Diversity of gut methanogens and functional enzymes associated with methane metabolism in smallholder dairy cattle

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Abstract
Methane is a greenhouse gas with disastrous consequences when released to intolerable levels. Ruminants produce methane during gut fermentation releasing it through belching and/or flatulence. To better understand the diversity of methanogens and functional enzymes associated with methane metabolism in dairy cows, 48 samples; 6 rumen fluid and 42 dung samples were collected from Kenyan and Tanzanian farms and were analyzed using shotgun metagenomic approach. Statistical analysis for species frequency, relative abundance, percentages, and P values were undertaken using MS Excel and IBM SPSS statistics 20. The results showed archaea from 5 phyla, 9 classes, 16 orders, 25 families, 59 genera, and 87 species. Gut sites significantly contributed to the presence and distribution of various methanogens (P < 0.01). The class Methanomicrobia was abundant in the rumen samples (~39%) and dung (~44%). The most abundant (~17%) methanogen species identified was Methanocorpusculum labreanum. However, some taxonomic class data were unclassified (~6% in the rumen and ~4% in the dung). Five functional enzymes: Glycine/Serine hydroxymethyltransferase, Formylmethanofuran—tetrahydromethanopterin N-formyltransferase, Formate dehydrogenase, anaerobic carbon monoxide dehydrogenase, and catalase–peroxidase associated with methane metabolism were identified. KEGG functional metabolic analysis for the enzymes identified during this study was significant (P < 0.05) for five metabolism processes. The methanogen species abundances from this study in numbers/kind can be utilized exclusively or jointly as indirect selection criteria for methane mitigation. When targeting functional genes of the microbes/animal for better performance, the concern not to affect the host animal’s functionality should be undertaken. Future studies should consider taxonomically categorizing unclassified species.

Keywords Ruminants · Species · Greenhouse · Enzymes · Kenya · Tanzania

Introduction
The livestock industry contributes to the subsistence of more than one billion of the world’s poorest people and supports approximately 1.1 billion people (Hurst et al. 2005). Rearing livestock is an effective risk mitigation strategy for vulnerable populations, and an important nutrient and traction source for smallholder farming systems (Thornton 2010). Despite there being great disparities between developed and developing countries, livestock products make up 33% of consumed protein and 17% of consumed kilocalories globally (Rosegrant et al. 2009). It is estimated that by 2050, milk and beef production will increase by 191–153%, respectively (FAO 2018). This will lead to an increased need to improve productivity to cater to the increased food demand. However, producing food intensively is likely to place more burden on the environment, as it may increase the amount of greenhouse gases (GHG) produced by the animals.

Methane (CH4) is a greenhouse gas with a 28 times global warming potential than that of carbon dioxide (CO2) (IPCC et al. 2014). The main sources of methane emissions from anthropogenic aided activities are carbon dioxide emissions and farming ruminants. The livestock sector contributes 14.5% of global GHG emissions (Gerber et al. 2013). In ruminants, CH4 is produced after the reduction of CO2 using H2 by methanogenic archaea (Danielsson et al. 2012). Methanogenic archaea are part of symbiotic microorganisms that aid in the breakdown of consumed complex carbohydrates...
from plants to simple molecules that can be utilized by animals such as ruminants (Danielsson et al. 2012; St- Pierre and Wright 2013). The other microorganisms in a such mutual association are bacteria, protozoa, and fungi. The host facilitates the microorganisms’ physical and chemical requirements (Stewart et al. 2018), while the microbes break down complex compounds to produce volatile fatty acids (VFAs) and varying amounts of formic acid, hydrogen (H₂) and CO₂ (Hook et al. 2010). Methane production by the methanogenic archaea process holds H₂ partial pressure down, leading to lesser fermentation of reduced end products like acetate (Moss et al. 2008). The produced methane is not utilized by the animal itself; rather it is eructed from the animal to the atmosphere. Methane is therefore not only harmful to the environment, it also represents a proportion of 2 to 12% gross energy loss to the animal (Johnson and Johnson 1995).

Methanogens occupy various diverse environments ranging from hostile environments to conditions such as those of the gut. They are either acidophilic, mesophiles, and/or psychrophiles (Evans et al. 2015). Methanogens have been found present in the gut of ruminants, termites, chicken ceca, and man (Meng et al. 2014; Saengkerdsub et al. 2007). Their vast environmental exposure would cause them to utilize different types of metabolism for their nourishment. Species such as those from the genus Methanocorpusculum that were initially identified from a wastewater bio digester and were thought not to reside in the gut of ruminants have been identified therein (Oren 2014). To date there are some phyla thrive well in high temperature requirement that the gut of the bovines do not offer as their optimal conditions range between 38.8 °C ± 0.5 °C (Chen et al. 2018) and their full information and categorization has not been fully documented. The phylum Bathyrarchaeota, for example, which was initially identified as Miscellaneous Crenarchaeotal group, has a small proportion of its species cultured and their genomic information completed, calling for a need to undertake advanced work around this area for orderly categorization (Dayu et al. 2020; Meng et al. 2014; Lloyd et al. 2013).

In the recent years, knowledge on gut microbiology has risen due to new molecular techniques such as next-generation sequencing, but the methanogens from the guts of crossbred dairy animals in developing countries have not been well characterized and their connection to CH₄ levels remains largely unaddressed. Moreover, given the differences in the physiological status of various sections of the cattle gut, it is unclear which microbial genes and functional enzymes are responsible for methane production in these various sections of the gut of cattle reared by small holder dairy cattle. To address these questions, we studied cows reared by small holder dairy farmers in the tropics that had different genotypes and were reared as different herds to identify gut methanogens from the rumen fluid and dung samples and functional enzymes associated with methane production and metabolism.

**Materials and methods**

**Description of the experimental sites.**

The research was undertaken in one experimental site in Kenya (University of Nairobi (UON) Faculty of Veterinary Medicine, Kanyariri farm, Kiambu County) and two sampling sites in Tanzania (Rungwe and Lushoto districts). The Tanzanian sampling sites and herd were under a Tanzanian led Project by International Livestock Research Institute (ILRI) dubbed “Maziwa Zaidi platform”.

**Sample collection from the experimental animals in Kenya and Tanzania**

Individual fecal samples were collected from 18 crossbred dairy cows from each district in Tanzania and from 6 animals reared at the UON Veterinary and teaching farm. The Tanzanian’s herd was purposefully selected based on the animal’s genotypes (Cheruiyot et al. 2018), and willingness of the dairy farmers to participate in the research project. The collection of the fecal samples was done by palpating the rectum using clean sterilized lubricated hand sleeves. About 250 g of fecal matter was hand grabbed and a proportion transferred into a sterile labelled 50 ml falcon tube as described by Kibegwa et al. (2020). About 250 ml of rumen fluid was collected using a flexible stomach tube from each animal of the six experimental animals at UON Veterinary and teaching farm immediately after collection of the fecal samples. The first 200 ml of the rumen fluid was then discarded to avoid salivary contamination and the remaining 50 ml transferred into sterile labelled falcon tubes. The samples from each sampling were immediately placed in a cool box with ice cubes and shipped to the Biosciences East and Central Africa (BecA-ILRI) Hub laboratories, Nairobi campus, where they were stored at −20 °C until further processing.

**Illumina sequencing library preparation and DNA extraction**

Before DNA extraction, samples were thawed at room temperature and then whirl wound thoroughly at maximum speed for at least 30 s as described by Habimana et al. 2018 for homogeneity. Whole genomic DNA was extracted separately from all individual samples using the QIAamp DNA Stool Mini Kit (Qiagen, USA) following the manufacturer’s instructions. Nucleic acid quality and quantity was assessed.
using NanoDrop® ND-2000 UV spectrophotometer (NanoDrop Technologies, Wilmington, DE) and 1% Agarose gel electrophoresis. Library construction was performed using the Nextera DNA Preparation Kit and Nextera index Kit (Illumina, San Diego, CA, USA), following the manufacturer’s instructions. In summary, the procedure involved two PCR procedures. In PCR one, 50 ng of the genomic DNA was initially fragmented and adapters added simultaneously. The second PCR was limited (5 cycles) to amplify the fragments of DNA to which adapters had been added from the first PCR. After the first PCR, the fragments were purified using the Zymo DNA Kit (Zymo Research Corporation, Irvine, CA, USA) while after the second PCR, the product was cleaned up and their size selected using AMPure XP beads (A63881, Beckam Coulter, Brea, CA, USA). The final library concentration and the average library size was measured using the Qubit® HS Assay Kit (Life Technologies Corporation, Grand Island, NY, USA), and Bioanalyzer tapestation 2200 (Agilent Technologies, Santa Clara, USA), respectively. Paired end (200 cycles) sequencing of pooled equimolar libraries was done using the Illumina MiSeq v3 (Illumina) System at the Biosciences eastern and central Africa (BecA)-ILRI Hub, Nairobi campus.

**Methanogens taxonomic characterization and identification of enzymes and pathways associated with methane metabolism**

All reads were uploaded to the ILRI research computing cluster (https://hpc.ilri.cgiar.org/), were analyzed and taxonomic assignment was done. Prior to taxonomic assignment, evaluation of sequence quality was done using FastQC software v.0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), poor quality reads were truncated using FASTX-trimmer, within the FASTX-toolkit v.0.0.14 (https://hannonlab.cshl.edu/ fastx.toolkit/). Sequences that had passed quality control were uploaded to MGRAST (Metagenomics Rapid Annotation using Subsystem Technology) for detection of taxa. Quality filtered sequences were then assembled into contigs using SPAdes version 3.13.0. (https://github.com/ablab/spades). SPAdes assembled contigs were uploaded to Kyoto Encyclopedia of Genes and Genomes (KEGG) reference database (Kenahisa and Goto, 2000). Functional genes were identified from the database with the KEGG Automatic Annotation Server (KAAS) version 2.1 (KAAS query list) as previously shown by Auffret et al. (2017). The contigs from each sample were separately blasted to a dataset of prokaryotes based on the assignment method of a bidirectional best hit and then computed and the structural genes checked via https://www.kegg.jp. The KEGG IDs were picked and imported to the Ipath3 (https://pathways2.embl.de) to visualize their involvement in metabolism of microbial metabolism among the diverse environment documented from various studies. The statistical analysis of frequencies, percentages and the relative abundance of various taxonomic classes was performed using MS Excel and IBM SPSS statistics 20 at \( P < 0.05 \).

**Results**

The relative abundance (%) of the methanogens from dung samples were 43.56% (Kenyan dung), 30.78% (Lushoto), and 9.76% (Rungwe). The five phyla identified according to the relative abundance during the study were Euryarchaeota (95.37%), Crenarchaeota (3.74%), Thaumarchaeota (0.52%), Korarchaeota (0.31%), and Nanoarchaeota (0.06%) (Supplementary File 1). Taxonomic classes Archaeoglobi, Thermococci, and Thermoprotei showed relative abundance only in the dung and not in the rumen for the case of Kenyan samples (Fig. 1a). The taxonomic class Methanomicrobia was dominant (~39%) from the rumen and from the dung samples (~44%) (Supplementary Files 2 and 3). Taxonomic classes: Methanomicrobia and Methanobacteria had high abundance of the species in the dung when compared to their presence in the rumen. The other taxonomic classes had a higher abundance in the rumen other than in the dung (Supplementary File 2). This study identified some species that were candidates for taxonomic classification (Fig. 1b and c). During this study, species who were candidates for classification have been referred to as unclassified while those that are already taxonomically placed are referred to as classified. A proportion of ~6% of the rumen samples were unclassified, while ~4% of the dung samples were unclassified. At the lower level of taxonomic classification, approximately 7.77% (at the genus level) of the species remained unassigned. Of the unassigned Archaeal methanogens (at the genus level), Kenyan samples had a collective relative abundance of 7.04% (dung) and 9.94% (rumen) while Tanzanian had a proportion of 7.7% (Lushoto) and Rungwe 7.75% (Fig. 1b). Most of the unassigned archaeal species were from the genera Cenarchaeum and Nitrosopumilus. The taxonomic genus Aciduliproducens was noted to have candidates for classification. The scholars Duya et al. (2020) and Wenheuer et al. (2019) during their studies identified the taxonomic family Nitrosopumilaceae being dominant, which was different during this study as members of the said family had species who were not fully classified. Methanogens from 5 phyla (Archaeal domains) related to methane release and metabolisms were identified. They were Euryarchaeota, Crenarchaeota, Thaumarchaeota, Korarchaeota, and Nanoarchaeota. Crenarchaeota, and Euryarchaeota phyla were statistically significant \( (P < 0.05) \) in the rumen and dung samples. Methanogens from the following 3 genera; *Methanocorpusculum*, *Methanosarcina*, and *Methanococcus* were statistically significant (Table 1). No variation
(P < 0.05) in the relative abundance of methanogens in the dung from the study areas was noted (Supplementary Files 4 and 5). Significant (P < 0.05) variation among methanogens (16 species) in the rumen and dung was also noted (Table 2).

Figure 2 shows the metabolic functions in the rumen and dung samples in which archaea functionality were derived from Ipath3 (https://pathways2.embl.de). The pathway contributed by the functionality of the rumen samples was denoted by a green color, that from the dung was red, and those that overlapped was shown by the blue color. (Fig. 3) More of the blue color was noted. Distinct functionality for the rumen and the rectum was observed.

Figure 3 shows the enzymes of importance that functioned and/or affected in the samples from the dung within the methane metabolism pathways.

The five (5) enzyme commissions (EC) were: ECs 1.11.1.6, 2.1.2.1, 1.2.99.2 (Now referred as 1.2.7.4), 2.3.1.101 (Now referred as 1.17.1.9), and 1.2.1.2. The specific enzyme commissions were denoted by red stars on the box ECs. Specific EC would facilitate specific end products and various substrates for the subsequent processes. Enzymes that were noted to have participated or had functionality in the rumen during methane metabolism were shown in Fig. 4 and the specific functional ECs (1.11.1.6 and 2.1.2.1) are shown by the red stars. The two functional ECs in the rumen and the KO0600 (methane metabolism) module pathway were common for both the rumen and dung samples. Without the activation of various enzymes along the reaction path would lead to unsuccessful yields of the various output.

The module KAAS database (KAAS query list, www.genome.jp/kaas-bin/kaas) also showed functionality for metabolism (09,100) and energy metabolism (09,102). Modules describing methanogenesis using methanol (MOO356), acetate (MOO357), and methylamine/ dimethylamine (MOO563) or Carbon (IV) Oxide (MOO567) as in the KEGG pathway were noted alongside the stated ECs above. KEGG Orthology (KO) in the rumen was 271 ID entries while the KO in the dung was 411 IDs. The entry IDs had different metabolic functions (KAAS query list
Table 1 Analysis for the presence of methanogens from the rumen fluid versus dung samples from Kenya

| Phylum/genus         | Rumen       | Dung        | P value |
|----------------------|-------------|-------------|---------|
| Crenarchaeota        | 4.6 ± 0.15  | 2.67 ± 0.17 | < 0.01  |
| *Sulfobulbus*        | 0.58 ± 0.1  | 1.05 ± 0.14 | 0.03    |
| *Pyrococcus*         | 0.45 ± 0.06 | 0.47 ± 0.13 | 0.91    |
| *Thermofilum*        | 0.35 ± 0.02 | 0.53 ± 0.1  | 0.13    |
| *Staphylothermus*    | 0.14 ± 0.03 | 0.28 ± 0.11 | 0.26    |
| *Caldiflora*         | 0.15 ± 0.05 | 0.17 ± 0.08 | 0.85    |
| Euryarchaeota        | 94.6 ± 0.3  | 96.61 ± 0.2 | < 0.01  |
| *Methanobrevisbacter*| 20.62 ± 2.31| 14.74 ± 1.34| 0.07    |
| *Methanocorpusculum* | 22.01 ± 2.51| 4 ± 0.22    | < 0.01  |
| *Methanosarcina*     | 10.25 ± 0.49| 14.05 ± 0.65| < 0.01  |
| *Methanococcus*      | 6.16 ± 0.11 | 8.49 ± 0.71 | 0.02    |
| *Methanothermobacter*| 3.39 ± 0.14 | 3.07 ± 0.17 | 0.19    |
| Thaumarchaeota       | 0.45 ± 0.2  | 0.52 ± 0.07 | 0.75    |
| *Cenarchaeum*        | 0.14 ± 0.05 | 0.1 ± 0.04  | 0.53    |
| *Nitrosopumilus*     | 0.38 ± 0.05 | 0.35 ± 0.19 | 0.88    |
| *Korarchaeota*       | 0.31 ± 0.13 | 0.15 ± 0.02 | 0.3     |
| *Nanoarchaeota*      | 0.04 ± 0.02 | 0.04 ± 0.02 | 0.97    |

Table 2 Significantly varying methanogen species between rumen and dung samples

| Phylum/species          | Rumen       | Dung        | P value |
|-------------------------|-------------|-------------|---------|
| Euryarchaeota           |             |             |         |
| *Methanobrevisbacter smithii* | 8.08 ± 0.92 | 12.35 ± 1.01| 0.02    |
| *Methanosarcina acetivorans* | 5.82 ± 0.38 | 4 ± 0.35   | 0.01    |
| *Methanocorpusculum labreanum* | 4 ± 0.22  | 22.01 ± 2.51| < 0.01  |
| *Methanosarcina mazei*  | 3.17 ± 0.31 | 2.31 ± 0.05 | 0.03    |
| *Methanospirillum hungatei* | 2.93 ± 0.14 | 1.91 ± 0.2  | 0.01    |
| *Methanococoides burtonii* | 2.93 ± 0.07 | 2.04 ± 0.15 | < 0.01  |
| *Acidaliphilum boonei*  | 2.6 ± 0.18  | 0.99 ± 0.04 | < 0.01  |
| *Methanococcus vannielli* | 2.55 ± 0.13 | 1.78 ± 0.09 | < 0.01  |
| *Archaeoglobus fulgidus* | 1.98 ± 0.42 | 0.86 ± 0.06 | 0.04    |
| *Thermococcus kodakarensis* | 1.75 ± 0.15 | 0.75 ± 0.1  | < 0.01  |
| *Thermoplasma acidophilum* | 0.93 ± 0.19 | 0.34 ± 0.05 | 0.02    |
| *Thermoplasma volcanium* | 0.83 ± 0.11 | 0.43 ± 0.03 | 0.01    |
| *Haloterrigena turkmenica* | 0.8 ± 0.13  | 0.35 ± 0.08 | 0.03    |
| *Methanothermococcus okinawensis* | 0.3 ± 0.04  | 0.12 ± 0.03 | 0.01    |
| Crenarchaeota           |             |             |         |
| *Hyperthermus butylicus* | 0.33 ± 0.05 | 0.14 ± 0.02 | 0.01    |
| *Ignisphaera aggregans* | 0.31 ± 0.03 | 0.13 ± 0.01 | < 0.01  |

www.genome.jp/kaas-bin/kaas). On the functional analysis level two (2) subsystems for selection criteria in MGRAS, Fig. 5 shows 5 statistically significant (P < 0.05) metabolisms both for the rumen fluid and dung samples that were generated. They are carbohydrate metabolism, biosynthesis of other secondary metabolites (not essential for growth and development), xenobiotics biodegradation and metabolism, nucleotide metabolism, and amino acid metabolism. Bolded values in Fig. 5 were statistically significant (P < 0.05) for the functionality level (two) 2 from the MGRAS analysis. The identified enzymes facilitated these processes.

Discussion

Gut methanogens

Methanogens are microorganisms which initially were exclusively thought to be from the phylum Euryarchaeota. From the recent documentation, there has been advancement of discovery of methanogens to other different phyla. Some of the phyla already agreed upon are Thaumarchaeota, Crenarchaeota, Korarchaeota (TACK), Nanoarchaeota, Bathyarchaeota, Geearachaeota, Marsarchaeota, and Verstraetearchaeota (Berghuis et al. 2019; Vanwonterghem et al. 2016; Evans et al. 2015; Kelly et al. 2011). This study identified species from Euryarchaeota, TACK, and Nanoarchaeota. Phyla Euryarchaeota and Crenarchaeota were noted to be dominant among archaeal methanogens and very few represented from the phyla Thaumarchaeota, Korarchaeota and Nanoarchaeota. The same methanogen abundance was noted by Jia et al. (2017). Jia et al. (2017) further noted that the phylum Euryarchaeota (specifically those from the genus *Methanosarcina*) was responsible for a variety of functionalities related to methane biosynthesis.

Methanogens have been extensively studied in comparison to other phyla. They have been recorded to have 155–200 isolated species which are clustered into 4 classes, 7 orders, 14 families, and 29–35 diverse genera (Singh et al. 2011). This study showed presence for 3 taxonomic classes, 6 orders, 12 families, 24 genera and 37 individual species of the methanogenic archaea. This is an illustration of a high representation of the methanogenic archaea in the study areas. Methanogens in the rumen and rectal area of the ruminant species would vary in their population’s organization, ecology, and their substrate sources (Knapp et al. 2011). Such variation could be as a result of the prevailing physico-chemical properties (Dayu et al. 2020). The most dominant species in the fore and hindgut was noted to be *Methanocorpusculum labreanum (~17%)*, followed by other hydrogenotrophic methanogens which is in agreement with a study by Auffret et al. (2017) and Chen et al. (2014). The dominance of *Methanocorpusculum* can be explained by their ability to utilize a wide range of substrates such as acetate, H2 + CO2, formate, ethanol, 2-propanol, 2-butanol, or cyclo-pentanol. This causes them to flourish in several environment where a suitable substrate is available. *Methanocorpusculum* relies on acetate as a growth feature and on peptone, tungstate, and...
nickel for their stimulatory (Rosenberg et al. 2014). Moreover, this genus can survive in a wide environment, temperature range of 15–60 °C and pH of 6.1–8.0 (Liu and Whitman 2008). These conditions can be achieved in the rumen.

Methanococcus are methanogens that were thought to be only isolated from the sea. However, that opinion since changed when Methanococcus were found in other environments that are not marine-related conditions (Tumbula and Whitman 2003). Methanococcus species are not associated with any disease on their hosts and are firmly anaerobic and hydrogenotrophic. They have distinct abilities to undertake sulfur metabolism (Liu et al. 2009). Most are mesophilic (require a temperature of between 20 °C and 45 °C), and others are thermophilic (41–122 °C) or hyperthermophilic (above 60 °C) (Stetter 2006). In this study, mesophilic species: Methanococcus aeolicus, Methanococcus maripaludis, Methanococcus vannielii, and Methanococcus voltae were identified and are especially the mesophilic type. The species identified in this study were similar to those identified by Goyal et al., (2016).

*Methanosarcina* methanogenic archaea utilize the substrates acetate, H$_2$ + CO$_2$, CO, methanol, methylamines, and methyl mercaptoproprionate dimethylsulfide. They grow on a variety of substrates because they are notably cytochrome bound (Buan et al., 2011). These methanogens operate in a temperature range of between 1 °C and 70 °C and a pH range of 4–10 and adapt to low hydrogen availability (Liu and Whitman 2008). *Methanosarcina* have a flexible metabolic pathway making their growth genes on one substrate easily deleted without affecting their subsequent growth on another available substrate(s). With this capability, *methanosarcina*’s genetic analysis can be used to investigate how methanogens grow and participate in methane production along the known methane metabolism pathways (Kulkarni et al. 2009). *Methanosarcina acetivorans*, *Methanosarcina barkeri*, and *Methanosarcina mazei* were identified during this study.

The methanogen *Methanocorpusculum labreanum* species that hailed from *Methanocorpusculum* was identified during this study. This species was found both among the rumen fluid samples and dung samples from the Kenyan dairy cattle. This was in contrast with a study by Liu et al. (2012) in a study on Chinese sheep that identified sequences related to *Methanocorpusculum* species from sheep droppings only but absent in the rumen of the same sheep. In another study by Luo et al. (2013), the authors found out that *Methanocorpusculum* were dominant (60%) in the hindgut of captive *Ceratotherium simum*. *Methanocorpusculum* utilizes the substrate acetate, H$_2$ + CO$_2$, formate, ethanol, 2-propanol, 2-butanol, or cyclo-pentanol. Identification of
this species in the rumen indicated that they may have a wider niche than previously thought.

**Functional enzymes associated with methane metabolism**

Enzymes catalyze chemical reactions that are key and important for life functionality such as metabolism and digestion (Blanco and Blanco 2017). Over the years, there is an advancement of knowledge on methane metabolism between methanogens and methanotrophic archaea with a universal display of the methyl-coenzyme M reductase complex as a main enzyme in their pathways (Evans et al. 2019). The study identified the function of the enzyme glycine/serine hydroxyl methyltransferase in the aspects of amino acid transportation and metabolism/biosynthesis from module entry K00600. This was identified from the enzyme commission EC: 2.1.2.1 and was associated with the structural gene glyA. Glycine/serine hydroxyl methyltransferase is a vitamin reliant enzyme that catalyzes the reversible and conversion of L-serine to glycine and tetrahydrofolate to 5, 10-methylenetetrahydrofolate. Upon completion of the enzymatic reaction, it leads to the delivery of substantial carbon units to the cell (Edgar 2005). Another study noted that this enzyme as well catalyzes glycine and acetaldehyde to form L-threonine with 4-trimethylammoniobutanal to form 3-hydroxy N6, N6, N6-trimethyl-L-lysine (Schweitzer et al. 2009). Methanogens like *Methanococcus jannaschii*, which was identified in this study, have been shown to use the enzyme for amino acid biosynthesis (Tsoka et al. 2004).

Formylmethanofuran–tetrahydromethanopterin–N-formyl transferase enzyme was noted from the EC: 1.17.1.9 (Formerly EC: 2.3.1.101). The module entry involved was K00672. The enzyme was notably involved in energy...
production and conversion. Entry K00123 that is involved in
the anaerobic selenocysteine-containing dehydrogenase was
also noted and needed for energy production and conversion.
The gene responsible for the enzyme is Ftr. Structural genes
associated with the functionality of the enzyme were fdgG,
fdhF, and fdwA. Formylmethanofuran–tetrahydromethanopterin–N-formyltransferase enzyme catalyzes two notable
substrates; methanofuran and 5-formyl-5–6-7–8-tetrahydro-
methanopterin (Wagner et al. 2016). Methanofuran is
vital for methane formation from CO₂ by methanogens. CO₂
as a methanogenic substrate is initially reduced and activated
to formylmethanofuran (Wagner et al. 2016). Mesophilic
methanogen (Methanosarcina barkeri) and thermophilic
methanogens (Methanopyrus kandleri), that were also iden-
tified in this study, have shown functionality for the enzyme
formylmethanofuran–tetrahydromethanopterin–N-formyl-
transferase (Enzmann et al. 2018).
Anaerobic carbon monoxide dehydrogenase enzyme
facilitates the metabolism of methanogens by the reversible
interconversion between carbon monoxide and CO₂.
The catalyzed reaction is vital for energy conservation and
carbon fixation among methanogens, especially during the
Wood–Ljungdahl pathway (King and Weber 2007; Bor-
rel et al. 2016). The enzyme anaerobic carbon monoxide
dehydrogenase of the catalytic subunit was noted during this
study from the EC: 1.2.7.4 pathway, formerly EC. 1.2.99.2.
The involved module entry was K00198. Hydroxylamine
reductases (hybrid-cluster protein) together with the enzyme-
matic function of anaerobic carbon monoxide dehydroge-
nase are vital for inorganic ion transport and metabolism
energy production and conversion. The key genes involved
were cooS and acsA.
Catalase–peroxidase enzyme is documented to be an
inconsequential material in the antioxidant system in metha-
nogens even for those aerotolerant, including species such as Methanosarcina acetivorans (Jennings et al. 2014). The
genus Methanosarcina would also possibly produce meth-
ane with the help of enzymes and protein constituents in
the methyl nutrient pathway, acetic acid, and CO₂ reduction
(Thauer 2011).
The catalase–peroxidase enzyme is a sturdy catalase with
H₂O₂ as the contributor which releases O₂ (Vlasits et al.
2010) and molecules of water in a two-step reaction (Nandi
et al. 2019). Methanogens in their environments are exposed
to oxygen occasionally in aerobic situations; this would call
in for the functionality of antioxidants to facilitate lower-
ing levels of oxygen (Angel et al. 2012). Ma and Lu (2011)
pointed out that some methanogens can withstand some
levels of oxygen for some hours. The enzyme possessed by methanogens (although they are noted to be limited in the numbers and are restricted to *Methanosarcina* and *Methanobrevibacter* species) notably acquired through a gene (*katG*) transferred laterally (Zamocky et al., 2012a). When catalase peroxidase enzyme has functionality in EC: 1.11.1.6, it acts on hydrogen peroxide as an acceptor and not the functionality under EC. 1.11.1.21 for both catalase and peroxidase. Module entry K03782 was involved. Specifically, the catalase–peroxidase I is involved in catalyzing inorganic ion transport and metabolism. The gene involved in the process is *katG*.

In conclusion, this study broadens our understanding on dominant methanogen species in Kenya and Tanzanian among smallholder cattle and functional enzymes associated with methane metabolism and production. The most abundant methanogen noted during this study was *Methanocorpusculum labreanum*. Two enzymatic pathways (EC 1.11.1.6 & 2.1.2.1) were common for both the catalyzed reaction from the rumen and the rectum. The methanogen species abundances from these study areas in numbers/kind can be utilized exclusively or jointly as indirect selection criteria for methane mitigation. This calls for interdisciplinary cohesion and collaboration for fruitful achievements. Studies should be carried out to taxonomically categorize species missing out of place. Furthermore, every part of the gut (either fore or the hindgut) was capable of hosting methanogens. Targets for methanogens should entirely be towards the whole gastrointestinal tract. Furthermore, there is a need to target functional genes of the microbes and those of animals to achieve a friendly environment without affecting the animal’s functionality. Animals that are less methane emitters should be bred to cut on their methane release from the gut. Further studies should be carried out to target pathways for tolerable methane emitter dairy cattle without hindering other necessary metabolic processes. The fraction of the methanogens that are yet to be fully classified should be carried out and a thorough study of their temperature, substrate(s), and pH should be noted.
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Author contributions FMK, RCB, and CKG conceived and designed the experiment. FMK conducted the experiment. FMK and DKN analyzed the data. DKN wrote the manuscript. All authors read and approved the manuscript.

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Availability of data and materials Most data that were generated and/or analyzed during the study are included in the article that has been published and those that are contained in the supplementary files. More data related to this article can be viewed via https://www.mcg-rast.org/linking.cgi?project=mgrp81260.

Declarations

Conflict of interest The authors of this piece of work declare no conflict of interest.

Research involving human and animal rights This research study was performed under the University of Nairobi (UON), Faculty of Veterinary Medicine Institutional Animal Care and Use Committee (IACUC) accepted procedures. Experimental animals were handled professionally while exercising restraint to reduce on any discomfort and injury.

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