-specific bacteria and discuss their implications in the context of probiotics development and detection of foodborne pathogens.

Life-long dynamics of the swine gut microbiome

In a test animal trial, we followed 18 pigs from birth to market and found that their gut microbiome

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structures significantly shifted during different growth stages: lactation, nursery, growing, and finishing. Dramatic changes in gut microbiome structure occurred during weaning, when the pigs were separated from the sows and provided solid food rather than sow milk. Gradual but significant changes in swine gut microbiome structures were also observed during the different growth stages when the pigs were on a solid diet. Different signature bacterial taxa for these growth stages that drive the shifts in swine gut microbiome structure are listed in Figure 1.

Prevotella is the most abundant genus in the swine gut microbiome. Eleven of the top 30 features belong to this genus. Although three of these *Prevotella* features belong to the “core” gut microbiome (Table 1), especially Feature 9, which showed the highest abundance at lactation, most of these *Prevotella*-associated features are stage-specific (Figures 1 and 2). One of them (F29) started appearing at the grower stage whereas eight of these *Prevotella* features emerged only after weaning when the pigs were introduced to solid food (Figure 2). *Prevotella* is one of the most abundant genera in humans and its role in human health has been controversial.¹³ *Prevotella* species

are associated with dietary carbohydrates in humans,¹⁴ and produce acetate, an energy source for some butyrate producers such as *Ruminococcus*, *Clostridium*, and *Blautia*. In fact, members of these genera such as *Blautia* also emerged together with *Prevotella* at the beginning of nursery stage (Figure 2), supporting the co-occurrence and possible cross-feeding between these bacteria in pigs.

The swine core microbiome

The core microbiome has been well-researched in different species and ecological niches.^{1,15–18} Identifying a core microbiome is vital to understand its function in the gut to enable manipulation of microbial communities that are beneficial for human health. In general, a core microbiome refers to the common group of bacteria present in all or most (e.g. >90%) of the communities of a special habitat.^{16,19} However, it has been challenging to define a core microbiome given the many factors that affect the human gut microbiome such as diet, genetics, age, and antibiotics.^{2,5,19–23}

There have also been efforts in defining a core swine microbiome.^{24,25} In a recent study, Holman

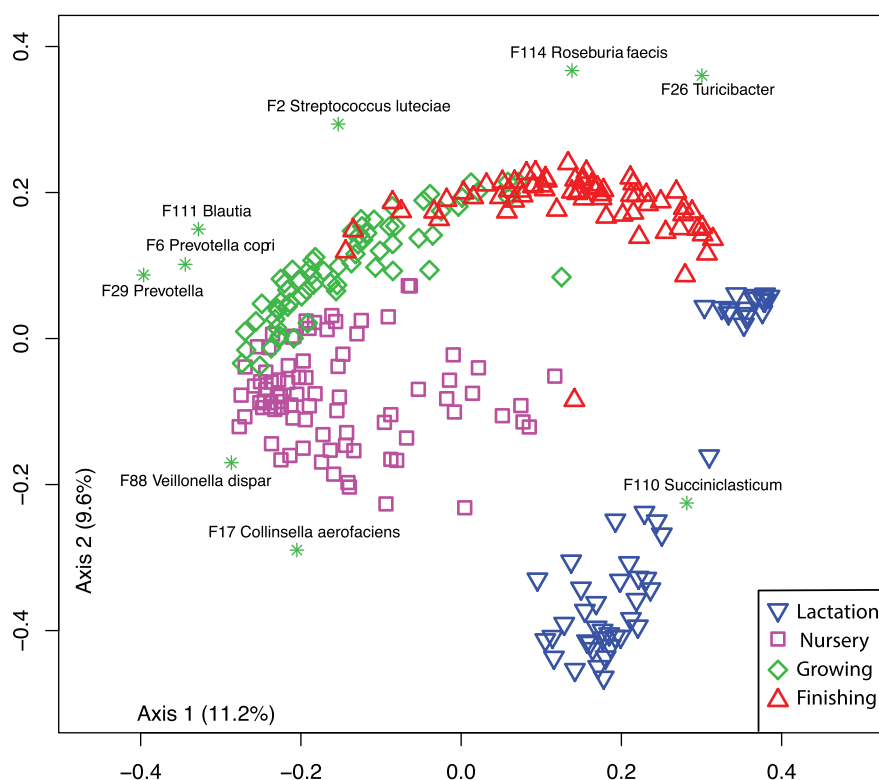


Figure 1. Bi-plot showing the life-long dynamics of the swine gut microbiome and the stage associated *Prevotella*.

Table 1. The swine core gut microbiome.

Feature#	Feature ID	Phylum	Order	Family	Genus	Species
F1	77560703da191 f21e7d250845229fe06	Firmicutes	Clostridiales	Veillonellaceae	Megasphaera	
F2	e11db671d9c36b550f08a6ee36ba2cef	Firmicutes	Lactobacillales	Streptococcaceae	Streptococcus	luteciae
F3	1919b6828724477c2ab08fd9efe3bcd9	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella	copri
F5	002109bc8b4bcf7a97a7794f4cfdad2b	Firmicutes	Lactobacillales	Lactobacillaceae	Lactobacillus	
F6	9c4260f79a8007b4d15a1e6fe1129ce1	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella	copri
F7	15caa2d41590f4361bd4ef0b6453fe1 c	Proteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia	coli
F8	230f858e6622e1a686ad91373adc20b9	Firmicutes	Clostridiales	Veillonellaceae	Phascolarctobacterium	
F9	38ec373490dc98851951b38c50961207	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella	
F10	21709f541225b3d1e30e195bcd4322b	Bacteroidetes	Bacteroidales	[Paraprevotellaceae]	YRC22	
F12	8f194030281f1b235fa5874aa0426bb0	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella	
F13	48ea88e5e788894c86b04d558c7ea12d	Firmicutes	Clostridiales	Veillonellaceae	Anaerovibrio	
F17	7ef3b00cc85b6d0730685b757440e392	Actinobacteria	Coriobacteriales	Coriobacteriaceae	Collinsella	aerofaciens
F20	20a99a2686aca6a717b01361cac773046	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella	stercorea
F21	f0e2244687ff7f51926d7b54adaec4b	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella	
F23	522ba9d695541c9ea478db7cafeb4355	Bacteroidetes	Bacteroidales	S24-7		
F28	fa9cbb7e45355cb861070b68d9a5653b	Firmicutes	Clostridiales	Ruminococcaceae	Oscillospira	
F46	b88939309e235a04fdcff68d166139ab	Bacteroidetes	Bacteroidales	[Paraprevotellaceae]	[Prevotella]	
F48	27a8a06bc2b03e2ee032a7aeef16dbe3	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella	stercorea
F50	36585850033948d7966edf6717266cc0	Firmicutes	Clostridiales	[Mogibacteriaceae]	Mogibacterium	
F52	f324a15262fda881e9e0f6b59a20b21d	Bacteroidetes	Bacteroidales	[Paraprevotellaceae]	CF231	
F53	f2c72e7978ae955c780882f1f689e9b	Firmicutes	Clostridiales	Christensenellaceae		
F62	2307b48380cd51b78c43a1da261bdde9	Bacteroidetes	Bacteroidales			
F63	fdf37b54fb9f5a82616d903886ade16d	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella	
F70	47ee99688649fa7c2937db076f2039de	Firmicutes	Lactobacillales	Lactobacillaceae	Lactobacillus	reuteri
F77	55d98aa0513d69baf52b439bb4e4207a	Proteobacteria	Campylobacteriales	Campylobacteraceae	Campylobacter	
F82	1e3a3bda59a3e703d9ebadfb122655d7	Firmicutes	Clostridiales	Ruminococcaceae	Oscillospira	
F86	4539eb8d334b124b12ef2f840be6c5d7	Firmicutes	Clostridiales	Ruminococcaceae	Oscillospira	
F87	fd2a145204502d5d1453ac09704619ad	Firmicutes	Clostridiales	Ruminococcaceae		
F93	1f75f87fb55993516a97af66507a0f08	Bacteroidetes	Bacteroidales	[Paraprevotellaceae]	[Prevotella]	
F112	04e3a222e2655f35b175436f9cb29844	Bacteroidetes	Bacteroidales			
F116	c0e706eff2fbc0db84755141796f11af	Bacteroidetes	Bacteroidales			
F120	fd4bd6f441389fd712ee14af66eaf217	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella	
F121	52f6ab8a13bead00126a83f61d959ab6	Firmicutes	Lactobacillales	Lactobacillaceae	Lactobacillus	delbrueckii
F123	f212b75172ddc5a1ef357a425ea33482	Firmicutes	Clostridiales	Christensenellaceae		
F131	c3a1361961c649a26927a4e6b6eae0c4	Firmicutes	Lactobacillales	Lactobacillaceae	Lactobacillus	mucosae
F132	4b922c405bf97b57647a7f6323092ce1	Actinobacteria	Coriobacteriales	Coriobacteriaceae		
F136	d3ea744c5bb301b99658befe3e1c162	Firmicutes	Lactobacillales	Lactobacillaceae	Lactobacillus	
F144	562f555227af77f2f0d0cc3e6462c8d8	Firmicutes	Clostridiales	Ruminococcaceae		
F149	795b1744a93f0c030c7cac5ed1573 c20	Firmicutes	Clostridiales	Ruminococcaceae		
F153	76f995a642c756e4baef9ba3588be99a	Bacteroidetes	Bacteroidales	Prevotellaceae	Prevotella	
F156	c26fda9fbfb7235e29a45dad8554c06 c	Bacteroidetes	Bacteroidales			
F157	f2f55ee5a197eaf6b65a1c22bedc016d	Firmicutes	Erysipelotrichales	Erysipelotrichaceae	[Eubacterium]	biforme
F169	b5dcb92aff75a84ef7f76c34e644b4dc	Firmicutes	Clostridiales	Christensenellaceae		
F171	6fa1b042db7e367356a0e6b600ce46a0	Firmicutes	Clostridiales			
F189	990ed356cd9dae5b83314355569514e3	Bacteroidetes	Bacteroidales			
F198	b05ae994010ddd94626d03a8c4ceafe1	Firmicutes	Erysipelotrichales	Erysipelotrichaceae	[Eubacterium]	biforme
F199	179a2e52862950c20141c0e376152624	Firmicutes	Clostridiales	Ruminococcaceae		
F201	27e548475cd61c016aac05c28e95223	Firmicutes	Clostridiales	Ruminococcaceae		
F214	5503fc4199485012d18108218ba0c589	Proteobacteria	Desulfufovibrionales	Desulfufovibrionaceae	Desulfufovibrio	
F215	deea65343744187b8e911e87975c8979	Actinobacteria	Coriobacteriales	Coriobacteriaceae		
F229	fec7ae85682e4fb767970f4dab35bbb5	Spirochetes	Spirochaetales	Spirochaetaceae	Treponema	
F235	d102b79fac474290436a96751aacb59	Proteobacteria	GMD14H09			
F241	3e2fe19948fdefb2fb179e6f8f1def05	Firmicutes	Clostridiales	Ruminococcaceae	Oscillospira	
F271	125a703172cdf3ac7155e51b904d6546	Firmicutes	Clostridiales	Ruminococcaceae	Oscillospira	
F275	5947530ad170bbc67f9af778d42f8dc7	Bacteroidetes	Bacteroidales	Porphyromonadaceae	Parabacteroides	
F302	f361d0070bf833c26d64b95fe512847a	Bacteroidetes	Bacteroidales	Prevotellaceae		
F310	413d7371b0955033e463b538fbaf0cfc	Firmicutes	Clostridiales	Ruminococcaceae		
F312	829d3bd6a7df03802fbb4a076f614b5	Firmicutes	Clostridiales	Ruminococcaceae		
F319	fc3d8267a88365a43202affb1b1c0d1f	Firmicutes	Clostridiales	Ruminococcaceae	Oscillospira	
F357	71903137448067873e3ffbf70dea44eb	Firmicutes	Clostridiales			
F394	1c4985419653025d3a4a1be178f4676 c	Firmicutes	Erysipelotrichales	Erysipelotrichaceae	p-75-a5	
F410	6d3a987e101712b3302f6e8e5beee7ea	Firmicutes	Clostridiales			
F413	02c93fa4666bd94365b522152d56bb3c	Firmicutes	Clostridiales	Lachnospiraceae		
F491	ebf50bcde4063f1d5e957907a2879929	Firmicutes	Clostridiales			
F502	0007527ae916772430dd7897aeeb0e8	Firmicutes	Clostridiales	Ruminococcaceae		
F518	547cc82727e9e688a34faefcedd5c11	Proteobacteria	Burkholderiales	Alcaligenaceae	Sutterella	
F521	ba2b96b172b40e9f509571929d9a082b	Actinobacteria	Coriobacteriales	Coriobacteriaceae		
F524	65732a9995f5007582ebb500e7df4ea5	Firmicutes	Clostridiales	Ruminococcaceae	Oscillospira	
F561	d76796a7df0d1f21a46610474a17f2e4	Firmicutes	Clostridiales	Ruminococcaceae	Ruminococcus	

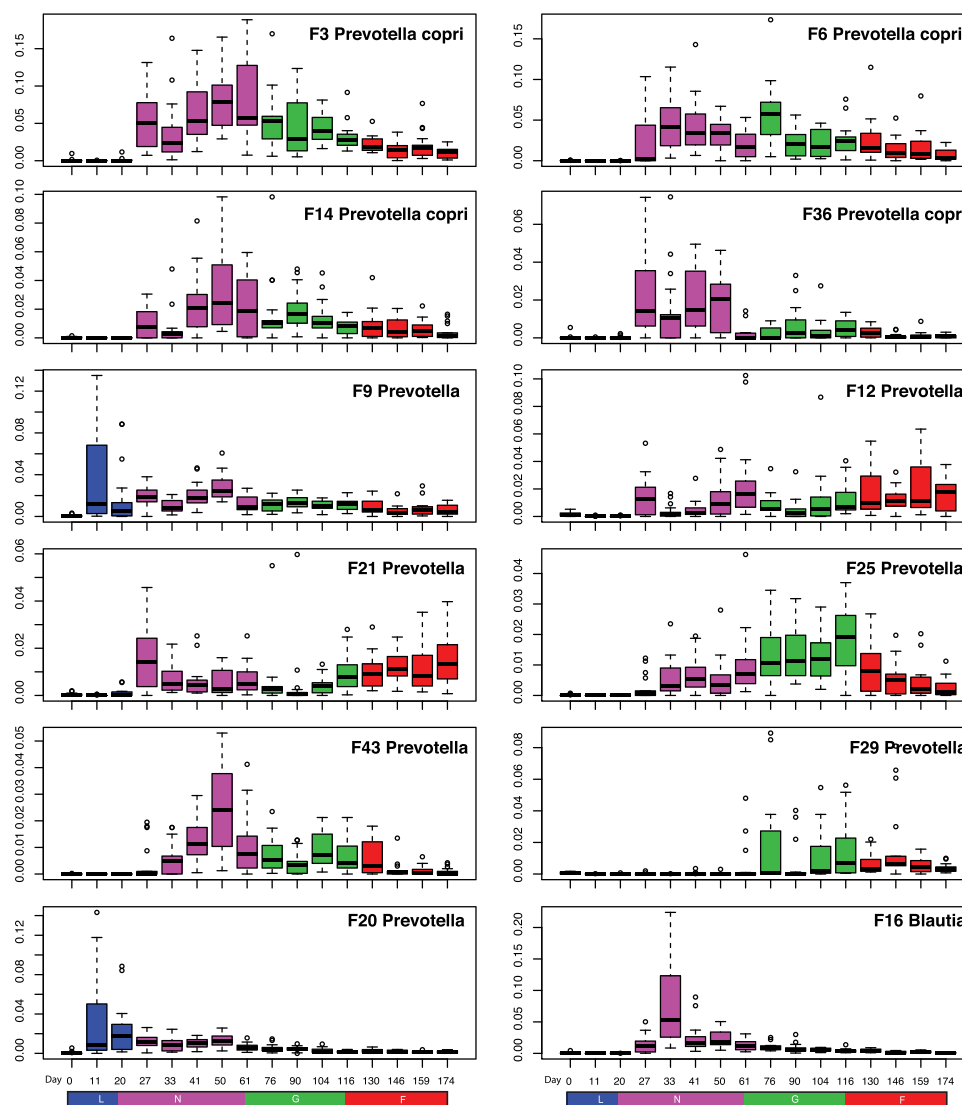


Figure 2. Boxplots showing the dynamics of the top 11 bacterial features associated with *Prevotella* and one feature associated with *Blautia* during different growth stages of pigs.

and colleagues performed a meta-analysis of 20 data sets and found several shared genera such as *Prevotella*, *Clostridium*, *Alloprevotella*, and *Ruminococcus*. However, the most striking conclusion from the analysis is that, other than gastrointestinal (GI) tract location, the “Study” factor was also significant in shaping the swine gut microbiota. It is not surprising that different niches along the GI tract harbor distinct gut microbiomes given the unique ecological environments and physiological conditions in each niche. The significant effect of “Study” on the swine gut microbiome underscores the importance of standardizing experimental techniques, e.g. sample collection and storage,^{26,27} DNA extraction,^{28–30} hypervariable regions of the 16 S

rRNA gene,³¹ and bioinformatics pipelines,³² to allow cross-study comparisons aiming to identify a core swine gut microbiome.

In our recent report, we identified a “core” microbiome of 69 bacterial features that were present in all the growth stages and shared by three groups of pigs in the test and validation trial (Table 1). Consistent with the findings of Holman et al,²⁵ most of these features are associated with the order Clostridiales (n = 28), Bacteroidiales (n = 22), and Lactobacillales (n = 6). At the family level, the top three families are Prevotellaceae (n = 11), Ruminococcaceae (n = 16), and Lactobacillaceae (n = 5). A total of 10 features were not classified to the family level. Although some of the top

features such as *Megasphaera* (F1) and *Prevotella* (F3) were present during all the growth stages, others such as F4 (unclassified Clostridiaceae) and F10 (*Bacteroidetes* YRC22), rarely noticeable at lactation and nursery stages, emerged rapidly and became the dominant taxa at the growing and finishing stages. The sequence and taxonomy of these features are listed in Table 1.

Our study has several contributions to the effort of identifying a core microbiome. We used ASVs (amplicon sequence variants) or ESVs (exact sequence variants) to define bacterial features that differed with a single nucleotide. This approach enables cross-study comparisons to determine whether the same bacterial features are shared among different studies.³³ For core microbiome studies, our data show that it is important to take into account the stage-specific bacterial taxa. For example, the finishing-stage bacteria (e.g. F4) might be missed in the “core” gut microbiome if only nursery-stage samples were used for comparison.

Swine probiotics development: does one size fit all?

Due to pressures from a public health standpoint, many countries have banned the use of antibiotics for growth promotion in animals. Alternatives to antibiotics are critical to improve animal health and growth performance in the swine industry. Probiotics serve as an important alternative to sub-therapeutic antibiotics.³⁴ As a first step in developing probiotics, we performed a regression-based random forest analysis to identify potentially beneficial bacteria that correlate with growth performance at each growth stage and at the end of the test trials. Apparently, different sets of bacteria associated with growth performance were observed from each stage. In a validation trial, we inoculated 12 post-weaning pigs with fecal samples from a healthy pig at growing stage. Fecal microbiota transplantation (FMT) improved the growth performance of the recipient pigs. Although not statistically significant likely due to the small sample size ($n = 12$), FMT did increase the body weight of recipient pigs by 4.9 kg on average compared to their litter mates at the end of the validation trial. The fecal sample from the donor is representative of the mature pigs from growing stages regarding the microbiome composition and structure.¹² FMT didn't change the overall

gut microbiome structure of the recipients, however, it did enrich several groups of bacterial taxa. Random forest identified these stage-associated bacteria that were enriched in the FMT group and correlated with growth performance in the validation trial. A comparison of the test and validation trial identified shared bacterial features that might serve as potentially beneficial bacteria promoting animal growth performance (Table 2).

Figure 3 shows the relative abundance of these bacteria in the pigs of the control and the FMT group in the validation trial. Bacterial features associated with *Bulleidia* (F336) and *Lacobacillus mucosae* (F454) were more abundant in the FMT group with greater growth performance at the nursery stage, whereas features affiliated with *Acidaminococcus* (F100) and *Prevotella* (F73) were over represented in the FMT group at the late nursery and growing stages. Members of *Turcibacter* (F26), more abundant in the FMT group, didn't emerge until the finishing stage. Of note, features associated with growth performance, calculated based on the final body weight, were mainly late colonizers of the swine gut. For example, F4 and F18, which were more abundant in the FMT group, started appearing in the gut at the growing stages. Our data show different sets of potentially beneficial bacteria associated with superior phenotypes with a stage-specific pattern. We propose that a mix of probiotics tailored to growth stages of pigs, rather than to a single bacterial strain, should be developed

Table 2. Stage-specific potentially beneficial bacteria shared by the two animal trials.

Lactation	Nursery	Growing	Finishing	Overall
F77	F222	F100	F26	F55
F363	F604	F73	F19	F4
F182	F7	F40	F27	F27
F876	F233		F333	F75
F162	F339		F100	F19
F1	F336		F61	F26
F166	F301		F394	F234
F502	F454		F134	F18
F21				F336
F53				F165
F247				F127
				F103
				F377
				F196
				F433
				F231
				F376
				F100
				F17
				F330
				F307

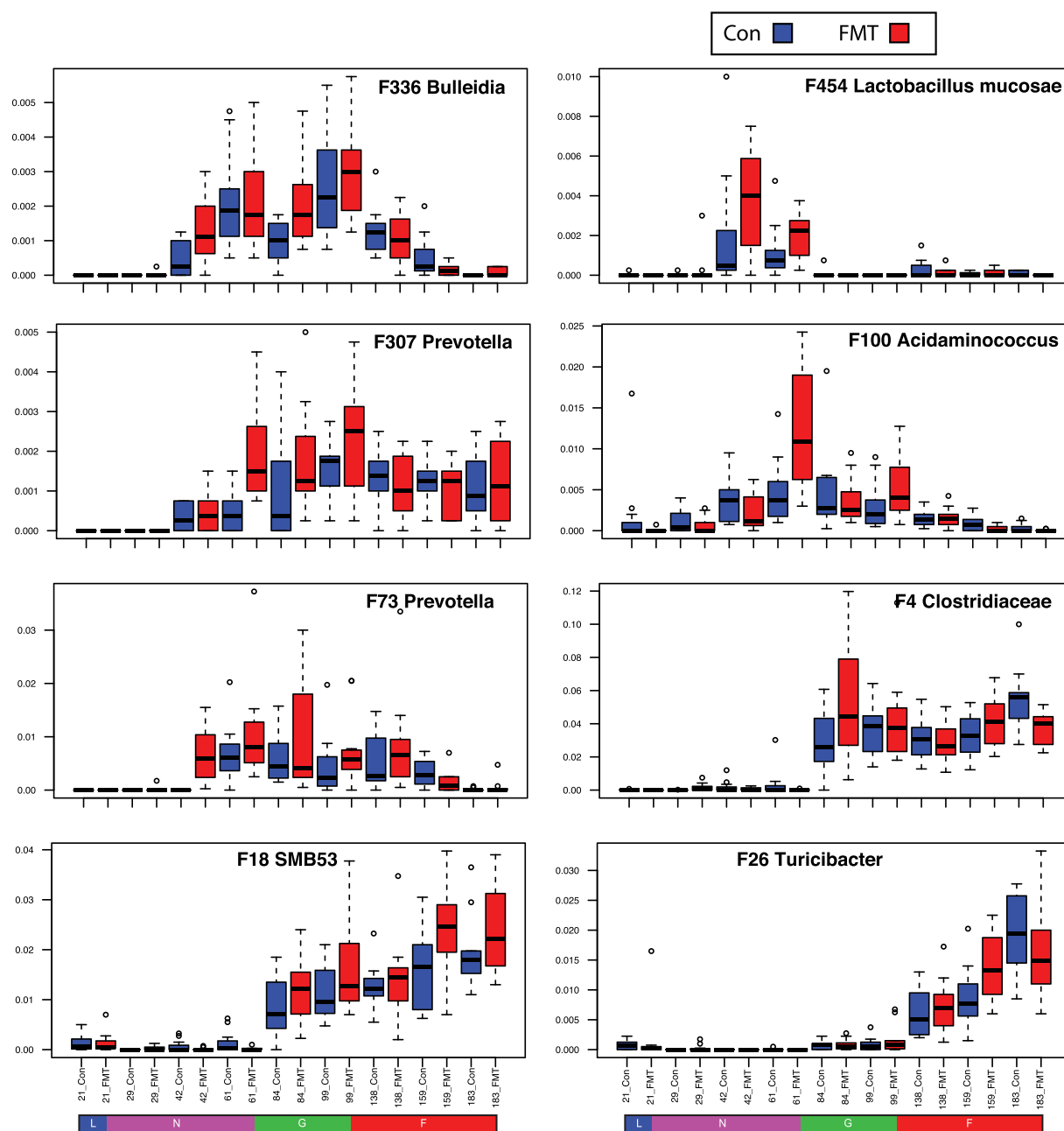


Figure 3. Stage-associated potentially beneficial bacteria in the control and fecal microbiota transplantation (FMT) pigs. FMT remarkably improved swine growth performance. Bacterial features identified by random forest that positively correlate with growth performance were more abundant in the FMT group.

to optimize their beneficial effects on swine health and production.

Foodborne pathogens: the best window for intervention

Pork, the most consumed meat worldwide, is a major source of foodborne pathogens, which are a major cause of human morbidity and mortality every year.³⁵ Most of the studies in the food safety area focus on the

post-harvest section of the swine industry with very limited information about the live animals on farm during the pre-harvest season. In fact, live pigs are not only the source of foodborne pathogens, which are passed along to the post-harvest supply chain, they also shed these pathogens on the farms, thus exposing farm workers as well.³⁶ Therefore, understanding the dynamics of these pathogens on farm provides information on the best window and strategy to manage these pathogens.

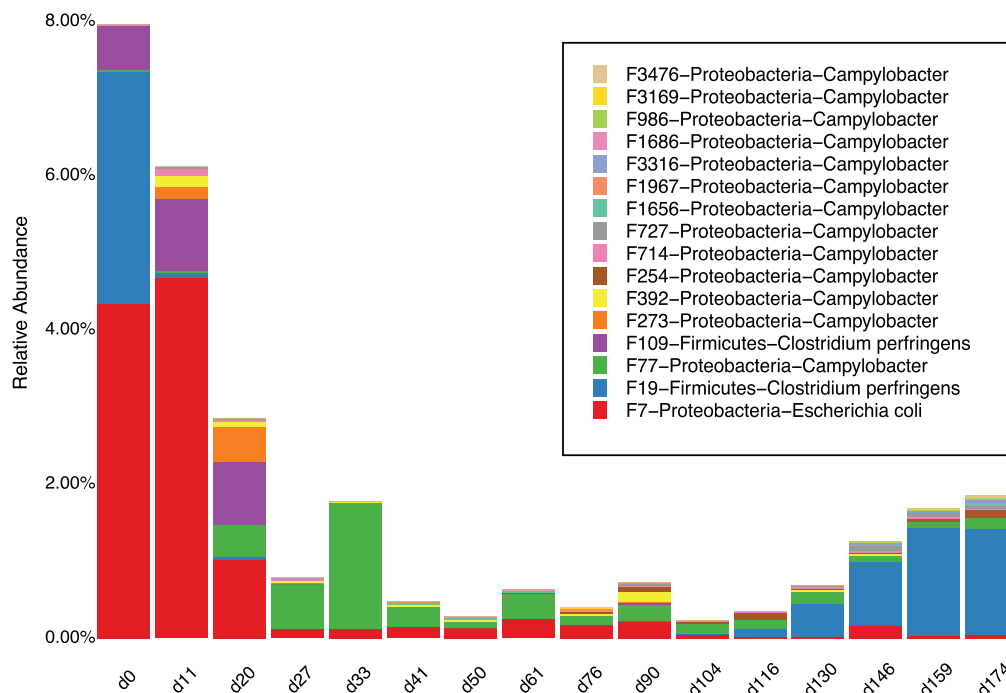


Figure 4. The dynamics of foodborne pathogens (members of *Campylobacter* and *E. coli*) in pigs during the pre-harvest section of the pork supply chain.

Campylobacter is one of the major foodborne pathogens in the swine industry.³⁵ Our longitudinal study shows the relative abundance and dynamics of 13 bacterial features associated with *Campylobacter* and one bacterial feature associated with *E. coli* (Figure 4). *E. coli* was abundant during the lactation stage and faded out after weaning. Different features of *Campylobacter* showed different dynamic patterns. Future study is needed to track the dynamics of these pathogens in the whole pork supply chain to determine which features are transmitted from farm to fork so that a treatment window based on this essential information could be determined.

Conclusions

In conclusion, this longitudinal characterization of the swine gut microbiome provides the foundation for translational research aiming to improve animal health and production. This study not only contributes to our understanding of many key biological and ecological concepts, but also identified potentially beneficial bacteria and pathogens. Characterization of the growth-stage-associated swine gut microbiome emphasizes the importance of optimization of

probiotics based on different stages. It also provides insights on the best window to manage foodborne pathogens during the pre-harvest season of the swine industry. We also advocate for standard protocols in swine gut microbiome studies (e.g. sample collection, DNA extraction) to improve reproducibility and cross-study comparability for translational research investigations.

Materials and methods

All the animals and sequencing data were from our previous study¹². No additional pigs or sequences were included. Sequence process and analysis were performed as described previously¹². The biplot in Figure 1 was generated by the `corr.axes` function (setting: `method = spearman`; `numases = 2`) in `mothur` software package (v.1.40.5).³⁷ Boxplots (Figures 2 and 3) and stacked bar chart (Figure 4) were generated by the `ggplot2` package of R.

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