Microbiome Analysis Exploring Taxonomic Diversity in Kasaragod Dwarf and Holstein Crossbred Cattle

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Research Article

Keywords: Hindgut, Kasaragod Dwarf cattle, Microbiota, Hostein crossbred cattle, Microbial community

Posted Date: November 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1031096/v1

Abstract

The indigenous cattle are efficient in converting low quality feeds and forage into animal products. Kasaragod Dwarf cattle, a unique non-descriptive native cattle of Kerala, India, are noted for their unique qualities, such as low feed intake, thermotolerance, greater resistance to diseases and A2 allelic variant milk. However, owing to the higher milk yield, Holstein crossbred cattle are given more importance over Kasaragod Dwarf. The hindgut microbiota plays a major role in various biological processes such as the digestion, vitamins synthesis, and immunity in cattle. In this study, we compared the hindgut microbiota of the Kasaragod Dwarf with the highly found, Holstein crossbred utilizing 16S rRNA high-throughput sequencing for a better understanding of the relationship between the host and microbial community. Four replicates of each 20 samples comprising two cattle type (n=10) were sequenced and analyzed. Marker gene-based taxonomic analysis affirmed variations in their microbial composition. Principle Coordinate Analysis (PCoA) using weighted and unweighted UniFrac distance matrices showed the distinct microbial architecture of the two cattle type. Random Forest analysis further confirmed the distinctness and revealed the signature taxa in K-Dwarf. The study observed the predominance of feed efficiency associated genera viz., *Anaerovibrio, Succinivibrio, Roseburia, Coprococcus, Anaerostipes, Paludibacter, Elusimicrobium, Sutterella, Oribacterium, Coprobacillus,* and *Ruminobacter* in Kasaragod Dwarf cattle. The study highlights the abundance of unique and beneficial hindgut microflora found in Kasaragod Dwarf, which may attest its importance over exotic cattle breeds viz., Holstein. To our knowledge, this is the first report of Kasaragod Dwarf cattle gut microbiome profiling. This study is pivotal towards developing genetic resources for the microbial population in K-Dwarf and how it could be differentiated from Holstein crossbred cattle.

Introduction

Ruminants make up a significant proportion of the domesticated animal species in both developing and developed countries. They play an indispensable role in maintaining the livelihood of millions of people. India has the largest cattle population accounting for 12% of the global population, and is richly endowed with indigenous cattle breeds with several unique adaptive characteristics (Parmar et al. 2017; Ramesha et al. 2016). Cattle play a significant role in the sustenance and development of the economy by aiding the marginalized people's subsidiary income and meeting the local population's nutritional diet.

Kasaragod Dwarf (K-Dwarf), *Bos indicus,* is a non-descriptive indigenous dwarf variety of zebu cattle endemic to the mountain ranges of Kasaragod (12.5°N 75.0°E), Kerala, one of the southernmost states in India. Some of the characteristics that distinguish them from the exotic cattle are high milk quality having therapeutic attributes, high disease tolerance, thermotolerance and increased feed efficiency (Srivastava et al. 2019). While exotic breeds show vulnerability to infectious or parasitic diseases, dwarf cattle exhibit a robust response to infectious diseases and even to different climatic conditions (Ramesha et al. 2016). Their dietary needs are met alone by the roughage from domestic cooking, which renders them feed-efficient variety (Srivastava et al. 2019). Despite possessing several valuable characteristics attributes,

native breeds have been unable to grab scientific attention for their conservation. Their domestication is affected by factors such as lower population density and decreased milk production potential.

In recent years, gut microbial ecology has gained enormous attention due to its impact on host health and performance. The intestinal gut microbiota in cattle encompasses a very complex ecosystem that interacts symbiotically with the host and assists in various metabolic processes, such as the production of volatile fatty acids, vitamin synthesis, microbial protein synthesis, xenobiotic metabolism, and immune regulation (Malmuthuge et al. 2017; Myer 2019; Ríos-Covián et al. 2016). High-throughput sequencing related studies on cattle have corroborated such findings. Studies following metagenomics approach revealed that the diversity of gut microbial community influences productivity traits and other phenotypic characteristics (Li et al. 2019; Xue et al. 2020). The bacterial communities in the gut of two genetically distinct cattle breeds, Holstein and Jersey, have been shown to be significantly different (Paz et al. 2016). Another comparative study on cattle breeds Vechur and crossbred has also reported genetic distinctness based on the gut microbial composition (Sadan et al. 2020). While rumen is the primary center, harboring microbial community for the conversion of ingested dietary components into energy to the host (Auffret et al. 2020; McGovern et al. 2020; Sadan et al. 2020; Xue et al. 2020), the hindgut (also referred as large intestine) microflora plays a significant role in deciding the host health and physiological characteristics of the mammalian host (Amin and Seifert 2021; Lopes et al. 2019; Sanz-Fernandez et al. 2020).

The hindgut is home to a diverse and densely populated microbiota, regarded as a fermentation center with less buffering capacity than the rumen (Gressely et al. 2011; Khafipour et al. 2016). The hindgut microbiota aids in the functioning of various biological processes such as the immune system, produces vitamins, and can inhibit pathogenic bacteria (Cho and Blaser 2012; Ley et al. 2008; Turnbaugh and Gordon 2009). A few recent studies on ruminants have established that the microbial diversity in the hind-gut had a direct effect on the productivity traits and cattle physiology (O'Hara et al. 2020; Sanz-Fernandez et al. 2020). Fermentation of undigested nutrients in the hindgut produces volatile fatty acids, are an important source of energy in cattle (Gressely et al. 2011).

Investigating the differences in microbial community composition across the hindgut microbiota can provide further insights linking animal phenotypes and production traits with variation in microbial colonization (Lopes et al. 2019; Qiu et al. 2019). Several studies have revealed the importance of the fecal microbiome as an alternative approach to explore the association between hindgut microbiota and host productivity (Fan et al. 2020; Fan et al. 2021; Lopes et al. 2019; Uchiyama et al. 2020; Wang et al. 2018). In our study, we performed a comparative analysis between the two the native cattle, i.e., Kasaragod dwarf, and the Holstein crossbred exotic cattle, to elucidate the hindgut microbiota compositions. The selected cattle type represents different genetic background with an equal distribution of age and gender. Both the study groups were raised under the same environmental conditions and fed a common herbivore native diet to minimize non-genetic influences. The study employed16S rRNA gene short variable tags of the V3 region generated through high-throughput sequencing to compare the hindgut bacterial communities' phylotype in K-Dwarf and Holstein cattle. This study provided a contrasting view of the microbial profile in two cattle types and highlights the association of particular microbial taxa with K-

Dwarf cattle. To our knowledge, this is the first comparative study demonstrating the differences in microbial compositions between these two cattle, i.e., the *Kasaragod Dwarf*, non-descriptive native cattle of Kerala, India, and Holstein crossbred.

Materials And Methods

Sample collection

The study cohort belonged to two major groups, Kasaragod dwarf (K-Dwarf), non-descriptive native dwarf cattle (n=10), and Holstein crossbred cattle (Holstein), (n=10). The average age of K-Dwarf cattle and Holstein crossbred cattle were 4.4 ± 1.07 years and 4.2 ± 2.09 years respectively. Samples for both the study groups were collected from Kasaragod, Kerala, India (12˚33'41" NL & 75˚9'59" EL). K-Dwarf cattle samples were collected from the cattle pure breed rich region in Kasaragod i.e., Badiadka. Samples were collected from dairy farms belonging to nonpublic individuals with their due consent. Holstein cattle dung samples were collected from the Kanhangad region of Kasaragod District. Prior to sample collection, all the subjects' samples were examined and certified fit by a veterinary specialist. Breed purity based on the morphological characteristics described by Anilkumar and Raghunandan (2003) was carried out with a certified veterinarian's assistance. All subjects included in the study were fed a common herbivore native diet (including green grass, hay, concentrate mixture, and *ad libitum* water supply), and their metadata is provided in Table S1. It was ensured that only subjects with no prior history of having any antibiotics for the past two months were considered part of the cohort. Before the morning feed, fecal samples were collected in a sterile container just after defecation. To avoid contamination, cattle were made to defecate into a sterile tray, which was then quickly transferred into a small sterile container. The samples were transported to the laboratory in cooler boxes (4°C) and processed immediately to minimize systematic bias that can be introduced during preprocessing step (Choo et al. 2015).

DNA Extraction and amplicon Sequencing

Metagenomic DNA was extracted from about 180 mg of the fecal sample using the QIAamp stool mini kit (Qiagen, USA) as per the manufacturer's instructions. The Quality of extracted genomic DNA was evaluated by electrophoresis in 1% agarose gel, and the 260 nm/280 nm ratio was determined by Nanodrop (Thermo Fisher Scientific, Waltham, USA). The V3 region of the 16S rRNA gene from each genomic DNA sample was amplified using a unique barcoded universal primer (341F and 534R) as described previously (Saxena et al. 2017). After examining the amplified products on 2% agarose gel, the amplicons were purified using the Ampure XP kit (Beckman Coulter, Brea, CA USA). Amplicon libraries were then prepared by following the Illumina 16S metagenomic library preparation guide and evaluated on 2100 Bioanalyzer using the DNA1000 kit (Agilent Technologies, Santa Clara, CA, USA). The libraries were further quantified using a Qubit dsDNA broad-range assay kit (Life Technologies, United States). Equal concentration of the libraries was sequenced using the Illumina NextSeq 500 platform in Next-Generation Sequencing (NGS) facility at the Indian Institute of Science Education and Research (IISER),

Bhopal, India. In total, 80 samples comprising four technical replicates for all 20 samples (10 samples from each cohort) were sequenced and analyzed in this study.

Sequence data processing

The amplicon reads obtained from the Illumina sequencing platform were quality trimmed using the NGS QC tool kit (Patel and Jain 2012), and the reads with ≥3 ambiguous bases were discarded. The paired-end reads were joined together using FLASH V1.2.11 (Magoc and Salzberg 2011). The sequences were quality filtered using Quantitative Insights Into Microbial Ecology (QIIME) standard protocol (version v1.9.1) (Caporaso et al. 2010). High-quality reads were clustered into Operational Taxonomic Units (OTUs) at 97% identity (Caporaso et al. 2010). Taxonomy was assigned based on the alignment against GREENGENES database (DeSantis et al. 2006) following a current study in cattle (Hagey et al. 2019).

For each analysis, the samples included were rarified to the sample with the lowest number of sequences (Weiss et al. 2017). Data were normalized using cumulative sum scaling before any statistical comparisons (Paulson et al. 2014). The sequences from each sample were analyzed together, and the sequences undetected at least 20 times were not included in the analysis (low variance and low count filtering) (Weiss et al. 2017). These pre-processing steps help to avoid sequencing depth bias for better comparative analysis.

Comparative microbiome analysis

Comparative analysis of microbiome between K-Dwarf and Holstein samples was prepared at alpha and beta diversity levels. At the alpha level, analysis was carried out based on Observed OTUs, and Chao 1. Mean alpha diversity estimates for the study groups were compared and statistically tested using the Mann-Whitney test. A significant difference level was set at p-value <0.05. Subsequently, beta diversity was calculated based on the weighted and unweighted Unifrac distance metrics (Lozupone et al. 2011), and plotted by Principal Coordinate Analysis (PCoA) to identify the microbial community associated with each cattle type. This was followed by Analysis of similarity (ANOSIM) (Clarke et al. 2006) test to statistically evaluate the significant differences or similarities between the cattle type's bacterial communities. Dendrogram clustering was carried out using the Bray Curtis index and ward clustering algorithm at the OTU level. MetagenomeSeq (Paulson et al. 2014), which uses a zero-inflated Gaussian fit model with an adjusted p-value cut off at 0.05, was used to classify genera differed significantly in abundance between K-Dwarf and Holstein group.Volcano plots were generated to illustrate significant differences between individual genera in K-Dwarf and Holstein communities, using the relative abundance data from the 16S rRNA gene surveys with the R-code supplied with the METAGENassist software (Arndt et al. 2012). Random Forest analysis was performed to identify signature microbes in the K-Dwarf and Holstein groups (Dinsdale 2013). The mean decrease in accuracy was estimated for each variable and the variable (genus) with a mean decrease in accuracy >0.01 was considered to be essential for the classification (Pulikkan et al. 2018).

Detection of Association of beneficial bacteria

A list of potentially beneficial bacterial genera was made following previous studies (Caro-Quintero et al. 2020; Gardiner et al. 2020; Løvendahl et al. 2018; McGovernet al. 2020; Yang et al. 2017). Beneficial bacterial genera were compiled and utilized for data mining from the annotated 16S rRNA sequence database.

Correlation of bacterial genera

Correlation between the genera in K-Dwarf and Holstein samples was determined based on spearman's rank correlation. For the Spearman rank correlation, the significant correlation was considered with coefficient values ranges -1 to 1, and FDR adjusted *p*-value < 0.05.

Data Availability

The 16s rRNA high-throughput sequencing data generated in this study have been deposited at NCBI under the accession number: SRR11213013 – SRR11213032.

Results

Statistics for 16S rRNA data

Output data for microbiota sequencing included a total of 7.5 million raw reads with an average value of 2,57,096.85 ± 83008.43 reads per sample (Table S2). After eliminating both short and low-quality reads, adaptors, and other redundant sequences, a total of 5.1 million high-quality reads (~77.3% of total raw sequences) were obtained. To minimize the variation, the high quality reads were further rarefied to the minimum library size of 1,43,291 sequences. After quality filtering and rarefaction, the reads were classified into 1,54,790 unique OTUs by a 97% sequence similarity cutoff. For all samples, rarefaction curve showed an increase in OTU numbers as a function of the number of samples. The curve became asymptotically stable along with the OTU number being saturated, and an increasingly smaller number of new OTUs were added in each sample (Fig S1), indicating an adequate sequencing depth to obtain an accurate estimate of the OTU richness (with the Good's coverage > 99.7%). After eliminating low abundance and low variance features, a total of 3,847 OTUs were considered for downstream analysis.

Comparative microbiome analysis between K-Dwarf and Holstein samples

Alpha and Beta diversity analysis

Significant difference in the alpha diversity (Chao1 and Observed species*)* between the K-Dwarf and Holstein samples (Mann-Whitney, p< 0.0001) was observed that revealed a notable higher bacterial richness in K-Dwarf, compared to Holstein cattle (Fig 1a-b, Table 1). Further, the beta diversity projection on the PCoA plot revealed that the K-Dwarf and Holstein groups' fecal microbial communities were significantly different (ANOSIM, p-value < 0.001, Fig 2a-b).

The beta diversity analysis results were further supported by dendrogram clustering. Based on the individual samples taxon composition, the two cattle groups differentiated into two distinct clusters (Fig 3).

Metrics	K-Dwarf cattle	Holstein Cattle	p-value(using Mann-Whitney)
Observed Species	3433.6 ± 55.62	3273.3±53.11	0.0001
Chao1	3554.79 ±43.83	3417.26± 52.49	0.0002

Table 1 Comparison of alpha diversity metrics (Observed species and Chao 1) between K-Dwarf and Holstein cattle. The observed index was calculated as the actual number of unique taxa observed in each sample. While the Chao1 was estimated as the richness by inferring the number of rare organisms, that may have lost due to undersampling.

Taxonomic composition

According to the sequence similarity searches against the GREENGENES database, obtained OTUs were categorized into 22 phyla. Dominant phyla (>0.01) in both the K-Dwarf and Holstein groups were represented by Firmicutes, Bacteroidetes, Proteobacteria, Spirochaetes, and Cyanobacteria and accounted for approximately 97% of the community (Fig S2a & b). Interestingly, a significant differential abundance of these four major phyla, including some minor contributing phyla, was observed across the cattle variety. For instance, Proteobacteria, Firmicutes, and Spirochaetes were significantly abundant in K-Dwarf, while Bacteroidetes were abundant in Holstein cattle. Also, TM7, Cyanobacteria, and Elusimicrobia lying under minor phyla were abundant in K-Dwarf, while phylum Actinobacteria and Tenericutes were significantly high in Holstein cattle. Dominant genera in K-Dwarf samples included *Ruminobacter* (20.9%), *Oscillospira* (10.3%),*Clostridium* (10.2%), *Treponema* (8.8%), and *Succinivibrio* (8.6%) (Fig 4a). Whereas, in the Holstein samples, the dominant genera were *Oscillospira* (20.7%), *Clostridium* (19.8%), *Ruminococcus* (10.8%), *Phascolarctobacterium* (9.8%), and *Prevotella* (7.5%) (Fig4b).

Differential taxonomic abundance and signature taxa analysis

To demonstrate the differential abundance analysis of microbial taxa and the degree to which the study groups differed at the phylum and genus level, the metagenomeSeq (fitZIG) approach was employed. Significant variations were observed at the phylum and genus level abundance between the K-Dwarf and Holstein groups (FDR adjusted p-value < 0.05).

At the phylum level, the relative abundance of nine phyla varied significantly between the study groups (MetagenomeSeq, FDR adjusted p-value < 0.001, Table S4). The phylum Proteobacteria, TM7, Cyanobacteria, Elusimicrobia, Firmicutes, and Spirochaetes were predominantly found in K-Dwarf, whereas in the Holstein group, Bacteroidetes, Actinobacteria, and Tenericutes were abundant.

At the genus level, 28 significant genera were differentially abundant between K-Dwarf and Holstein (MetagenomeSeq, FDR adjusted P < 0.05, Table S5) and highlighted through the heat map (Fig S3). The

volcano plot displayed the existence of different community dynamics between K-Dwarf and Holstein samples with fold changes >2 and p values <0.05 (Fig 5, Table S3). Following Random Forest analysis, signature taxa were identified that potentially differentiate the K-Dwarf from Holstein groups (Fig 6). The signature genera investigated for K-Dwarf included *Succinivibrio, Roseburia, Coprobacillus, Anaerovibrio, Anaerofustis, Paludibacter, Elusimicrobium, Candidatus-Azobacteroidetes, Sutterella, Ruminobacter, Oribacterium,* and *Coprococcus*. On the other hand, *Bifidobacterium, Prevotella*, and *L7A* were found to be the marker taxa for the Holstein group. The mentioned features were ranked by their contribution to classification accuracy. The noticeable coherence observed in both the study groups showed the critical role of these genera in forming a distinct gut microbiome.

Association of beneficial bacterial genera with cattle type

Data mining results reveal 45 beneficial bacteria, out of which 27 were found differentially abundant between K-Dwarf and Holstein samples (MetagenomeSeq, FDR adjusted P < 0.05). Of 27 differentially abundant genera, 11 genera (9 in K-Dwarf and 2 in Holstein) were marked as signature taxa in Random Forest analysis. High beneficial bacterial genera were observed in K-Dwarf compared to Holstein samples. Bacteria that enhance the fermentation capacity, such *as Ruminobacter, Sucinivibrio, Roseburia, Coprococcus, Dorea, Anaerovibrio, Blautia, Coprobacillus, Paludibacter, Sutterella,* and *Treponema* were observed significantly abundant in the K-Dwarf group. However, in the Holstein samples, relative abundance of only five beneficial bacterial genera i.e., *Oscillospira, Prevotella, Ruminococcus, Bifidobacterium,* and *Clostridium*, was observed as predominant.

Correlation analysis of taxa between cattle type

Using the Spearman rank correlation coefficient (*Rs)*, correlation analysis of highly abundant genera in K-Dwarf and Holstein cattle was performed (Fig 7). The results showed the taxa exhibiting a significant relationship with cattle type at the genus level. More than 10 beneficial bacterial genera such as *Sutterella*, *Succinivibrio, Paludibacter*, *Coprobcillus, Anaerovibrio, Roseburia, Dorea, Oribacterium, Blautia, Anaerofustis,* and *Coprococcus* was found to have a positive correlation with the K-Dwarf, (FDR adjusted p-value 0.05). Whereas, in the Holstein only three beneficial bacterial genera such as *Ruminococcus*, *Oscillospira*, and *Prevotella* were observed to be positively correlated (FDR adjusted p-value < 0.05).

Discussion

High-throughput sequencing of 16S rRNA gene has greatly contributed to the appraisal and understanding the diversity of gut microbiome. This approach has been widely used to provide a deep understanding of fecal microbiome in different cattle type (Hagey et al. 2019; Lopes et al. 2019; Lu et al. 2018). In this study, we acquired 16SrRNA gene datasets to determine bacterial taxa in cattle hindgut. Statistically significant differences in microbial composition were observed between K-Dwarf and Holstein cattle, with the K-Dwarf cohort harboring microbial taxa potentially contributing to high feed efficiency.

Compositional variance of microbial communities between K-Dwarf and Holstein cattle

Comparison of the K-Dwarf and Holstein cattle gut microbiome based on diversity analysis and differential abundance analysis indicated substantial differences in their microbiome structure. The results implied that the cattle type significantly contributes to the variation in fecal microbiome composition.

Alpha diversity metrics showed higher microbial diversity in K-Dwarf than Holstein cattle, suggesting a microbial diversity-wise wealth in K-Dwarf over Holstein. Supporting our results, a previous study conducted in a native cattle variety, Vechur, reported a higher alpha diversity index than the crossbred cattle. Various studies suggested that an increase in microbial diversity contributing to a stable microbial ecosystem and improved host health (Blaut et al. 2007; Kiros et al. 2018). The clustering analysis using the weighted and unweighted unifrac distance method classified the two cattle types into distinct groups, plausibly suggesting their unique characteristics in correspondence to their micro-ecology. Distinct cluster in dendrogram also resonated the unique microbial characteristics between the K-Dwarf and Holstein samples. Similar studies comparing the two distinct cattle varieties e.g., Holstein and Jersey cattle variety (Paz et al. 2016), Vechur and crossbred cattle (Sadan et al. 2020) have also reported a significant difference in their gut-microbial composition. Recently, Fan and team revealed the role of host genetics in diversity of the hindgut microbiota in cattle (Fan et al. 2020). The genomic disparity between the K-Dwarf and Holstein could be the plausible reason for the difference in gut microbial architecture observed (Kovacs et al. 2011; Li et al. 2019).

Based on our taxonomy analysis results, Firmicutes were the most dominant phylum, followed by Bacteroidetes, Proteobacteria, and Spirochaetes, which were in accordance with previous reports in cattle hindgut microbiota (Mao et al. 2015; O' Donnell et al. 2017; Song et al. 2017). Significant dissimilarities in the microbial structure were also observed at a lower taxonomic level between the two cattle groups. Twenty-eight significantly abundant bacterial genera were identified, illustrating a wide variation in K-Dwarf and Holstein cattle's gut microbial activity. A significantly higher proportion of beneficial gut bacterial genera was observed in the K-Dwarf compared to the Holstein based on the Random Forest classification. Some genera having a potential role in fermentation were highly abundant in K-Dwarf. For instance, *Anaerovibrio* was predominantly high in K-Dwarf aids in the synthesis of lipase which has role in digestion (He et al. 2016). In addition, bacterial genera such as *Succinivibrio*, *Paludibacter*, and *Coprobacillus*, contribute to feed-efficient phenotypes in cattle (Myer et al. 2017), were identified as signature taxa in the K-Dwarf. *Succinivibrio* ferments glucose to synthesize acetic and succinic acids and contributes to the metabolism of different fatty acids (Sun et al. 2016). Members of these genera help enhance feed efficiency by assisting in the complete metabolism of starch (Danielsson et al. 2017; Stolze et al. 2015). *Succinivibrio* is readily absorbed from the rumen for hepatic-gluconeogenesis and helps to improve feed efficiency (Hernandez-Sanabria et al. 2012). It assists in trapping metabolic hydrogen and lowering down methane emissions (Stolze et al. 2015). Their higher prevalence has been reported in

cattle producing less methane; besides, their abundance has been directly correlated to the high feed-efficiency of cattle (Løvendahl et al. 2018; Stolze et al. 2015). Thus, the predominance of *Succinivibrio* observed in K-Dwarf may be proposed to have a major role in their high-feed efficiency.

In comparison to the Holstein, the K-Dwarf has a higher abundance of other specific genera such as *Coprococcus*, *Paludibacter*, *Sutterella*, *Roseburia*, and *Oribacterium. Coprococcus* plays a pivotal role in energy homeostasis (Laydenet al. 2013) and was observed significantly higher in K-Dwarf. *Paludibacter*, a propionate producing fermentative bacteria that helps enhance the production of inositol phosphate metabolism, is abundantly found in feed-efficient animals (Gardiner et al. 2020). Also, *Sutterella* plays an essential role in the biosynthesis of amino acids, pantothenate, and biotin metabolism (Zhang et al. 2017). *Roseburia* is a major bovine gut bacterium that produces beneficial butyrate required for a healthy gut (Diao et al. 2016). The genus *Oribacterium* plays a role in the synthesizing amino acids like serine and oxoproline, an essential metabolite for glutathione metabolism (Mardinoglu et al. 2015; Zeng et al. 2019). Glutathione plays a significant role in many biological processes and provides antioxidant protection (Morris et al. 2014). Given the role of those identified bacterial genera in energy metabolism, their abundance in K-Dwarf further attests to their importance over the Holstein group.

Correlation Between Beneficial Bacterial Genera And Cattle Type

The symbiotic relationship between cattle and their inhabiting microorganisms is of great importance. Mutualism benefits the host in nutrition, development, physiology, and immunity (Gomez et al. 2019). Cattle have a remarkable ability to utilize feed for maintenance and productivity that varies according to the cattle type (Liu et al. 2021; Shabatet al. 2016). Several studies conducted in cattle have shown the association of cattle guts microbiota with varying feed efficiency (Auffret et al. 2020; Bergamaschi et al. 2020; Dvergedal et al. 2020). Unsurprisingly, our result showed a significant correlation (positive and negative) of microbial communities with both the cattle type. Several beneficial bacteria *viz., Sutterella*, *Succinivibrio*, *Paludibacter*, *Coprobcillus*, *Anaerovibrio*, *Roseburia*, *Ruminobacter*,

Dorea, *Oribacterium*, *Blautia*, and *Coprococcus*, were found in K-Dwarf cattle. Gardiner et al. (2020) reviewed the genera with high feed efficiency and illustrated their importance in complex dietary metabolism. All these same taxa associated with K-Dwarf, overlapped with Random Forest analysis results except for genera, i.e., *Blautia* and *Dorea*; however, they do have a role in nutrient assimilation (Eren et al. 2015; Myer et al. 2017).

Unlike the K-Dwarf, Holstein cattle were found to have a correlation with bacterial genera viz., *Ruminococcus*, *Oscillospira*, *Prevotella, Bifidobacterium, Anaeroplasma, Akkermansia, BF311, Lachnospira, Turicibacter, rc4_4, CF231, and L7A*. Here bacterial genera such as *Prevotella*, *Oscillospira*, *Ruminococcus*, and *Bifidobacterium* have been reported to have an essential role in feed efficiency (Ramayo-Caldas et al. 2016). However, a few isolated studies reported an association of these bacteria with low feed efficiency in mammals (Lu et al. 2018; McGovern et al. 2020; Yang et al. 2017).

Our study advances our knowledge about the composition of the hindgut microbiota in K-Dwarf and Holstein samples. Subsequent translational validation is warranted to determine whether changes in

bacterial community composition lead to differences in production-related phenotypic measures.

Conclusion

Utilizing 16S rRNA high-throughput sequencing approach, our study performed a comparison between the gut bacterial community of native Indian cattle, K-Dwarf, and the crossbred variety Holstein. The study demonstrates the differential abundance analysis of microbial taxa and the degree to which the study groups differed. A considerable microbial diversity-wise wealth in K-Dwarf over Holstein cattle was highlighted. The study has also attempted to depict the association of potential gut microbes with the higher feed efficiency of K-Dwarf. The study highlighted specific taxa that are potentially associated with different fermentation products and feed efficiency phenotype. The potential of the cattle gut microbiome observed in this study could be used as a resource to improve feed efficiency in exotic cattle breeds for both economic and environmental benefits. Our study lays a foundation for further research to precisely determine the compositional variations in microbial communities that bring up differences in production-related phenotypic measures.

Declarations

Conflicts of interest

The authors declare that they have no conflict of interest.

Data Availability

The raw data files (reads in FASTQ format) were deposited at NCBI SRA database under accession number: SRR11213013 – SRR11213032.

Competing Interests

The authors declare that they have no competing interests.

Authors' contribution

TG conceived and designed the study. DM carried out samplings. JP contributed to samples collection. DM carried out the experiments. VKS facilitated the sequencing of samples. RS performed the library preparation and sequencing. KA and DM carried out data analysis. DM wrote and drafted the original manuscript. TG, VKS, JP and KA edited and reviewed the manuscript. The final manuscript was read and approved by all authors.

Acknowledgment We are extremely grateful to the Kerala State Council for Science, Technology and Environment (KSCSTE) for providing financial assistance to the first author.

We are thankful to Dr. Jayaprakash, Assistant Director, Kasaragod dwarf cattle Conservation farm, Bela, Badiaydka, Kasaragod, Kerala, India.

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Figures

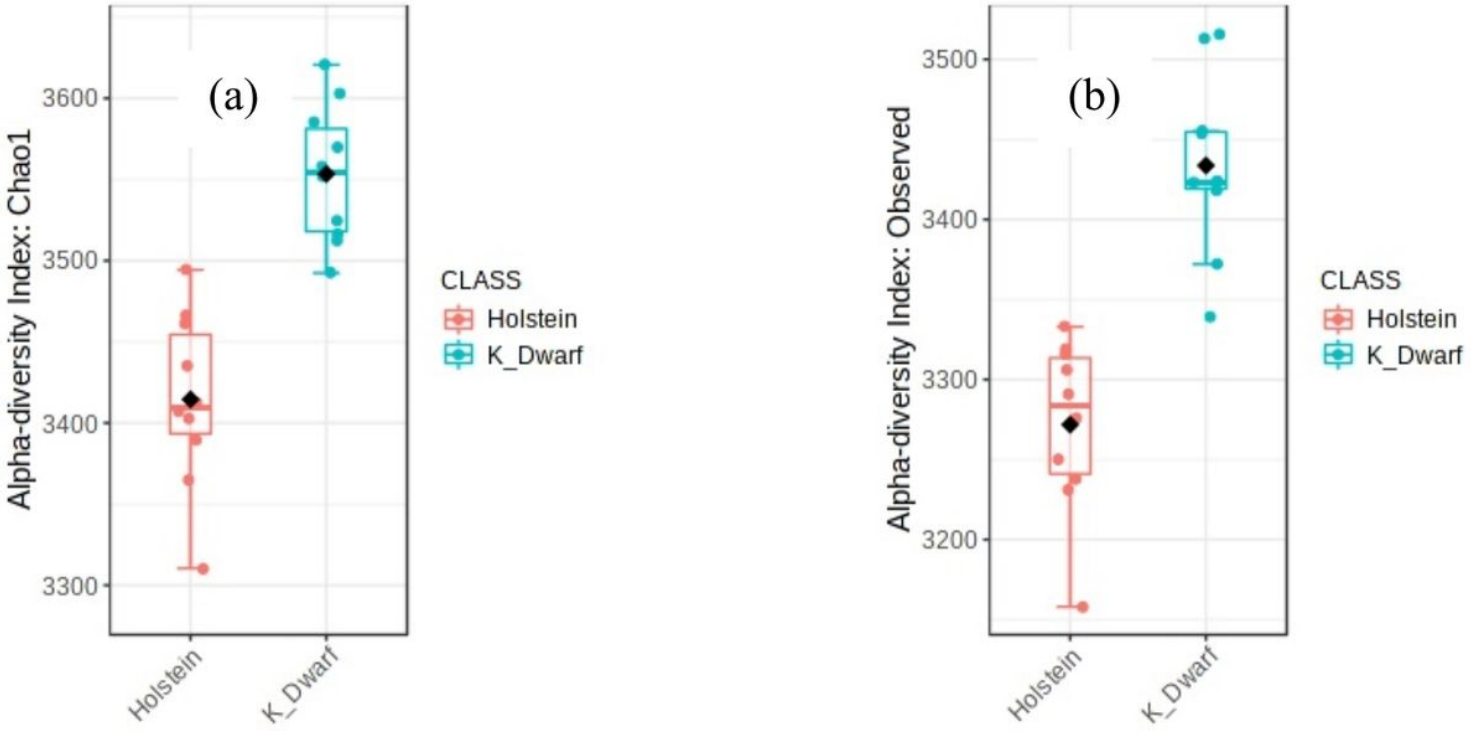


Figure 1

Alpha diversity Box plot (a) Chao1 and (b) Observed species indices reflect OTU diversity in samples.The greater the Chao 1 index, the higher the expected species richness of the microbiota. Boxes represent the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively), and

the horizontal line inside the box defines the median.The averages were compared by Mann Whiteny Test (P< 00.05)

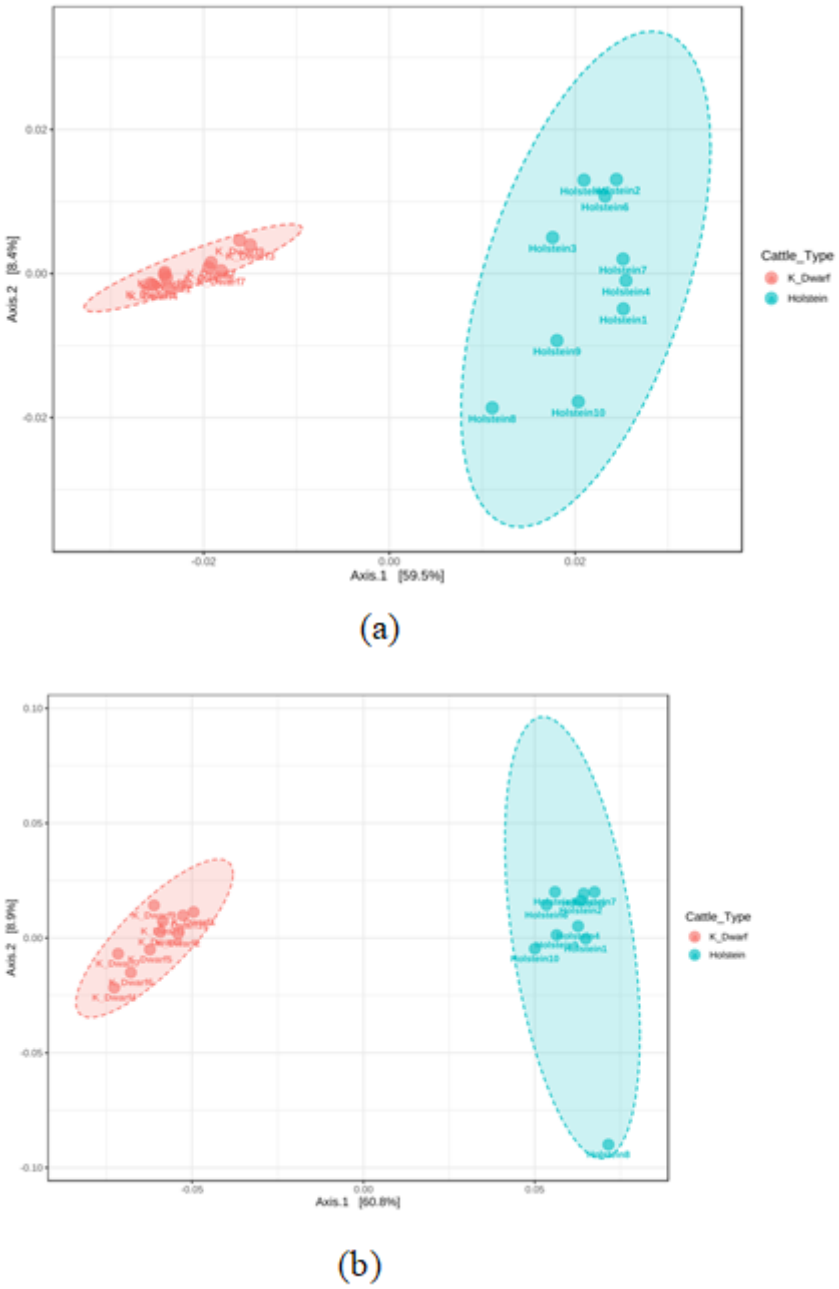


Figure 2

Beta Diversity. Principal Coordinate analysis (PCoA) plot of weighted and unweighted unifracdistance matrix for K-Dwarf and Holstein samples. The plot depicts a significant difference in bacterial community structure in two cattle types. PCoA plots each sample as a point in multidimensional space based on the composition of the bacterial population. Closeness of two points in the PCoA denotes similar bacterial population composition between the samples. K-Dwarf microbiota (red dot) and Holstein microbiota (blue dot) are separated with a p- value < 0.001

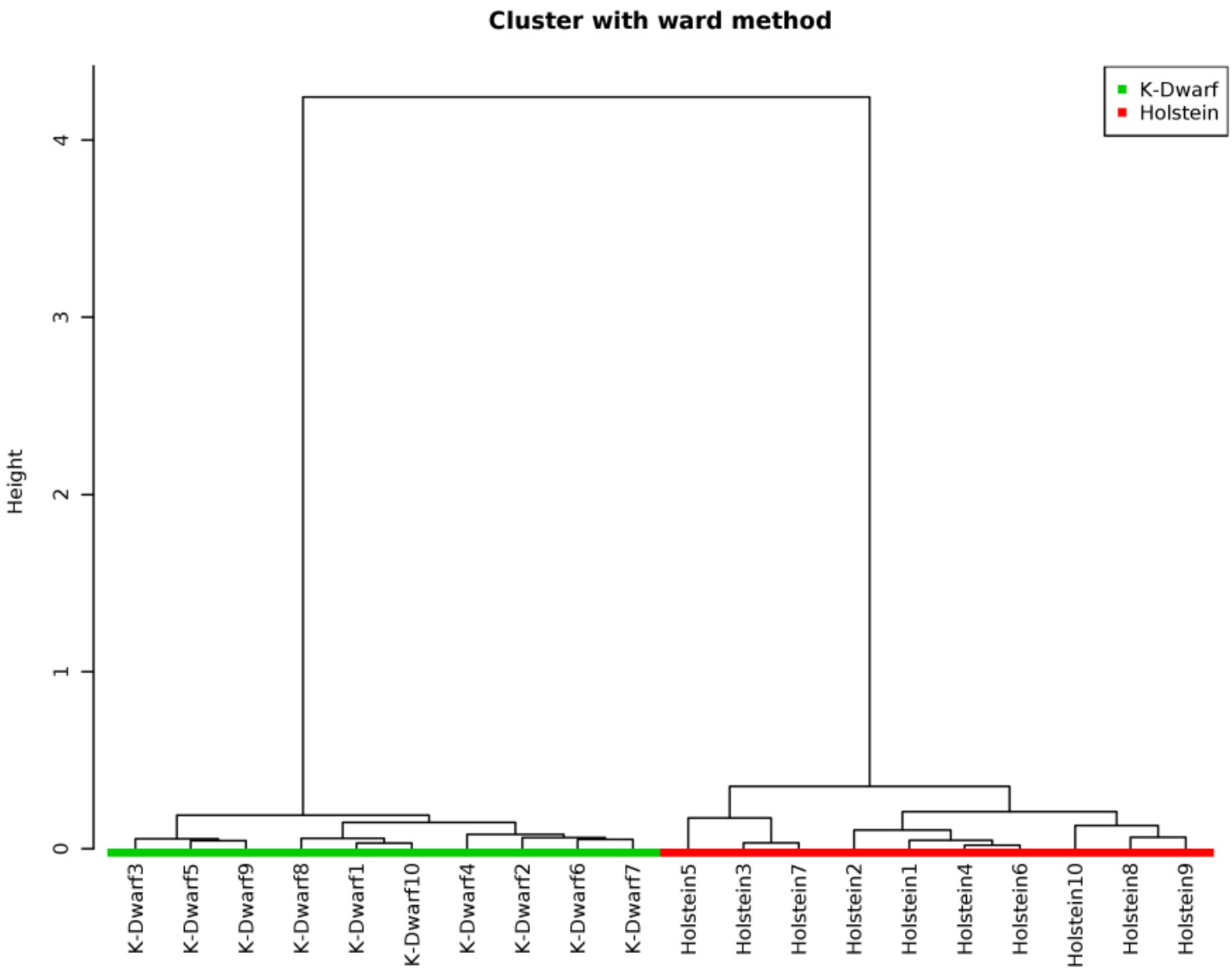
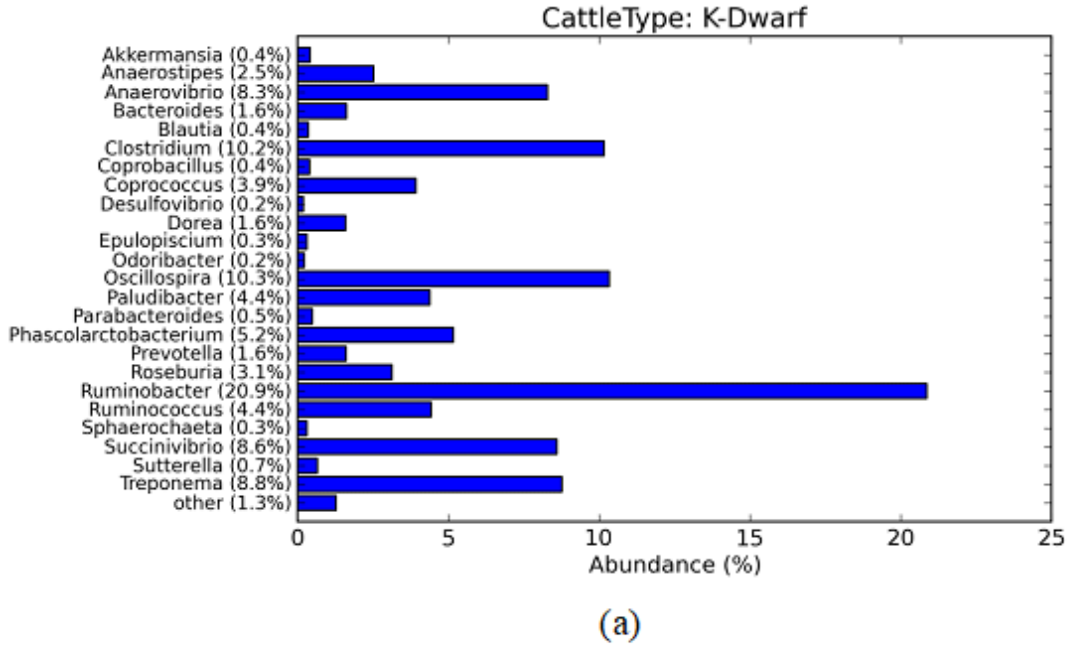


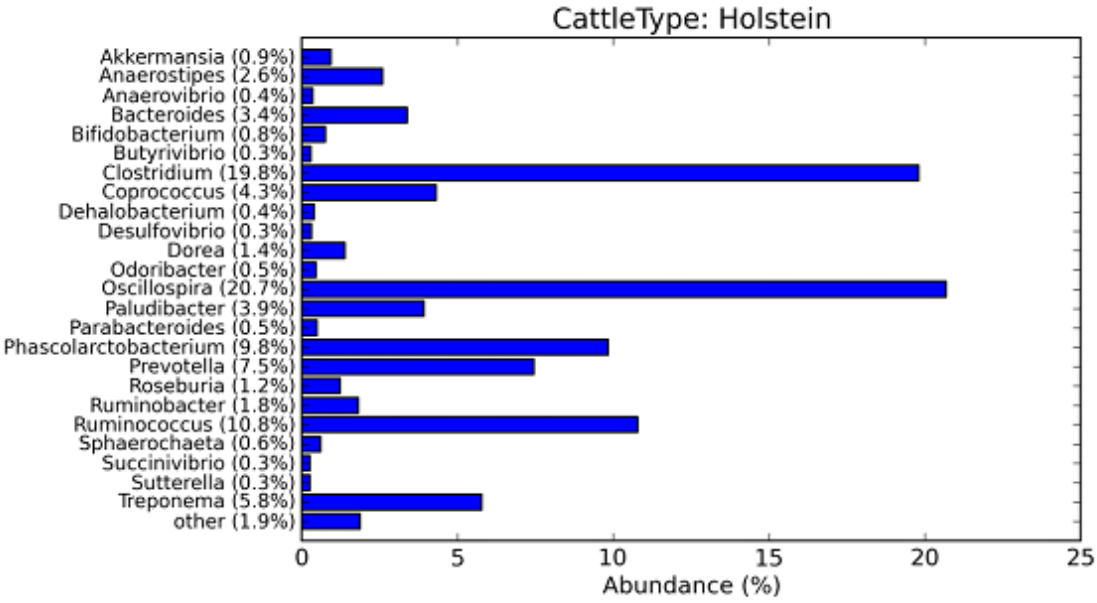
Figure 3

Dendrogram showing the hierarchical cluster analysis using Bray Curtis distance and ward clustering algorithm for bacterial abundance data at the OTU level. Two main clusters are found, K-Dwarf (green) and Holstein (red), each one represents cattle type.



(a)

c



(b)

Figure 4

Relative abundance of bacteria at the genus level (a) K-Dwarf and (b) Holstein in fecal microbiota

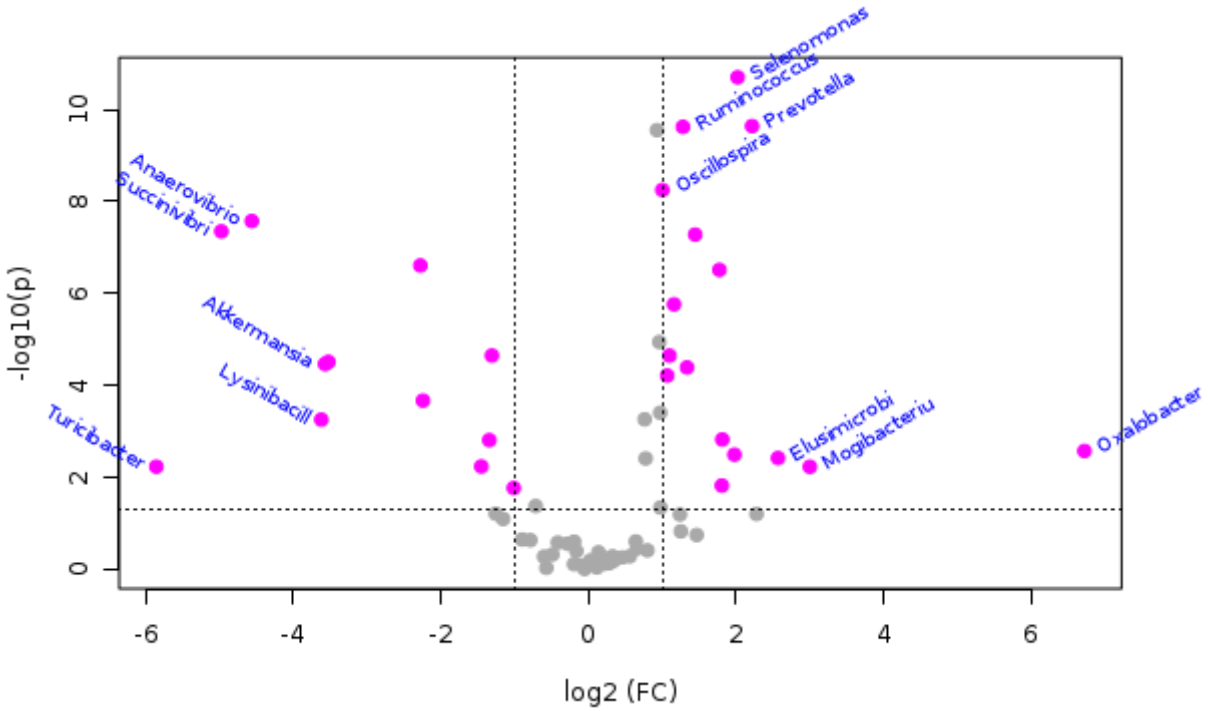


Figure 5

Volcano plot suggests the significant changes in the gut microbiome community in the K-Dwarf and Holstein samples. Dashed vertical and horizontal lines reflect the filtering criteria (Fold change threshold 2, p-value threshold 0.05). Red dots represent Genus entities that are significant based on specific test (Wilcoxon) at each group. In both A and B plots, the X-axis represents the abundance fold change on log2 scale, and the Y-axis represents the negative log10 of the calculated p-value

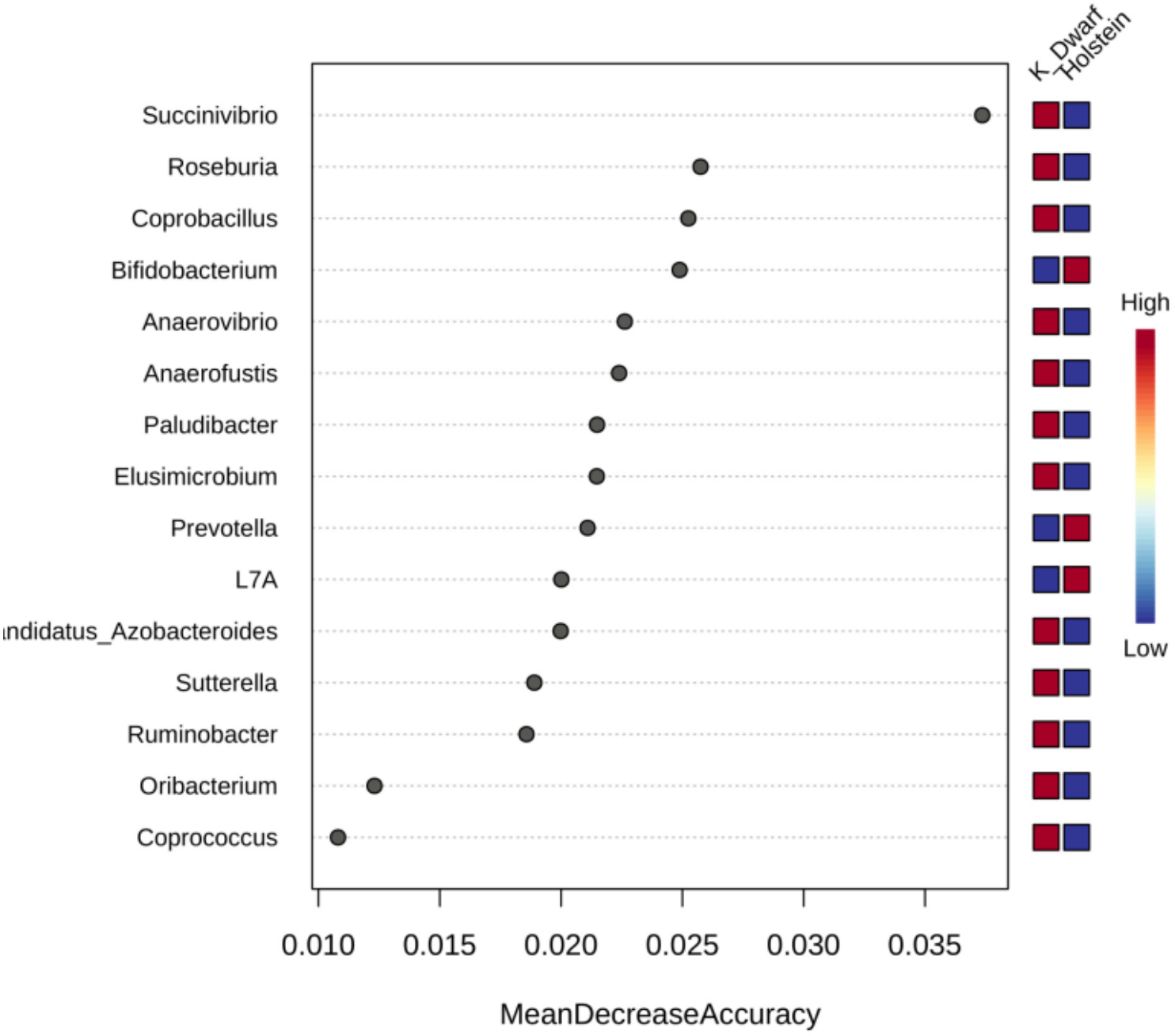


Figure 6

Random Forest The figure shows genera that are responsible for the differences between groups of K-Dwarf and Holstein. Red fields show higher abundance, blue fields a low abundance of the particular genus based on cattle type. The plot shows each variable on the Y-axis and its importance on the X-axis. Random forest calculates feature importance by removing each feature from the model and measuring the decrease in accuracy

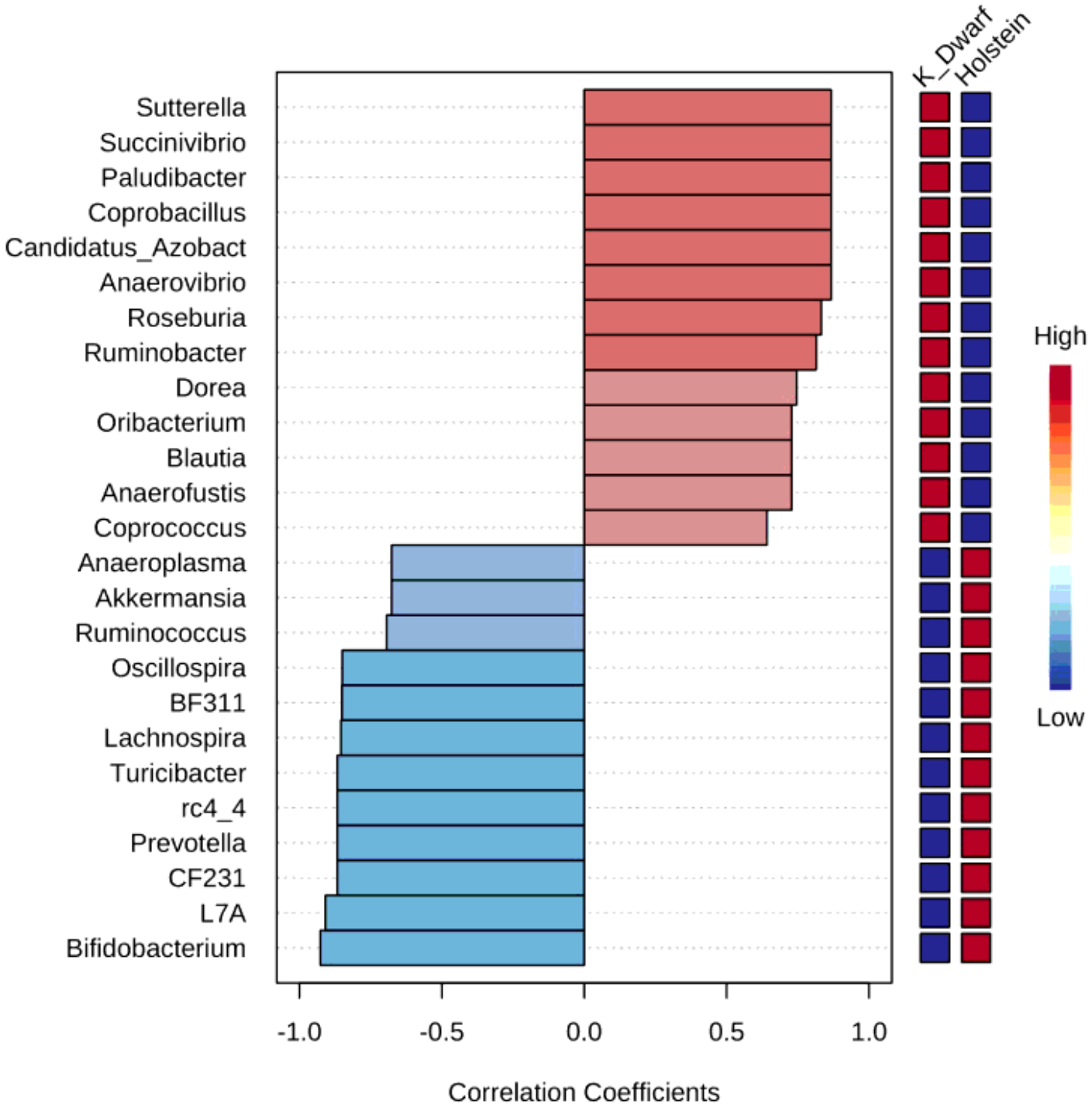


Figure 7

Correlation between cattle type and genus abundance. (a) The cladogram shows the correlation occurring between bacterial genus and cattle type. X-axis represent genus and y axis represent correlation coefficients. Taxa associated with the K-Dwarf are represented with the red and Holstein with the blue color. The darker color represents stronger correlations. The bar size represents the effect of the size of specific taxa in the particular group at genus level. (b) Heat map with color key shows differential abundance of feed efficient bacteria. X-axis represent the samples and y- axis represent the genus

associated with the feed efficiency. There is a histogram in the color key showing the number of coefficient values within each color bar. The dashed lines were the reference value zero.