Shifts in ileal mucosa microbiota throughout the production stages of swine

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ABSTRACT:

The intestinal microbiota shifts over the life of a pig due to physiological maturation, immune development and change in diet from milk to solid feed increasing in fiber over time. To better understand how the commensal microbiota is established, ileal mucosa samples were collected from pigs at 6, 18, 56, 110, and 154 days of age, representing early lactation, lactation just before weaning, as well as the end of nursery, grower and finisher phases, respectively. TRFLP was performed to determine the ileal microbiota present at each time point. One-way ANOVA analysis was performed on TRF peak area, peak abundance and Simpson diversity indices to determine differences over time. Cluster analysis was performed to indicate microbiota structure relatedness. Samples from days 6 and 18 had fewer TRF peaks per enzyme ($P < 0.001$) than samples from later time points. The TRFs present at day 6 and 18 were putatively identified as uncultured bacteria of the order Clostridiales. More TRFs were present in samples starting at 56 days of age ($P < 0.001$) compared to days 6 and 18, however, many of the TRFs were not more abundant on day 56 ($P > 0.10$) than in samples from days 6 and 18. TRF abundance was greatest ($P < 0.05$) on days 110 and 154. Day 56 was the only sampling day where most samples clustered together as a subset of day 110 and 154 with 55 % similarity, whereas days 6 and 18 formed a major separate cluster with 2 % similarity to the animals of 56 days of age and older. Among the TRFs becoming more abundant at day 56 were TRFs identified as *Clostridium* cluster XIVa which are associated with dietary fiber degradation. By day 56, there was an increase in diversity ($P < 0.001$) with as many as 83 TRFs being more abundant than at earlier time points which indicated an increase of *Bacillus*, *Bacteroides* and *Prevotella*. These data illustrate that the ileal mucosa is largely uncolonized during the early life of a pig. The nursery phase after weaning was determined as a transition phase in which the diversity of bacteria in the gut increased and started to resemble that of an adult pig. By 110 and 154 days of age, a diverse commensal microbiota was established. The increase of cluster XIVa *Clostridium*, *Bacteroides* and *Prevotella* later in life are an indication of the role of diet in helping to form the commensal microbiota as they are instrumental in the breakdown of nutritional fiber.

INTRODUCTION:

There are four management phases, each with a unique diet, in the life of a commercially raised pig (1). The lactation phase is from birth to weaning, which occurs at 20 days of age. After weaning, pigs are transitioned onto solid feed during the nursery phase which lasts from 21 to 56 days of age. Nursery diets are easily digestible, contain low fiber contents and are high in energy and protein. The grower and finisher phase typically lasts from 57 to 110 and 111 to 155 days of age, respectively, with diets consisting of decreasing amounts of energy and protein, and increasing amounts of dietary fiber (1). The intestinal microbiota in the pig post partum throughout the early grower phase has been well studied (2, 3, 4) due to the drastic changes in gut microbiota early in life as well as due to the diet change from milk to solid feed during weaning. Changes in early gut microbiota have been described to impact microbial colonization later in life with a strong impact on host immunology (5). However, no study is known to describe swine gut microbiota in late grower and finisher pigs. In this study, the microbiota is examined at each phase of a commercially raised pigs life to determine how the microbiota is established and shifts with each change in diet.

OBJECTIVE:

To determine how the commensal microbiota in the ileum mucosa changes over the complete life span of a commercially raised pig.

MATERIALS AND METHODS:

Sample Collection

- 10 pigs were sacrificed each at days 6, 18, and 56, 5 pigs were sacrificed each at days 110 and 154
- The ileum was defined as the 15 cm of the terminal small intestine proximal to the cecum
- Ileal samples were rinsed with sterile peptone to remove luminal contents, cut longitudinally and masticated
- Samples were centrifuged, and cells re-suspended in TSB + 10% glycerol, and stored at -80 °C before DNA extraction

Terminal-Restriction Fragment Length Polymorphism (TRFLP) Analysis

- DNA isolations were performed using the Roche Genomic DNA Isolation Kit (Roche Diagnostics Corp., Indianapolis, IN)
- PCR amplification reactions were carried out using a 5'-tetrachlorofloure sciene labeled eubacterial 16S forward primer 8F (AGAGTTTGATYMTGGCTCAG) and the universal reverse primer 1406R (CCGTCAATTCCTTTRAGTTT)
- Triplicate PCR reactions were pooled and purified using the Qiagen PCR Clean Up Kit (Qiagen, Valencia, CA).
- Equal volumes of PCR product were digested with 10 U of either *Bfa*I (C^TAG) or *MSP*I (C^CGG; both New England BioLabs, Inc., Ipswich, MA) and then purified with the Qiagen Nucleotide Removal Kit (Qiagen)
- Samples were analyzed using an ABI PRISM 337 Genetic Analyzer (Applied Biosystems, Los Angeles, CA) in Genescan mode.
- Relative peak areas were averaged for each sample and areas below 1 % removed to reduce background noise
- TRFs were putatively identified at the genus level using the resources available through the Microbial Community Analysis (MiCA) at the University of Idaho (http://mica.ibest.uidaho.edu) and diversity Indices were calculated (6)

Statistical Analysis

- Cluster analysis was performed in BioNumerics v. 6.6 (Applied Maths, Austin, TX) using the Pearson correlation and the UPGMA method
- TRF data were analyzed using the PROC MIXED procedure of SAS (version 9.1.3, SAS Institute Inc., Cary, NC, USA) to determine the quantitative difference in TRFs over time.

RESULTS:

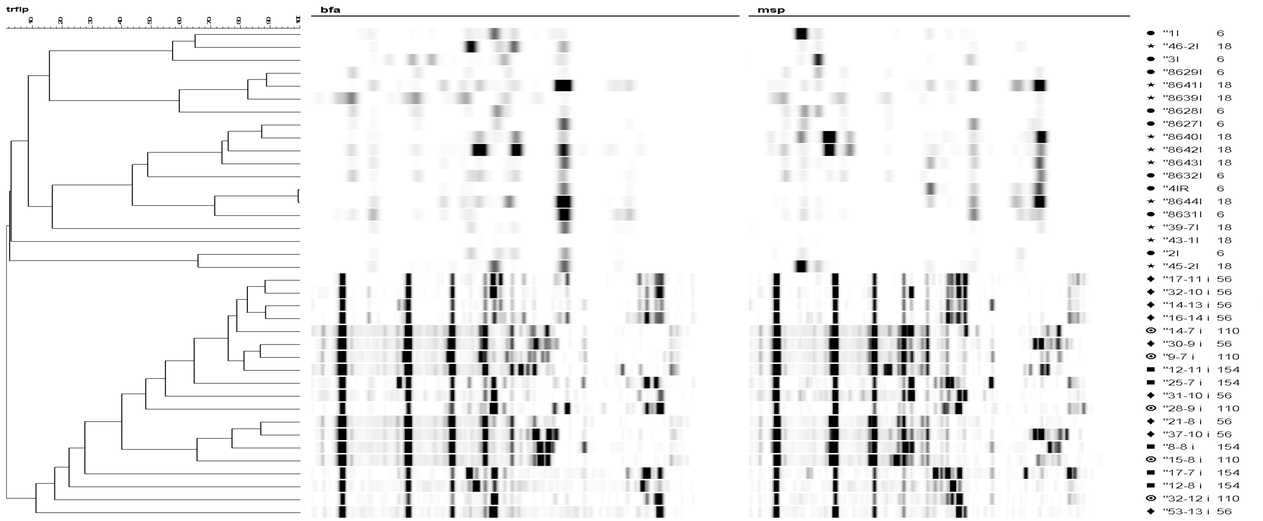

Figure 1: Dendrogram showing microbial relatedness between ileal mucosa microbiotas at five time points. Samples taken at day 6 and 18 cluster together but separately from samples taken at day 56, 110 and 154 which also cluster together.

RESULTS (CONTINUED):

Table 1: TRF peak abundance, evenness, and diversity indices by restriction enzyme calculated for each day sampled.

Index	Day 6	Day 18	Day 56	Day 110	Day 154	P-value	SEM
MspI							
Peak #	3.20^{b}	3.40^{b}	12.22^{a}	12.80^{a}	13.20^{a}	<0.001	1.00
Evenness	0.882	0.683	0.782	0.773	0.802	0.295	0.073
Shannon	0.334^{b}	0.309^{b}	0.842^{a}	0.846^{a}	0.895^{a}	<0.001	0.078
Simpson	0.403^{b}	0.393^{b}	0.793^{a}	0.782^{a}	0.809^{a}	<0.001	0.085
*Bfa*I							
Peak #	2.10^{b}	1.90^{b}	13.78^{a}	12.60^{a}	13.20^{a}	<0.001	0.79
Evenness	0.962^{a}	0.929^{a}	0.811^{b}	0.776^{b}	0.796^{b}	<0.001	0.033
Shannon	0.257^{b}	0.200^{b}	0.919^{a}	0.853^{a}	0.889^{a}	<0.001	0.062
Simpson	0.381^{b}	0.301^{b}	0.824^{a}	0.782^{a}	0.815^{a}	<0.001	0.075

^{a,b} Averages with differing superscript within a row are significantly different at $P < 0.05$, separation of means using Student-Newman-Keuls test; SEM, standard error of the mean.

Table 2: Putative genus level identification using the MiCA database of TRFs that were associated with different days.

TRF	Day 6	Day 18	Day 56	Day 110	Day 154	Putative TRF ID
M:62	0^{b}	0^{b}	16837^{a}	17472^{a}	16842^{a}	*Bacillus*
M:97	0^{b}	0^{b}	773^{b}	603^{b}	2593^{a}	*Bacteroides*/*Prevotella*
M:99	0^{c}	0^{c}	12007^{ab}	13797^{a}	6883^{b}	*Bacteroides*/*Prevotella*
M:133	0^{b}	0^{b}	1944^{a}	422^{ab}	1643^{ab}	*Bacillus*
M:134	0^{b}	0^{b}	1061^{ab}	2028^{a}	1406^{a}	*Bacillus*
M:153	0^{b}	0^{b}	36^{b}	72^{ab}	128^{a}	*Lactobacillus*
M:155	0^{b}	0^{b}	68^{b}	470^{a}	73^{b}	*Lactobacillus*
M:194	0^{b}	0^{b}	71^{ab}	197^{a}	75^{ab}	Enterobacteriaceae
M:203	0^{b}	0^{b}	480^{a}	359^{a}	113^{b}	*Clostridium* cluster XIVa
M:219	0^{b}	0^{b}	181^{b}	188^{b}	672^{a}	*Clostridium* cluster XIVa
M:433	8.2^{b}	10.6^{b}	143^{b}	34^{b}	1561^{a}	*Clostridium*

^{a,b,c} Averages with differing superscript within a row are significantly different at $P < 0.05$, separation of means using Student-Newman-Keuls test.

CONCLUSIONS:

- The ileal mucosa showed little colonization during the lactation phase compared with later phases (Figure 1 , Table 1).
- The number of genera colonizing the ileal mucosa increased throughout the progression from nursery to grower and finishing phases (Table 1).
- Samples taken at day 6 and 18 formed a major separate cluster with 2 % similarity to samples taken from animals that were 56 days of age and older. Most samples from day 56 of age clustered together with 55 % similarity as a subset of samples taken at day 110 and 154 (Figure 1).
- *Clostridium* cluster XIVa, *Bacillus*, *Bacteroides* and *Prevotella*, which are all involved in fiber digestion, were all increased during the nursery, grower and finishing phases as compared to the microbiota during the lactation phase (Table 2).
- Dietary components, especially nutritional fiber, impact the establishment of the commensal microbiota in the ileum of adult pigs.
- Though the microbiota shifts between later phases, the microbiota that is established during the nursery phase remains predominant in the commensal microbiota for the remainder of the pigs life (Table 2).

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