High-throughput Phenotyping of Rumen Microbial Contents Using Buccal Swabs

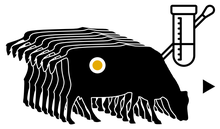
Juliana Young,[a] Joseph H. Skarlupka,[b] Madison S. Cox,[b] Rafael Tassinari Resende,[c] Amelie Fischer,[d] Kenneth F. Kalscheur,[a] Jennifer C. McClure,[a] John B. Cole,[e] Garret Suen,[b] Derek M. Bickhart[a#]

[a]US Dairy Forage Research Center, USDA-Agricultural Research Service, Madison, Wisconsin, USA; [b]Department of Bacteriology, University of Wisconsin, Madison, Wisconsin, USA;
[c]School of Agronomy, Universidade Federal de Goiás (UFG), Goiânia, GO, Brazil; [d]Institut de l'élevage, Beaucouse, France;
[e]Animal Genomics and Improvement Laboratory, USDA-Agricultural Research Service, Beltsville, Maryland, USA;
[#]Address correspondence to Derek Bickhart, derek.bickhart@usda.gov

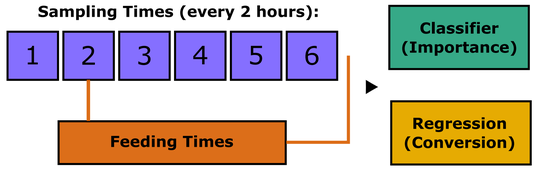
Introduction

The rumen microbiota has been inextricably linked to production traits in cattle as it is the primary means by which the host animal digests feed. However, gold-standard methods to assess the rumen microbial community are either laborious (ie. Stomach tubing) or require invasive surgeries (ie. Rumen cannulation). This often limits surveys of the rumen microbiota to smaller sample sizes and reduces the power necessary to identify small effects that microbial composition has on other traits. Recent work has suggested that buccal swabs could serve as suitable proxies for rumen microbial contents [1,2]. However, this work notes that the contamination of the oral microbiota is a major issue among several other factors. Our study uses a time-course experimental design and Random Forest models to identify both (a) the putative oral- and feed-associated taxa that contaminate buccal swabs and (b) the best time to collect buccal swabs with respect to their correlation with rumen samples.

Methods

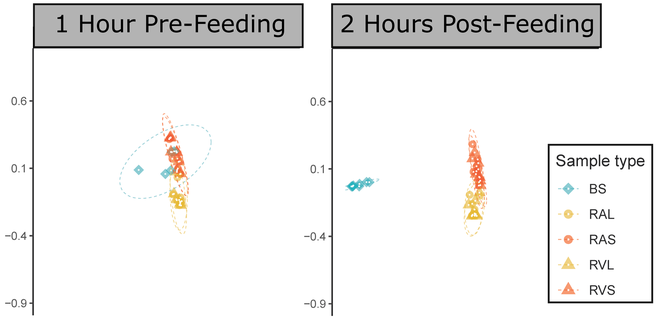


Rumen Cannula & Buccal Swab Sampling

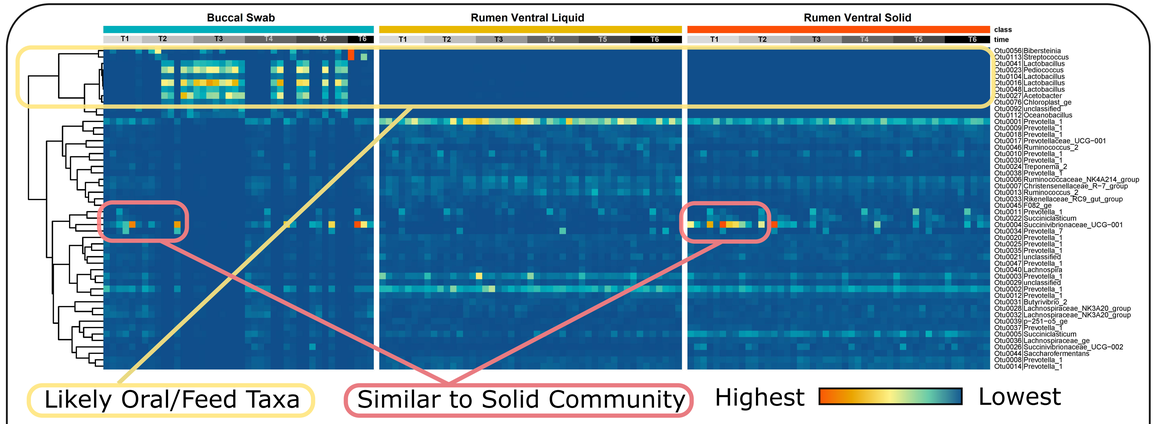


Buccal swabs (BS) and rumen contents (separated into solid (RAS, RVS) and liquid (RAL, RVL) fractions) were taken from 8 cannulated cows at six timepoints (T1-6) separated by 2 hours each. The first timepoint started 1 hour prior to morning feeding and continued until just prior to evening feeding. DNA was extracted from samples and the V4 region of the 16S rDNA gene was amplified for sequencing. Sequences were processed using Mothur [3] and filtered OTU tables were used in a Random Forest Classifier and Regression.

PCoA suggests best time to sample

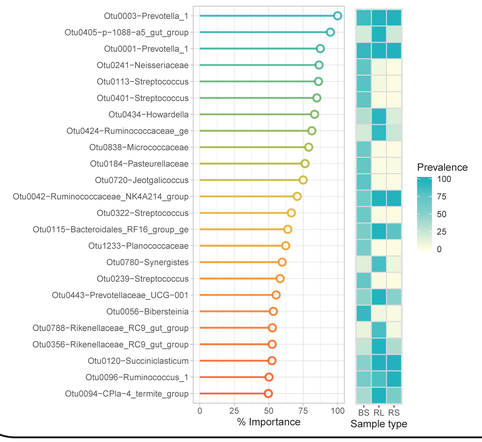


PCoA plots of OTUs from buccal swabs (BS) and Rumen samples (RVS, RAS, RVL, RAL) show the greatest similarity at the first timepoint (T1; pre-feeding). The differences between sample types is most pronounced shortly after feeding (T3) due to contamination with putative feed- and oral-associated microbes.



A heatmap of OTU relative abundances shows clear visual patterns to help distinguish between contaminants. Columns represent individual cows separated by timepoints (T1-6) and sample type (BS, RVS and RVL) and each row is an identified OTU, with each cell colored by the relative abundance of that OTU in the sample. The first obvious pattern is the presence of high relative abundance of OTUs in the upper clades of rows in the buccal swab (BS) samples that are likely oral in origin. Furthermore, there are examples of taxa that are abundant to similar degrees in rumen solids and buccal swabs in specific animals, pre-feeding. This may be a consequence of rumination, where the animal depletes liquid from the bolus before chewing the solid fraction.

Random Forest Classifier identifies oral taxa



A Random Forest Classifier model was trained on the data to classify samples into buccal swabs (BS), rumen solids (RS) and rumen liquids (RL). After training the model, we identified the top 20 taxa that were most informative to the model's decisions as a function of the mean decrease GINI (% importance). A heatmap of average relative abundance of each OTU is presented to the right of each taxa. The Classifier model clearly identified oral- and feed-associated taxa, but also identified rumen taxa that were underrepresented in BS samples.

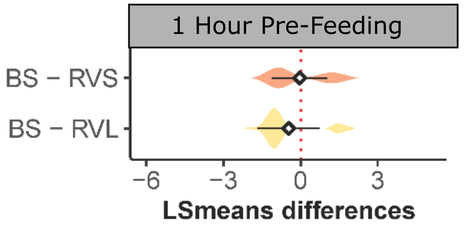
Conclusions

Swabs taken pre-feeding were correlated with rumen microbial profiles

Contaminants from the oral or feed communities were identified

Regression of buccal swab data to rumen microbial profiles still needs further tuning

Regression Analysis



We used a Random Forest Regression model to attempt to derive the relative abundance of rumen (RVS, RVL) OTUs from the OTU counts of buccal swab samples (BS). Unfortunately, the model only had a maximum r2 of 0.45 for specific OTUs and we found high degrees of collinearity among others. We suspect that the prevalence and abundance of certain rumen taxa are highly dependent on several variables including the time since last rumination.

References & Acknowledgements

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