Comparison of DNA Extraction Methods in 16S rRNA Sequencing using Equine Fecal Material

[1]Alexandra Meyer, [1,2,3]Aaron C. Ericsson, [1,4]Philip J. Johnson

[2]MU Metagenomics Center, [3]Department of Veterinary Pathobiology, [4]Department of Veterinary Medicine and Surgery,
[1]University of Missouri CVM, Columbia, MO

Background

Changes in the human intestinal microbiome have been associated with diseases such as cancer, asthma, diabetes, obesity, and immuno-senescence. The microbial population of the equine GI tract also plays a role in health and disease susceptibility. As a non-ruminant, equids depend on the colonic and cecal microbes to assure critical nutrient availability. The identification and quantification of this dynamic community can provide a clue to the pathogenesis of a wide array of clinical conditions. Culture-independent methods such as Next-Generation Sequencing (NGS) offer a means of characterizing the gut microbiota non-invasively. NGS opens the door to the study of important equine diseases such as colic, colitis, and laminitis in the horse. This method requires extraction of high-quality DNA from fecal material to accurately characterize the microbial population. Equine feces contain a hitherto unidentified inhibitor of the PCR process. Therefore, we elected to test several available DNA extraction protocols for equine feces.

Aim

- To compare five different methods of DNA extraction from equine feces via "next-generation" sequencing (NGS)

Methods

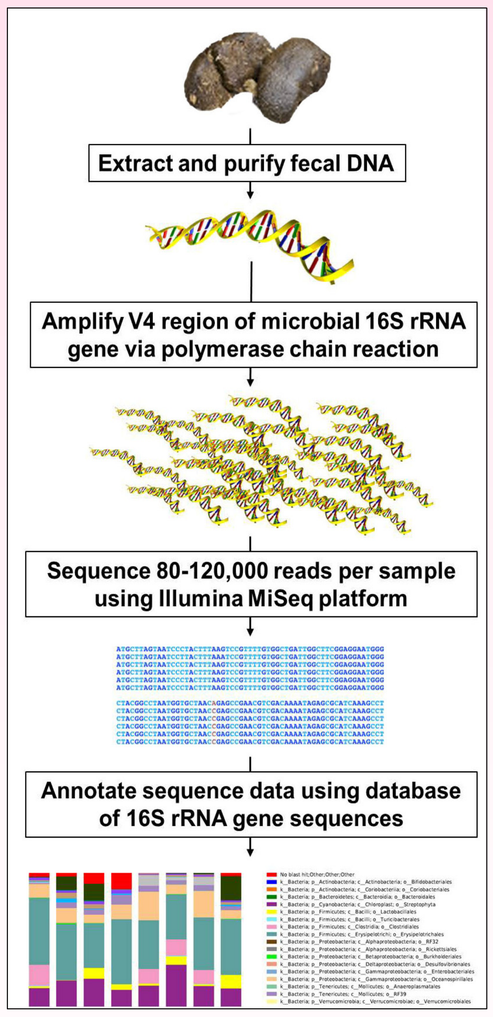

Figure 1: Depiction of 16S rRNA, or "next-generation" sequencing (NGS)

- Feces were collected from 8 different equids in the MU Veterinary Medical Teaching Hospital that were not receiving antimicrobials or affected with gastrointestinal disease
- Extraction methods performed:
  - Qiagen DNeasy kit
  - MoBio PowerFecal Kit
  - Qiagen Cador Mini-pathogen kit
  - QIAamp DNA stool minikits
  - Manual isopropanol precipitation protocol adapted from literature
- Sequenced using Illumina MiSeq

Results

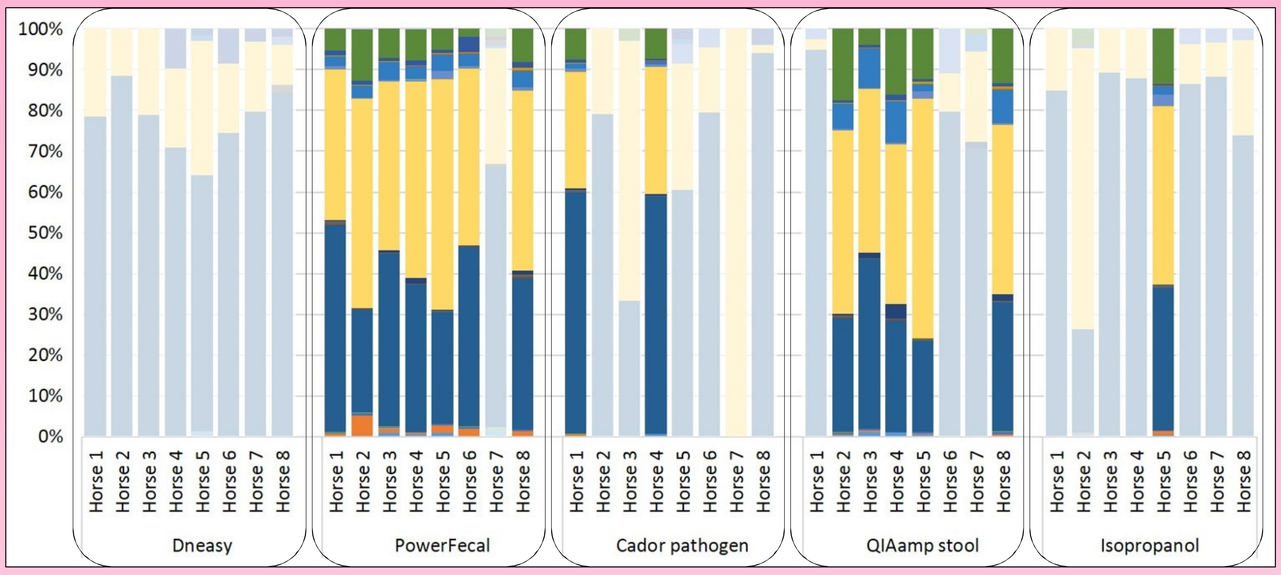

Figure 2: NGS analysis of 8 samples subjected to 5 extraction methods, annotated to the **phylum** level. Faded bars returned fewer than 10,000 reads. Predominant phyla include *Bacteroidetes* (dk. blue), *Firmicutes* (yellow), *Actinobacteria* (light blue), and *Proteobacteria* (green).

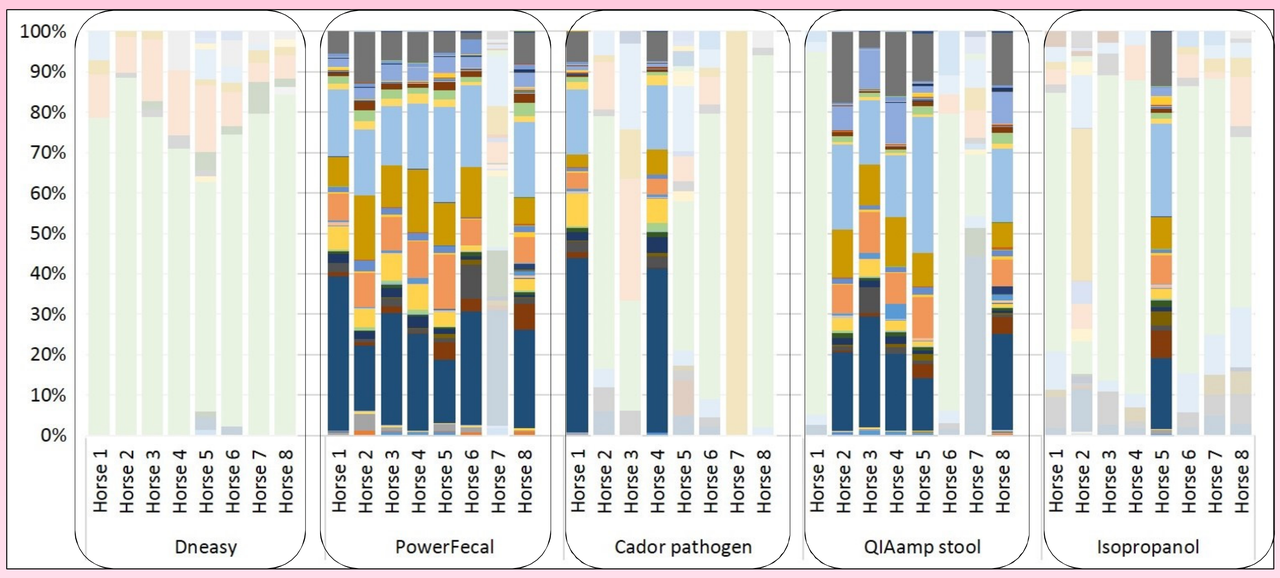

Figure 3: NGS analysis of 8 samples subjected to 5 extraction methods, annotated to the **family** level. Over 150 microbial families, comprising close to 300 operational taxonomic units, were detected.

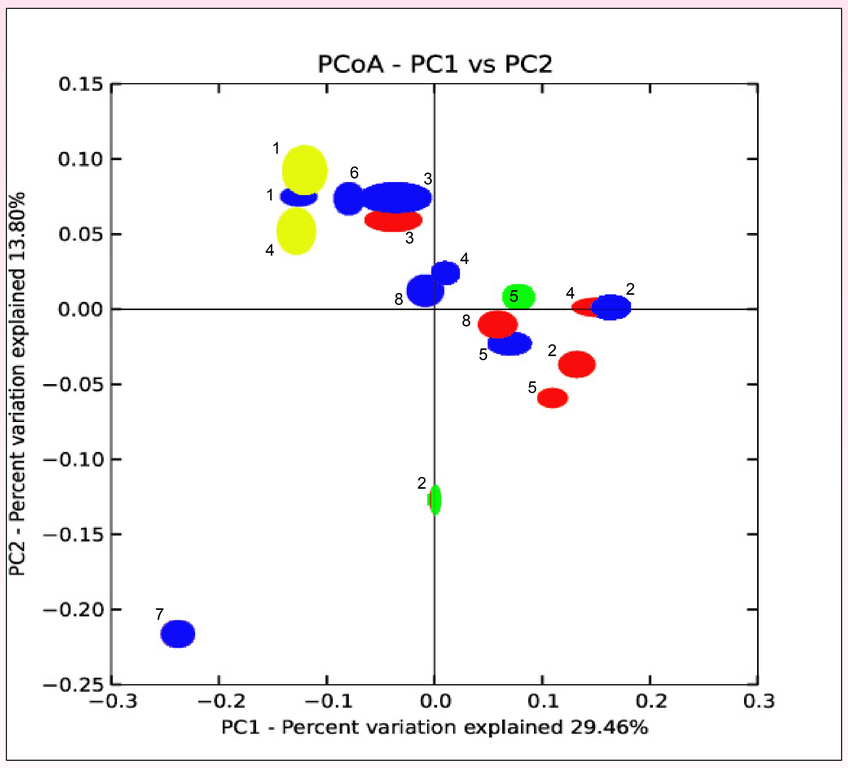

Figure 4: Principal Component Analysis (PCA) of all samples returning greater than 1000 sequences. Colors indicate extraction method and numbers indicate animal ID. PowerFecal = blue, QIAamp stool = red, Cador pathogen = yellow, isopropanol = green.

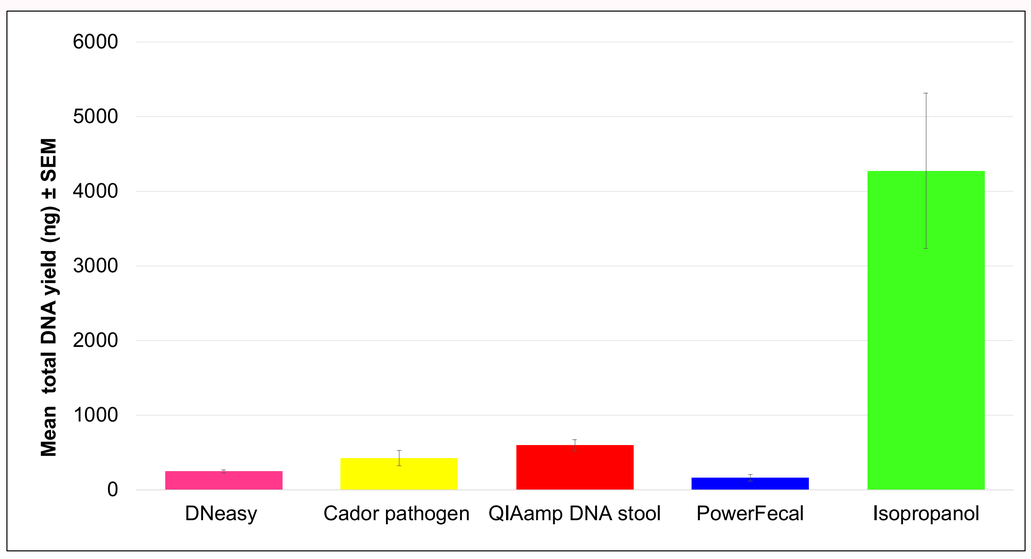

Figure 5: Mean total DNA eluted by each extraction method.

Conclusions

- DNA extracted from 8 different equine fecal samples using each method was used as template for NGS (**Figure 2, 3**). Samples with less than 10,000 reads were discarded, as they are considered unreliable.
  - The PowerFecal method consistently yielded the highest species richness.
  - Under the experimental conditions used, the DNeasy method did not yield greater than 10,000 sequences for any sample, and species richness was minimal.
  - The consistency between methods with samples above 10,000 reads indicates that if PCR is not inhibited, the extraction method used will likely yield a representative profile of the microbial population.
- PCA (**Figure 4**) suggests that there are minimal differences in the microbiota detected in identical samples extracted via different methods. The samples taken from the same horse often cluster together, indicating that detected microbial communities are very similar, regardless of extraction method.
- The total amount of DNA eluted does not correlate with the reads per sample (**Figure 5**). For example, the isopropanol method yielded the greatest amount of DNA, but only one sample yield reads above 10,000 k.
- Equine feces are difficult and problematic for DNA extraction. Kits designed for DNA extraction from feces, such as the DNeasy and QIAamp stool minikit, did not perform as expected and yielded poor results.
  - Equine fecal material contains unknown inhibitors.
  - If the process is uninhibited and yields over 10,000 reads per sample, the method should be successful.

Next Steps

Future studies will investigate whether amplification facilitators could be developed for the purpose of overcoming PCR inhibitors in equine feces

Acknowledgements

Funding was provided as a gift by Boehringer-Ingelheim Vetmedica and the Animal Health Foundation of Pacific, Missouri.

VRSP

College of Veterinary Medicine
University of Missouri

Animal Health Foundation