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Characterizing the microbiota across the gastrointestinal tract of a Brazilian Nelore steer

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ABSTRACT

The gastrointestinal tracts (GIT) of herbivores harbor dense and diverse microbiota that has beneficial interactions with the host, particularly for agriculturally relevant animals like ruminants such as cattle. When assessing ruminant health, microbiological indicators are often derived from the rumen or feces. However, it is probable that ruminal and fecal microbiota do not reflect the microbial communities within the GIT of ruminants. To test this, we investigated the compartments of the GIT from a Brazilian Nelore steer and performed a 16S rRNA pyrosequencing analysis on the collected samples. Our results showed high intra-individual variation, with samples clustering according to their location in the GIT including the forestomach, small intestine, and large intestine. Although sequences related to the phyla Firmicutes and Bacteroidetes predominated all samples, there was a remarkable variation at the family level. Comparisons between the microbiota in the rumen, feces, and other GIT components showed distinct differences in microbial community. This work is the first intensive non-culture based GIT microbiota analysis for any ruminant and provides a framework for understanding how host microbiota impact the health of bovines.

1. Introduction

Symbioses that occur in the gastrointestinal tract (GIT) of animals have long been recognized as essential factors in the structural development of this anatomical feature. Importantly, development of the GIT likely facilitated the evolution of the animal lifestyle (Ley et al., 2008), and thus has essential implications for overall animal health. The microbiota in the rumen and feces of ruminants have been used as a diagnostic tool for assessing animal health (Dowd et al., 2008; Lettat et al., 2012; Van Baale et al., 2004; Van Donkersgoed et al., 1999), but the role of microorganisms in other segments of the GIT, such as the small and large intestine, have received little attention.

To address this, we investigated the microbial community across the GIT of the Nelore (*Bovinus indicus*) breed of cattle, an indicine species that has been genetically improved through crossbreeding (Dani et al., 2008). This species represents over 72% of the bovine herds in Brazil and is the top exported beef cattle in the world (United States Department of Agriculture [http://www.usda.org], Ministerio da Agricultura do Brasil [http://www.agricultura.gov.br]). Understanding the structure of the microbial community and the factors that affect microbial assemblage in the GIT of this bovine may be useful for developing new livestock management technologies, particularly in nutrition and sustainability systems.

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Here we describe for the first time, the composition and phylogenetic distribution of the microbiota present within the different compartments of the GIT for any ruminant, and in particular, from a Nelore steer from a Brazilian commercial farm. We show that the microbiota across the GIT is localized, with distinct communities in the forestomach, small intestine, and large intestine. Importantly, we show that the microbiota across the GIT is different from that of feces and the rumen. Given the importance of the Nelore breed in global cattle production, this study not only provides the first intensive non-culture based study of the Nelore-associated microbiota, but further increases our understanding of the localization of GIT-associated microbial communities in ruminants.

2. Methods

2.1. Study animal

A two-year old, healthy, purebred Nelore beef steer, weighting 460 kg, was used in this study. This animal was reared and maintained in Uberaba City, Minas Gerais, Brazil, using standard livestock management practices, which includes pasture feeding followed by a diet of corn silage 40 days before sacrifice. This animal was slaughtered according to institutional animal care guidelines, which includes anesthetization followed by an intramuscular injection of 2% xylazine chlorhydrate. Euthanasia was performed using 2% lidocaine injected in the cistern magna.

2.2. Gastrointestinal and feed sampling

Our sampling approach included collecting luminal contents from three regions of the GIT, namely the forestomach, small and large intestine. All segments from these three regions were sampled as follows: forestomach: rumen, reticulum, omasum, and abomasum; small intestine: duodenum, jejunum, and ileum; and large intestine: cecum, colon and feces. Luminal samples of the gastrointestinal tract were obtained as follows. Following sacrifice, the animal was transferred to a biopsy table. Samples from the forestomach, including the rumen, reticulum, omasum, and abomasum, were collected and pooled out separately. Rumen samples were filtered through sterile gauze to obtain both liquid and solid (fiber-adherent) portions. Intestinal sampling was performed from the beginning of the duodenum in the small intestine through the end of the colon in the large intestine. The duodenum was 1 m in length and was sampled at the beginning, middle, and end sections. Sixteen samples were collected from the jejunum by sampling every five intestinal handles (1.5 m each). Due its small length (30 cm), the entire ileum luminal contents were collect as one sample. For the large intestine, the voluminous cecum was delimited into three sections (top, medium, and bottom) and separately sampled. Four anatomical points of the colon were sampled: internal and external handles of the ascendent colon, the transverse colon, and the descendent colon. Fecal samples were also collected during animal defecation before animal slaughter.

In order to prevent shifting of luminal contents from one site to another during intestinal sampling, a 30 cm section from each sampling site was isolated from the rest of the intestine by tying off each section using thread. This strategy was used to ensure that the sampled contents came from each specific anatomic location in the GIT. Animal feed was also sampled, including grass from five random sites in the pasture field and five random locations in the corn silage trough provided to the animal prior to sacrifice. All samples were kept at −80 °C until processing.

2.3. DNA extraction

Genomic DNA was isolated from all samples using a QIAamp DNA mini kit (QIAGEN, Valencia, CA) with 50 mg of luminal samples or 100 mg of feed samples and resuspended in 180 μL of ATL buffer that was subsequently incubated for one hour at 37 °C with 250 μg of lysozyme (Sigma–Aldrich) for cell lysis. Subsequent steps were followed according to the manufacturer's manual for bacterial DNA isolation from biological fluids. DNA concentration and purity were determined using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE) at wavelengths of 230, 260 and 280 nm. Different samples from the same GIT segment (3 samples from the duodenum, 16 from the jejunum, 3 from the cecum and 3 from the colon) were separately pooled in equimolar amounts prior to PCR.

2.4. PCR and pyrosequencing

A fragment spanning the V6–V8 variable region of the 16S rRNA gene from bacteria (positions 906–1406 in the *Escherichia coli* numbering system) was PCR amplified using custom-designed primers containing the Roche 454 A or B Titanium sequencing adapters, a unique five base-pair barcode sequence (only in the reverse primer) and the primer of interest. These included 926F-5′-*CCTATCCCCTGTGTGCCTTGGCAGTCTCAG*AAACTYAAAKGAATTGACGG-3′ and 1392R-5′-*CCATCTCATCCCTGCGTGTCTCCGACTCAG*-XXXXX-ACGGGCGGTGTGTRC-3′, where X represents the barcode sequence location. A total of 25 ng of DNA was used in a 50 μL reaction containing 1× HF buffer, 200 μM of dNTPs, 0.125 μM of each primer, 3.5% of DMSO, and 1 unit of Phusion® High-Fidelity DNA Polymerase (New Englands Biolabs Inc., Ipswich, MA). PCR conditions were: initial denaturing of 94 °C for 2 min; 30 cycles consisting of 94 °C for 30 s, 50 °C for 45 s and 68 °C for 1.45 min; and final extension step of 68 °C for 10 min. Amplicons were purified twice using the Agencourt AMPure XP system (Beckman Coulter, Inc., San Diego, CA) and quantified using a Qubit® Fluorometer (Invitrogen, San Diego, CA). All subsequent amplicons were pooled out in equal amounts for emulsion PCR.

Amplicon sequencing was performed using the manufacturer's protocols (Roche Applied Science, Indianapolis, IN) for Titanium sequencing on a Roche 454 GS Junior Titanium sequencer. Emulsion PCR, recovery, and sequencing were done according to manufacturer's protocols using the Lib-L kit with an initial emPCR ratio of two molecules of DNA per bead. All sequences were submitted to the National Center for Biotechnology Information's