

Tracking Bacteria through the Entire
Gastrointestinal Tract of a Beef Steer

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Core Ideas

- 50–80% of operational taxonomic units can be attributed to the GIT's upstream compartment.
- Rumen and feces are dominated by firmicutes, 70% of which are in both compartments.
- Abomasum, small-, and large-intestine samples each had distinct taxonomic composition.
- Ingesta had more proteobacteria and bacteroidetes; tissue had more clostridia.
- One-third of the OTUs in the pre-gastric samples were also found in the rectum.

Abstract: Biological processes within beef cattle gastrointestinal tracts (GIT) influence animal health, and the output (feces) is an important vector for zoonotic pathogens and antibiotic resistant bacteria. Historically, outside of the rumen, little attention was paid to the bacteria along the GIT, despite their essential role in catabolizing feed into feces. Here we characterize bacteria from 15 GIT sites within a beef steer and examine the proportion of bacteria contributed by upstream compartments. This animal displayed characteristic differences between tissue and digesta communities in gastric and large-intestine, but not small-intestine, samples. The GIT sites shared between 50 and 80% of the operational taxonomic units (OTUs) with their neighboring upstream compartment, with the exception of the ileum. The ileum shared only 11% with the jejunum but displayed a similar phylum-level taxonomic distribution with the jejunum. It is unclear whether the observed changes between compartments represent a nonrandom decrease in bacterial number or rapid multiplication of certain taxa.

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FECAL-BORNE bacteria play a dynamic role in livestock agricultural systems. They cycle nutrients (Fernandez et al., 2016), emit gaseous, sometimes odorous, compounds (Varel et al., 2010; Miller et al., 2016), contribute to the animal's health by affecting the immune system status (Kogut and Arsenault, 2016), and serve as an indigenous source for zoonotic pathogens and antibiotic-resistant bacteria (Savage, 1977, Durso and Keen, 2007; Jacobsen et al., 2009; Durso et al., 2011). Although usually studied in isolation, the feces represents an end product of a series of sequential bioreactions occurring along the entire cattle gastrointestinal tract (GIT). The beef cattle GIT is a series of dynamic selective environments, from mouth to anus, where a wide range of fermentative biochemical reactions convert complex feedstuff into components necessary for animal growth (Mackie, 1996). The digestive tract is structured so that the output from one compartment serves as the input for the subsequent compartment. Both host animal physiological factors and substrate availability create a set of unique selective pressures in each of these compartments that influence which bacteria are enriched in each compartment.

Recently, Mao et al. (2015) characterized microbial communities along dairy cattle GITs and used metagenomics to determine GIT location of genes associated with carbohydrate and amino acid metabolism. However, the genotype, physiology, and metabolism of dairy cattle differs markedly from that of beef cattle (McSweeney and Mackie, 2012). Herein we examine GIT communities of a beef steer at 15 sites along the GIT from the mouth to the recto-anal junction, with 11 of 15 sites having paired ingesta- and tissue-associated samples ($n = 22$ paired samples + 4 unpaired).

Abbreviations: GIT, gastrointestinal tract; OTU, operational taxonomic unit.

Materials and Methods

Samples ($n = 26$) were collected in 2008 from a healthy steer euthanized due to a broken leg (Table 1). The animal's last meal was approximately 4 h before sample collection and consisted of high-moisture corn (17%), dry-rolled corn (17%), alfalfa hay (43%), corn silage (20%), and vitamin and mineral supplement (2.4%). All samples were harvested aseptically by veterinarians into sterile centrifuge containers (on ice) and stored at -80°C . Utensils were sterilized as samples were collected. The mouth swab consisted of moistened sterile gauze wiped on the inside surface of the animal's cheeks. Tissue samples were washed three times in sterile phosphate buffered saline to remove adhering ingesta prior to storage. Small intestinal digesta samples were collected using a sterile spatula. Total community DNA (including both living and dead cells, as well as extracellular DNA) was isolated using a

Mo Bio fecal isolation kit (Mo Bio Laboratories) with a bead beating step to facilitate bacterial lysis. 16S rRNA sequencing was performed as previously described (Dowd et al., 2008). Briefly, universal eubacterial primers 515F and 806R were used in a single-step polymerase chain reaction (PCR), followed by Ion Torrent PGM sequencing (Thermo Fisher). Barcodes, primers, and short sequences (<150 bp) were removed, as were sequences with ambiguous base calls and sequences with homopolymers greater than 6 bp in length. Chimeras were removed following denoising and operational taxonomic unit (OTU) generation (97%), and BLASTn was used against a curated GreenGenes database (DeSantis et al., 2006) to assign taxonomies (Dowd et al., 2008).

Results and Discussion

After sequence quality control and processing, 964,192 sequences from 26 libraries were assigned to 20,120 OTUs, with 13% (2581) present as only a single sequence from a single site. Within each library, 33 to 55% of the OTUs were singletons, and 17 to 51% of the OTUs contained at least 10 sequences (Table 1). *Succinivibrio*, *Ruminococcus*, *Turicibacter*, and *Methanobrevibacter* were identified in all 26 samples.

Pre-Gastric

The pre-gastric compartments include the mouth, pharynx, tonsil, and esophagus. They contain a mix of saliva, new feed, and fermented material that has been regurgitated and remasticated as part of the rumination process. There was a mean of 2804 OTUs in pre-gastric samples (Table 1). Mouth swabs are commonly used to sample for specific zoonotic and veterinary pathogens (McAllister et al., 2006; De Cooman et al., 2014), and data were examined to determine the viability of swabs as a useful overview of beef pre-gastric bacterial communities. At the phylum level, proteobacteria were over-represented in the swab compared with pooled pre-fermentation samples (57% vs. 38%) with concurrent declines in firmicutes and bacteroidetes. Actinobacteria numbers were similar for swabs and pooled pre-gastric samples for this animal.

Fermentation and Gastric Compartments

The fermentation and gastric compartments include the reticulum, rumen, omasum, and abomasum. Focusing specifically on the four-chambered fermentation chamber and gastric stomach, the majority of the beef steer OTUs associated with the tissue samples ($n = 9356$) were detected in the digesta ($n = 7880$ shared of 10,048 total in digesta). Between 38 and 49% of the OTUs in the reticulum, rumen, omasum, and abomasum of this steer were shared between tissue and digesta samples (Fig. 1).

Data from this animal provide insight into what proportion of OTUs in each of the fermentation and gastric chambers can be attributed to the previous, upstream compartment (Fig. 2). Between 60 and 81% of OTUs are shared between the pre-gastric, reticulum, and rumen compartments, reflecting the mixing of rumen bacteria with reticulum and pre-gastric substrates via regurgitation

Table 1. List of samples and basic sequencing results.

| | No. of reads† | H‡ | No. of OTUs | % <1:>10§ |
|---------------------------------------|---------------|-------|-------------|-----------|
| Pre-gastric | | | | |
| 1. Mouth swab¶ | 32,923 | 5.72 | 2888 | 43:34 |
| 2. Mouth tissue ^a | 14,678 | 3.10 | 2137 | 44:27 |
| 3. Pharynx tissue | 6,875 | 1.51 | 1179 | 49:27 |
| 4. Tonsil tissue | 14,875 | 3.33 | 2472 | 47:26 |
| 5. Esophagus tissue | 34,695 | 7.52 | 5346 | 41:29 |
| Gastric | | | | |
| 6. Reticulum tissue ^b | 49,842 | 9.99 | 5571 | 36:41 |
| 7. Reticulum digesta ^b | 68,308 | 13.55 | 6714 | 33:51 |
| 8. Rumen tissue ^c | 59,638 | 11.59 | 5792 | 35:48 |
| 9. Rumen digesta ^c | 50,708 | 10.37 | 5721 | 36:45 |
| 10. Omasum tissue ^d | 50,748 | 10.45 | 5943 | 36:41 |
| 11. Omasum digesta ^d | 33,680 | 7.21 | 4440 | 39:36 |
| 12. Abomasum tissue ^e | 44,794 | 7.43 | 3137 | 41:39 |
| 13. Abomasum digesta ^e | 58,071 | 10.91 | 5346 | 38:43 |
| Small intestine | | | | |
| 14. Duodenum tissue ^f | 11,379 | 2.42 | 1931 | 52:20 |
| 15. Duodenum ingest ^f | 21,983 | 4.36 | 3438 | 50:21 |
| 16. Jejunum tissue ^g | 12,289 | 2.77 | 2537 | 53:17 |
| 17. Jejunum digesta ^g | 23,797 | 4.61 | 3469 | 50:21 |
| 18. Ileum tissue ^h | 34,296 | 6.81 | 4254 | 42:31 |
| 19. Ileum digesta ^h | 56,779 | 8.76 | 3438 | 37:48 |
| Large intestine | | | | |
| 20. Cecum tissue ⁱ | 40,357 | 8.19 | 5451 | 42:28 |
| 21. Cecum digesta ⁱ | 43,420 | 8.67 | 5139 | 39:34 |
| 22. Spiral colon tissue ^j | 40,210 | 8.19 | 5249 | 41:30 |
| 23. Spiral colon digesta ^j | 34,750 | 7.10 | 4402 | 40:32 |
| 24. Rectum tissue ^k | 32,356 | 6.76 | 4286 | 40:32 |
| 25. Rectum digesta ^k | 45,212 | 8.38 | 4512 | 33:51 |
| 26. RAJ digesta# | 47,529 | 9.01 | 4670 | 36:46 |

† The number of reads that cleared quality control screening and were assigned to operational taxonomic units (OTUs).

‡ H, Shannon diversity index.

§ % <1:>10 reports the number of singleton OTUs in each sample and the number of OTUs with >10 assignments in each sample. OTUs were assigned at the 97% sequence similarity level.

¶ Samples with the same superscript letter are paired tissue and ingesta samples.

RAJ, recto-anal junction.

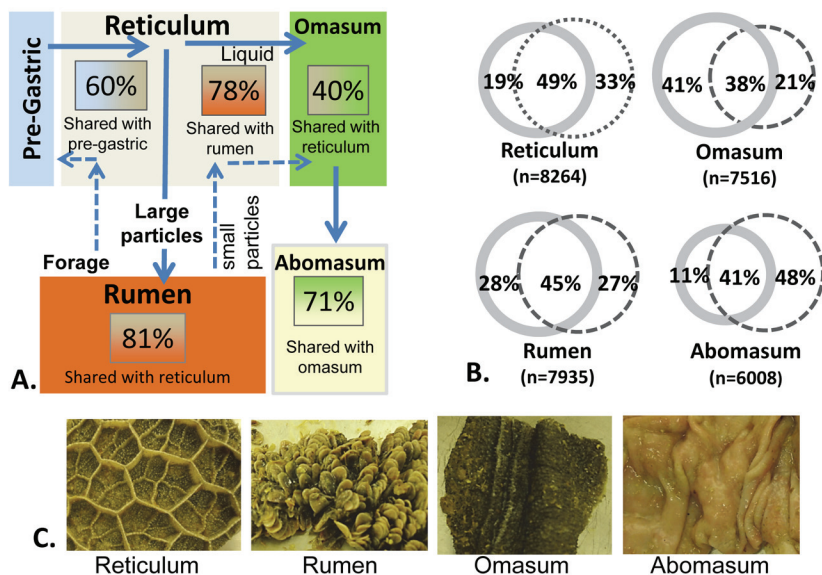


Fig. 1. Beef steer gastric bacteria. (A) Flow of digesta in the four gastric chambers and percentage of operational taxonomic units (OTUs) shared with neighboring compartments. (B) Percentage of OTUs shared between tissue (solid gray circles) and digesta (dashed circles) in each of the four gastric chambers. (C) Morphology of tissue in each of the four gastric chambers.

and rumination. In contrast, only 40% of the OTUs in the omasum were shared with the reticulum, indicating significant changes in the microbial habitat and ecosystem between these two compartments. The abomasum is the gastric, or true stomach. It shared 71% of its OTUs with the omasum. In the steer examined for this study, the number of OTUs in the abomasum is lower than the other gastric compartments ($P < 0.05$), reflective of the harsh acidic environment of the abomasum. Overall, each gastric chamber has its own distinct morphology, and the rough texture of the reticulum, rumen, and omasum that lack mucus layers contrasts with the mucus-containing acidic abomasum and almost certainly provides microhabitats that can support a broader selection of bacterial taxa (Fig. 2).

Small and Large Intestines

The number of OTUs dropped when moving from the abomasum to the small intestine (Fig. 1). Specifically, the number of OTUs was lower in the duodenum compared to the abomasum. This same trend was reported in recent dairy and sheep studies (Mao et al., 2015; Wang et al., 2017). In the current study, the number of OTUs increased again in the large intestine, whereas in Mao et al. (2015) no major increase in OTU numbers was observed (1024 in duodenum, 1086 in rectum). Overall, our Shannon diversity numbers for the beef steer are higher than those reported by Mao et al. (2015) for dairy. Because of differences in sample processing and depth of sequencing, direct comparisons between the two studies cannot be made. Nonetheless, relative diversity within each study reveals that beef and dairy cattle are similar in their spatial diversity from mouth through duodenum (Fig. 2), with the gastric samples displaying the highest diversity. Postduodenum, Shannon diversity increased in

the steer from the current study but remained the same in the dairy cattle pools (Mao et al., 2015).

Recent metagenomic work (Mao et al., 2015) characterized bacterial communities at 10 sites of the dairy cattle GIT and included an analysis of bacterial functional capabilities. Although this latter task is beyond the scope of the currently reported project, the heterogeneity in composition and diversity observed by Mao et al. (2015) along the length of the dairy GIT was also detected in this smaller study of 16 locations along the steer's GIT (Fig. 2). Taxonomically, there were characteristic differences between tissue and digesta communities within the gastric and large intestine samples. In both instances, digesta had higher proportion of proteobacteria and bacteroidetes, and tissue was associated with proportionally more clostridia. Differences between tissue and digesta were less pronounced in the small intestine. Additionally, there were distinctive patterns of taxonomic composition at the phylum level between abomasum, small-intestinal, and large-intestinal samples (Fig. 2).

Comparing Bacterial Composition at the Mouth and Rectum

Looking at the composition of the bacterial microbiome in the rectum and possible relationships to ingested feed or upstream bacteria, 597 OTUs first observed in the oral cavity were also identified in the rectum, representing 4% of the rectum OTUs. Of the 8662 OTUs present in the combined pre-gastric compartments, only 2771 (33%) were also present in the rectum. These OTUs possibly represent organisms that were ingested as part of the feed and that survived the journey through the entire GIT, resident taxa, or fecal organisms reingested by the animal. Like the rumen, the rectum was dominated at the phylum level by firmicutes (72 and 71%, respectively), and 70% of the rumen firmicutes OTUs were also found in the rectum. The 10 most frequently occurring OTUs found first in the colon/rectum in this steer are assigned to *Clostridium* ($n = 308$ assignments), *Roseburia* ($n = 129$), *Oscillospira* ($n = 106$), *Succinivibrio* ($n = 62$), *Ruminococcus* ($n = 61$), *Bacteroides* ($n = 53$), *Prevotella* ($n = 52$), *Blautia* ($n = 41$), *Coprococcus* ($n = 39$), and *Turicibacter* ($n = 31$). This study noted 1045 "new" OTUs in the colon and rectum not seen in any previous compartment. Because of experimental design limitations, the origin of these OTUs is unclear. It is possible that due to undersampling, these OTUs were present upstream but were not detected. Alternatively, they could represent resident bacterial populations that were established at an earlier time point. As with any study of this design, the OTUs identified represent the total DNA present in the sample at the time of collection, regardless of whether the DNA was contained in a cell, and regardless of whether the

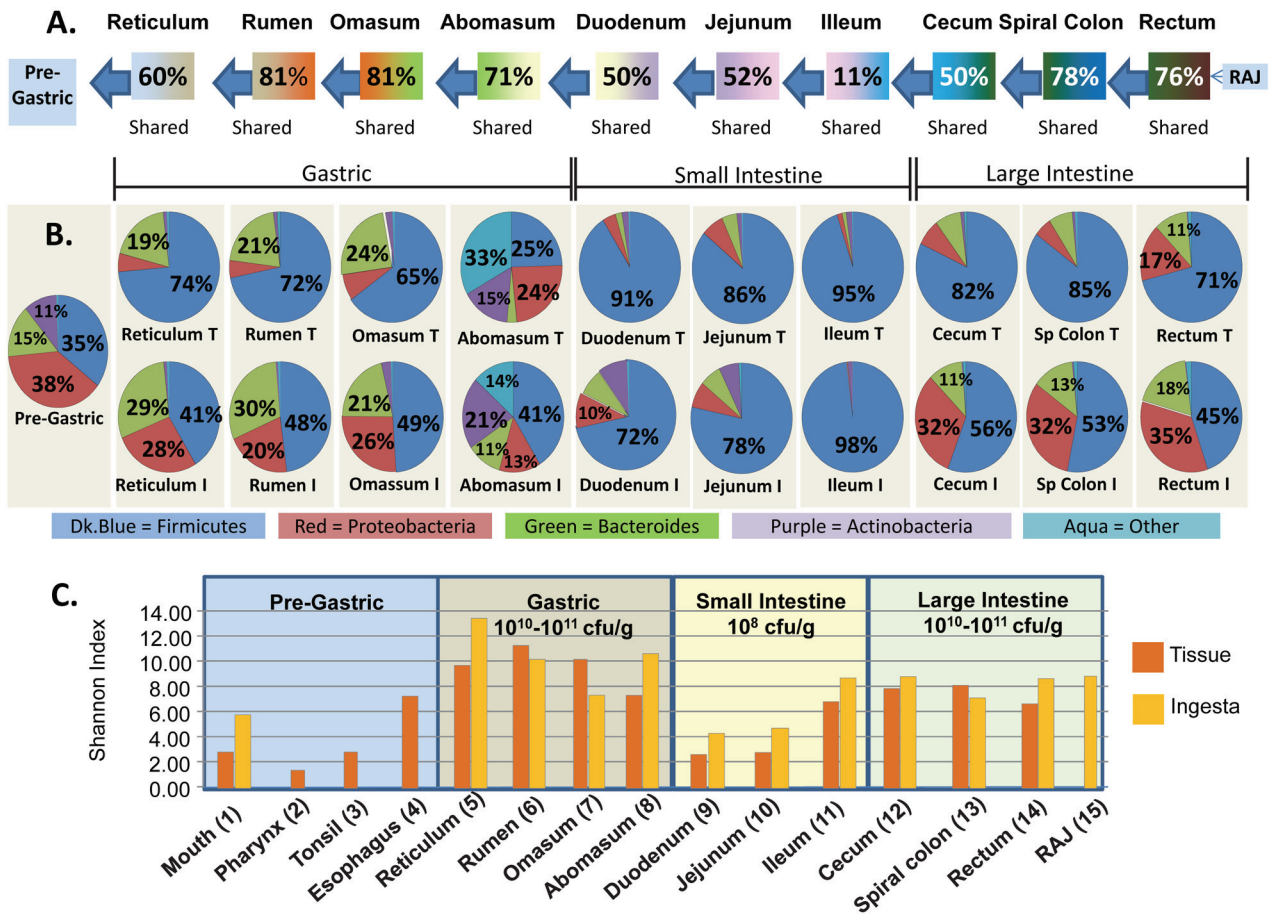


Fig. 2. Spatial distribution of bacteria along a beef steer gastrointestinal tract. (A) Percentage of operational taxonomic units from each sample that were shared with the compartment immediately upstream. (B) Pie graphs show the proportional distribution of the most frequently occurring phyla. “Pre-gastric” represents a pool of mouth, pharynx, tonsil, and esophagus. Rectal–anal junction (RAJ) data not shown in this figure. (C) Shannon diversity index for tissue and digesta samples along the length of a beef steer. cfu, colony-forming unit.

cells were viable. The dense concentration of microbes in the GIT and the active metabolism of substrates generally provide for quick catabolism of dead cells and free DNA; however, this was not tested for and cannot be assumed.

Our results, along with other sequencing-based ruminant GIT studies (Mao et al., 2015; Wang et al., 2017), reinforce the concept that there are distinctive and characteristic ecological differences throughout the beef cattle GIT with respect to bacterial diversity and taxonomic composition. These 16S rRNA sequencing studies provide additional resolution to the outstanding work of early culture-based efforts to characterize the microbial flora of cattle (Hungate 1960; Hinton et al., 1985). The microbial composition of the rumen and the feces remains a research priority for animal nutrition, animal health, food safety, and environmental quality, but the possibility exists that the bacterial community structure, and ultimately function, of the rumen and rectum are influenced by the other in-line GIT microhabitats. Although the design of this study does not allow for inferences beyond this one beef steer, these results provide a foundation for future work articulating taxonomic differences in GIT systems between beef and dairy animals.

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