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Shifts in Fecal Microbial Community Composition Associated with the Degree of Solubility of Dietary Fiber

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Abstract

Trillions of microorganisms colonize the human gastrointestinal tract. They exert a strong influence on human health and diseases. Genes and diet are important factors in modifying gut microbial community composition. The role of genetics versus the role of shared diet, however, remains ill-defined. In this study, two genetically identical pigs in a withdrawal study of gut microbiome across four 14-d periods under two diets with different solubility of dietary fiber were used to eliminate the influence of genotype, isolating the role of diet as the main cause for difference in gut microbiome composition. Fecal microbial community of each pig under different diets was characterized using 454-pyrosequencing of amplicons from the hypervariable V3 region of the microbial 16S rRNA gene. Distance matrix and taxonomic classification was performed using Compression-Based Distance and Ribosomal Database Project classifier, respectively. Distance matrix analysis revealed that the individual bacterial community composition was distinctly clustered by the degree of solubility of dietary fiber. Taxonomy analysis exhibited that *Firmicutes* was the predominant phyla. Low solubility of dietary fiber increased *Clostridia* and decreased *Spirochaetes* at class level; raised *Bacteroides*, *Oscillibacter* and *Succinivibrio* and reduced *Prevotella*, *Treponema* and *Escherichia* at genus level. High solubility of dietary fiber played an opposite role in changing intestinal microbiome.

Introduction

Trillions of microorganisms colonize the human gastrointestinal tract. They play a strong role in determining human health. Commensal gastrointestinal tract (GIT) microbes modulate nutrient uptake and utilization, promote the development and maturation of GIT, extract energy from indigestible non-starch polysaccharides, maintain a healthy immune system and regulate brain development and behavior. Perturbations in intestinal microbial community structure have been associated with a variety of diseases. The alleviation of disease symptoms has been correlated with treatments that alter the gut microbiome toward that of a healthy individual. Genes and diet are important factors in modifying gut microbial community composition. Scientists always devote themselves in finding contributions of genetics and dietary in altering gut microbiome. For instance, Zoetendal *et al.*[1] reported that the similarity between fecal DGGE profiles of monozygotic twins were significantly higher than those for unrelated individuals. Gut microbes structure in mice changed within one day of a change in diet from a low-fat diet to a high-fat diet.[2] The role of genetics versus the role of shared diet, however, remains ill-defined.

Objectives

To evaluate the effects of different solubility of dietary fiber on fecal microbiome composition in genetically identical pigs.

Materials and Methods

1. **Make sure that solubility of dietary fiber is only variation (Fig. 1)**
   Two female healthy cloned adult pigs created from Duroc gilt (2-14) using somatic cell nuclear transfer were used in a withdrawal experimental design across four 14-d periods under two different diets, soybean hull diet and wheat bran diet. In each period, the two pigs, who had the same genotype, were maintained in the same environment and ate the same diet. Soybean hull and wheat bran provided high solubility of dietary fiber and low solubility of dietary fiber, respectively. Feces were collected at the last day of each period. Samples were stored at -20 °C until DNA extraction.

2. **Characterize gut microbiome composition through using 454-pyrosequencing of amplicons from 16S V3 region (Fig. 1)**
   Genomic DNA was extracted using the RBB+C method[3] with minor modifications including the addition of 1000 µl ASL buffer to the samples following the Qiagen DNA Stoll Kit (Qiagen). The hypervariable V3 region of 16S rDNA was amplified in the PCR with specific primers for pyrosequencing analysis. After checked for size and purity, PCR products were subjected to pyrosequencing (454 GS FLX).

3. **Obtain information about similarity and taxonomy of gut microbiome (Fig. 1)**
   Similarity was analyzed using compression-based distance metric (CBD). All pairs of V3 16S rDNA sequences were compared using CBD. These pairwise distances were used to generate the distance matrix. Multidimensional scaling (MDS) in R language (v2.11.1) was used to convert distance matrix into low dimensional space which was visualized in a 2D graphics by matplotlib.[4]

   Taxonomy was assigned using Ribosomal Database Project (RDP) classifier (v10.2; ≥80% confidence).[5]

Materials and Methods

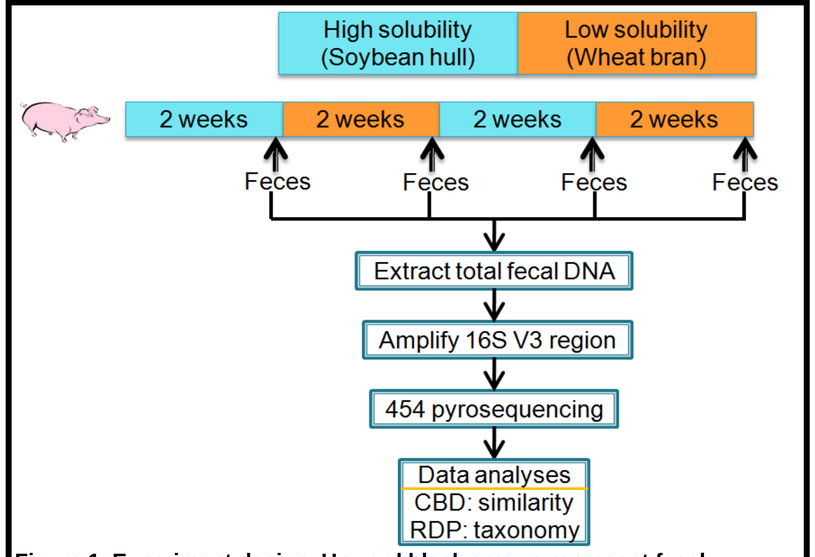

Figure 1. Experiment design. Upward black arrows represent fecal collection time points.

Results

1. Pyrosequencing analysis of V3 16S rRNA genes generated a total of 187,471 sequences from 8 samples of two pigs fed either high solubility of dietary fiber diet (soybean hull diet) or low solubility of dietary fiber diet (wheat bran diet) under four 14-day periods (Table 1). After removing sequences with low quality score < 25, ambiguous characters and read lengths beyond the main distribution (≥146 and ≤201),[6] we obtained 173,407 sequences from the 8 samples and the average sequence length was 176 nt (Table 1).

Phase	Sample	Total sequences number	Processed sequences number	Average processed sequence length (nt)
1st	#1-High	9291	8480	175
2nd	#1-Low	26147	25347	174
3rd	#1-High	41346	34955	176
4th	#1-Low	15324	13892	174
1st	#2-High	18133	17183	178
2nd	#2-Low	43805	41030	175
3rd	#2-High	13449	13122	177
4th	#2-Low	19976	19398	176

Table 1 Sequence statistics from fecal samples of two pigs fed diets with high solubility of dietary fiber or low solubility of dietary fiber across four two-week phases.

2. Comparison of the individual bacterial community composition using CBD-based MDS (Fig. 2) exhibited a disparate clustering by the degree of solubility of dietary fiber.
3. *Firmicutes* was the predominant phyla in each sample (43% - 58%) (Fig. 3). The rest of dominant bacteria was assigned to *Bacteroidetes* (9% - 14%), *Spirochaetes* (2% - 8%) and *Proteobacteria* (0% - 2%) at phylum level in each sample (Fig. 3). Low solubility of dietary fiber increased *Clostridia* and decreased *Spirochaetes* at class level (Fig. 4A); raised *Bacteroides*, *Oscillibacter* and *Succinivibrio* and reduced *Prevotella*, *Treponema* and *Escherichia* at genus level (Fig. 4B-D). High solubility of dietary fiber played an opposite role in changing intestinal microbiome (Fig. 4).

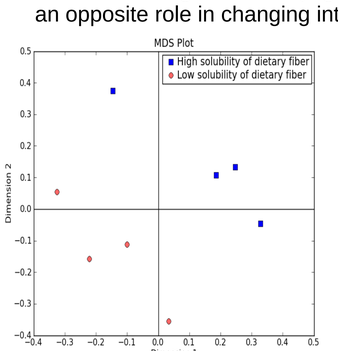

Figure 2. CBD-based MDS analysis showed clustering of microbiomes by diet.

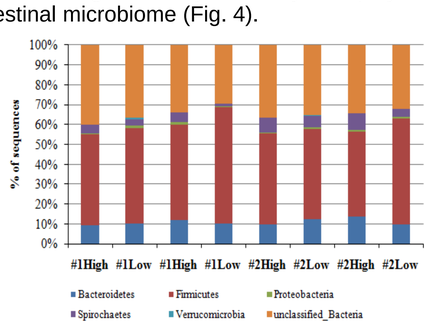

Figure 3. Distribution of phylum assignments of sequences from two cloned pigs fed diets with high or low solubility of dietary fiber.

Results

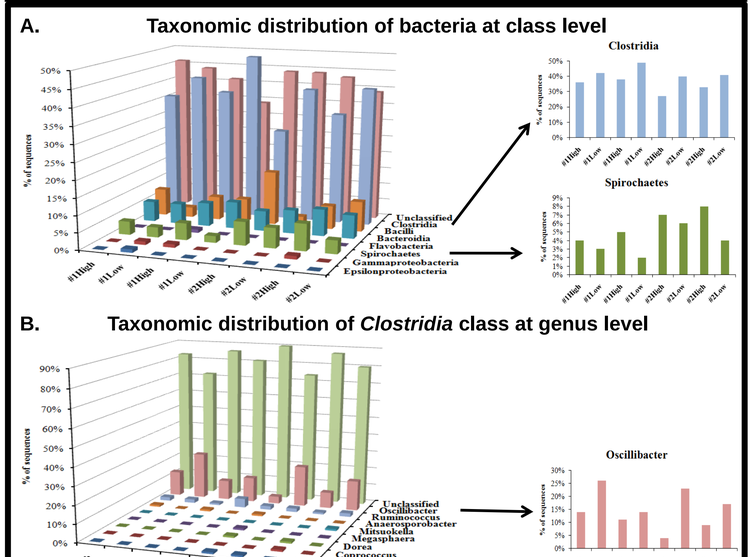

Figure 4. Distribution of class (A) and genus (B-D) assignments of sequences from two cloned pigs fed diets with high or low solubility of dietary fiber. The *Treponema* genus was the only one bacteria which was included in the calss *Spirochaetes* and was therefore not shown as a separate column.

Conclusions

Special gut bacteria were correlated with level of solubility of dietary fiber in genetically identical pig clones. These results imply interactions between diet and gut microbiome.

Acknowledgements

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