Influence of Host and Geographical Regions on the Rumen Methanogens Diversity in Indian Cattle and Buffaloes

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Abstract

Background Archaea belong to the domain Euryarchaeota, represents only 3-5% of the rumen microbiota; however, they play a central role in maintaining the H₂ pressure. Host species in conjunction with geographical regions may lead to the variable distribution of rumen methanogens. Indian cattle and buffaloes represent 13 and 53% of the global population, respectively. However, the influence of host species (bovine) and geographical regions on the methanogens distribution has not been investigated in Indian subcontinent. Therefore, the present study was undertaken to explore the rumen methanogens diversity in the cattle and buffaloes native to the two distinct geographical regions of India.

Results Next generation sequencing of 16S rRNA gene from 36 rumen fluid samples corresponding to cattle and buffaloes revealed that *Methanobrevibacter* (*Mbr.*) genus constituted largest fraction (55-62%) of the rumen archaeal community. Furthermore, *Methanobrevibacter gottschalkii* was the most prominent methanogens with the significantly higher abundance in cattle. *Methanomassiliicoccales* represented <10% of the total archaeal community and was found to be the most variable between these two host species. In north region, the distribution of *Methanomassiliicoccales*, such as *Methanosphaera sp.*, *Group 4*, *Group 8*, *Group 12 sp.* and *Group 9 sp.* was significantly higher in cattle. The distribution of prominent methanogens, such as *Mbr. gottschalkii* and *Mbr. ruminantium* did not differ between two host species in the north region. However, *Mbr. gottschalkii* was significantly higher in buffaloes as compared to cattle in south region. *Mbr. wolinii* was exclusively detected in the cattle. Inter-region comparison established that *Methanomassiliicoccales* were the most variable between the two regions.

Conclusions Host species did not affect the distribution of hydrogenotrophic methanogens except for *Mbr. gottschalkii*. Methylophilic methanogens, in spite of their limited representation, were most influenced by the host species and geographical regions. Overall, the *Methanomassiliicoccales* distribution was higher in cattle, and the environmental conditions in north region were conducive for their higher distribution. The variable distribution of methanogens indicated that host and geographical region oriented strategies needs to be developed for the reduction of enteric methane emission. Further studies are warranted to explore the impact of diet on the distribution of rumen methanogens between the host species within and across different environmental conditions.

Background

After CO₂, methane is the second most significant greenhouse gas [1] present in the atmosphere at a concentration of about 1830 ppb and increasing with an average of 10 ppb per year [2]. Agriculture sector annually contributes 188 Tg to the anthropogenic methane emission. Enteric fermentation is one of the largest sources of methane emission among the agriculture sector; wherein global livestock emits 80–90 Tg methane per year. Worldwide the respective contribution of cattle and buffaloes to the annual enteric methane emission is estimated 72.42 and 11.05 Tg. A recent report [3] from the country estimated annual enteric methane emission from the Indian cattle and buffaloes as 4.92 and 2.91 Tg, respectively. Thus, these two host species remained major emitters aggregately contributing 33.1% of the global enteric

methane emission. In spite of representing one of the production inefficiencies, methanogenesis is generally referred as a wasteful but necessary process in the rumen. Methanogenesis act as a sink for fermentative H_2 produced in the rumen. Rumen harbours millions of anaerobic microbes, which accomplish the feed fermentation and aid in removing waste products. Archaea belong to the domain Euryarchaeota represents only 3–5% of the rumen microbiota [4, 5]; nevertheless, their role in maintaining the H_2 pressure low within the desirable limits through methanogenesis is incredible [6, 7]. Unlike bacteria, archaea lack peptidoglycan in their cell wall [8]. A unique characteristic of methanogens is the presence of coenzyme F_{420} [9], which mediates redox transformations essential for the catabolic pathways [10]. Rumen methanogen community is complex and their substrate requirement is different as majority of them utilize carbon dioxide and formate; while others use methylated compounds for producing methane. Till date, about 155 species of the methanogens associated to six orders and twenty-nine genera have been isolated from different ecosystems [11]. However, only seven species of methanogens have been isolated so far from the rumen ecosystem [12]. It has been reported that the isolated methanogens from the rumen represents < 10% of the archaeal community [13]. Understanding of the rumen microbiome particularly of archaea is critical in order to develop effective strategies for enteric methane amelioration. Recent culture-independent studies have confirmed that the rumen methanogenic community is complex and a large fraction of it has not been cultured yet and thus remains unidentified [14, 15]. Recent metagenomics approaches enabled rapid community analysis without introducing the cultivation bias or primers associated variations [16, 17]. Recent next generation sequencing techniques certainly improved our understanding about the diversification and the metabolic capacity of the methanogenic archaea in the rumen [18–20].

Cattle and buffaloes with a corresponding number of 195 and 110 million, aggregately constitute more than half of the total livestock population in the country. India alone harbours about 13% and 53% of the world's cattle and buffalo population, respectively [21]. Cattle and buffaloes are accounted for 85% of the annual enteric methane emission in India [3]. A recent comparison among distantly located geographical regions revealed that livestock in north and south region respectively contribute to about 37 and 17% of the enteric methane emission [3]. Host species in conjunction with environmental conditions and distinct feeding practices could be accountable for the variation in the regional enteric methane emission from the livestock. Effect of host on the rumen microbiota establishment has been reported [22] and previous reports suggest an impact on the archaeal community composition [23–26]. Further, the body size of the host species also influences the abundance of rumen microbiota [27]. As buffaloes are generally heavier than the cattle in India, a variable archaeal community structure between cattle and buffaloes may exist. Moreover, the environmental conditions prevailing in different geographical regions influence the rumen archaeal community composition [28]. Two distinct geographical regions considered in the present study experience variable climatic conditions as north India encounters a cool and dry weather in winter and hot in summer; while relatively high temperature & humidity are the common features of south India weather. Present study was undertaken aiming to compare the rumen archaeal community structure between cattle and buffaloes belonging to the two distinct north and south geographical regions of the country. We hypothesized that the rumen archaeal community structure is controlled by the host species and

geographical locations. Although cattle and buffaloes significantly contribute to the food basket of animal origin and methane emission; nevertheless, there is no existing report, where the rumen archaeal community structure between these two important bovines considering the impact of distinct geographical locations has been investigated in Indian context.

Methods

Collection of rumen fluid samples

A total 36 rumen fluid samples were collected from the cattle (N = 19) and buffaloes (N = 17) native to two distinct geographical region of India. Uttar Pradesh, Punjab and Gujarat represented the north region; while south region was represented by the Karnataka and Tamil Nadu states (Fig. 1). About 50 mL of the rumen fluid samples were collected from the individual cattle and buffalo in each region. The samples were filtered through double layer of muslin cloth and placed in a box containing dry ice. Thereafter, the rumen fluid samples were transported to the laboratory and processed for the genomic DNA isolation.

Dna Isolation

To allow the sedimentation of dissolved micro feed particles, the rumen fluid samples were initially centrifuged (Kubota 6500, Japan) at 1500 rpm for five minutes. The supernatant obtained from the short spinning of rumen fluid samples was then transferred to another tube. About 2 mL supernatant was taken into an Eppendorf tube and centrifuged at 13400 rpm for 10 minutes at 4 °C. A thick pellet obtained by the centrifugation was retained while the supernatant fraction was carefully removed. Subsequently, 1 mL lysis buffer was added, and then pellet was dissolved through gentle pipetting. The mixed content was transferred to a 2 mL sterile screw cap tube containing 0.5 g (0.1 mm) pre-sterilized zirconia beads (Biospec USA). Repeat bead beating plus column method was followed for genomic DNA isolation in the present study [29]. The QIAamp DNA mini kit (Qiagen, Germany) was used as per the manufacturer's instructions. Quality of genomic DNA was checked using 0.8% agarose gel electrophoresis; while the concentration of DNA was confirmed with Qubit 4.0 (Invitrogen). Genomic DNA was used for generating 2 × 300 bp amplicons using Illumina MiSeq platform.

Library Construction And Sequencing Of 16s Rrna Gene

Amplicon libraries were prepared using Nextra XT kit (Illumina Inc.). Archaea specific primers *Arch-344F* 5'ACGGGGYGCGAGCAGGCGCGA 3' (Wemheuer *et al.*, 2012) and *Arch-806R* 5'GGACTACVSGGGTATCTAAT 3' (Takai and Horikoshi, 2000) were used for the amplification of V3-V5 regions of 16S rRNA gene. Illumina i5 and i7 adapters were added to the primers for generating the amplicons. Amplicon libraries were purified by AMPureXP beads (Beckman Coulter Life Sciences, USA) and analyzed on 4200 Tape Station system (Agilent Technologies, USA) using D1000 Screen Tape station. Approximately 10–20 pM of each library was loaded onto the MiSeq platform for cluster generation and sequencing.

Bioinformatics Analysis

Raw paired end reads were imported to the QIIME2 [30] for analysis. The reads were processed using the *q2-dada2* plugin. The DADA2 pipeline was used for detecting and correcting the amplicon sequence data, joining of paired end reads and removal of chimeric sequences. Thereafter, quality filtered non-chimeric sequences were clustered into amplicon sequence variants (ASVs) with 100% similarity. Representative sequences were classified using RIM-DB archaea specific database [31] with *q2-feature-classifier* plugin. Multiple sequence alignment and masking of the conserved regions of the representative sequences was performed using *q2-alignment* plugin. The filtered alignments were used for the construction of phylogenetic tree using *q2-phylogeny* plugin. The plugin *q2-diversity* was used for alpha and beta diversity estimates. Alpha diversity measures, such as Shannon index, Pielou's evenness, and Beta diversity indices, such as Bray-Curtis and UniFrac distances were calculated using the rooted phylogenetic tree and a sampling depth corresponding to the lowest number of sequences in any sample. The significance of the alpha diversity was estimated using the Kruskal-Wallis statistical test, whereas, beta-diversity significance was determined using the Permutational multivariate analysis of variance (PERMANOVA). Further, the OTU table was imported into MicrobiomeAnalyst [32] for differential abundance analysis. Sequences with low abundance were filtered out using default parameters (minimum count of 4 in at least 20% samples and interquartile range of 10%). Differential abundance was performed using metagenomeSeq [33] with zero-inflated Gaussian mixture model (*fitZig*), and a *P* value < 0.05 was considered statistically significant. The co-occurrence analysis was performed using the CoNet 1.1.1 tool in Cytoscape 3.7.0 [34]. The correlations with a Fisher's Z score *P* value threshold of 0.05 were considered as statistically significant. Heatmap of the significant taxa at genus level was constructed using ClustVis tool [35]. Core microbiome analysis was performed using microbiome V1.4.2 package [36] with 50% minimum prevalence for different geographical regions at genus and species level.

Results

A total of 11.3 million paired end reads were generated from 36 rumen fluid DNA samples collected from cattle (N = 19) and buffaloes (N = 17) native to two distinct geographical regions of India. The corresponding paired end reads in cattle and buffaloes were 6.99 and 4.31 million, respectively. Following the stringent quality filtering, about 1.55 million sequences (Table 1) were retrieved from both the cattle (0.8 million) and buffaloes (0.74 million). The sample wise filtering details and number of sequences retained at each step are provided in Additional file 1, Table S1. Rarefaction curve for Shannon index and observed OTUs confirmed that the archaeal diversity was adequately covered (Additional file 1, Fig. S1a and b). The non-chimeric sequences were clustered into 2,583 ASVs.

Table 1
Summary of raw and filtered sequences

Host species	Region	Raw sequences	Filtered sequences	% Filtered sequences
Cattle	North	4551909	445492	9.79
	South	2445566	363215	14.85
Total		6997475	808707	11.56
Buffaloes	North	2984245	457068	15.32
	South	1331570	287090	21.56
Total		4315815	744158	17.24
Total (Cattle + Buffalo)		11313290	1552865	13.73
Filtering of reads involved quality based filtering, denoising, merging of paired end reads and removal of chimeric sequences.				

The current study presents the findings on the comparative analysis of methanogen diversity in cattle and buffaloes at the country as well as intra- and inter-region level.

Country Level Diversity

Results from the study confirmed that the methanogens belonging to *Methanobacteriales*, *Methanomicrobiales* and *Methanomassiliicoccales* orders were present in the rumen of cattle and buffaloes (Fig. 2). Among the *Methanobacteriales*, *Methanobrevibacter* (*Mbr.*) constituted largest fraction of the rumen archaeal community in both cattle (≈ 55%) and buffaloes (≈ 62%), although the difference in its abundance between the two bovine species was statistically insignificant. *Mbr. gottschalkii* clade was the most prominent species in both cattle and buffaloes, however, their abundance was significantly higher ($P = 0.015$) in cattle. Methylophilic methanogens (*Methanomassiliicoccales*) represented approximately 2–4% of the total rumen archaeal community; nevertheless, they were significantly different between cattle and buffaloes (Additional file 2). Particularly, the abundances of *Group 8* and *Group 12* genera were significantly higher in cattle ($P = 0.0011$ and 0.0002 , respectively), whereas, the distribution of *Methanomicrobium* (*Mm.*) *Methanobacterium* (*Mb.*) was higher in buffaloes, but not statistically significant. Among the *Methanobrevibacter* species, *Mbr. wolinii* was exclusively identified in the cattle. As expected, a large fraction of the rumen archaea (20–30%) remained unclassified in the current study.

Inter-region Diversity

Similar to the country level, *Methanobrevibacter* was the most dominant genus in the cattle from both north and south regions (Fig. 3a and Additional file 3), although their distribution was statistically

insignificant. However, *Mbr. gottschalkii* clade was significantly higher ($P < 0.00016$) in the cattle from the southern region. The diversity of methanogens affiliated to *Methanobacterium*, *Methanomicrobium* and *Methanosphaera* was not significantly different in cattle across the geographical regions. Moreover, the distribution of methylotrophic methanogen species, including *Group 4* ($P = 0.001$), *Group 8* ($P = 0.011$), *Group 10* ($P = 0.001$) and *Group 9* ($P < 0.001$) was higher in the cattle from the northern region (Additional file 2). *Mbr. wolinii* and *Group 12 sp.* ISO4 H5 methanogens were exclusively identified in cattle from the south and north regions, respectively (Fig. 3a).

Comparative analysis of methanogen diversity between the two geographical regions revealed a significantly higher abundance ($P = 0.024$) of *Methanobacterium* in the buffaloes from the southern region. Unlike in cattle, the *Group 12* affiliated methanogens were identified in the buffaloes of both the regions (Fig. 3b and Additional file 3), but with a significantly higher ($P < 0.001$) abundance in the northern region (Additional file 2). The distribution of prominent rumen methanogen species, such as *Mbr. gottschalkii* clade, *Mbr. ruminantium* clade, and *Mb. alkaliphilum* was not significantly different between the two regions (Additional file 2). In contrast to cattle, the distribution of *Mm. mobile* was significantly higher ($P = 0.022$) in buffaloes from the southern region. Similarly, the abundance of *Group 4 sp.* MpT1 was higher in the buffaloes of southern region ($P = 0.001$), whereas, that of *Group 12 sp.* ISO4 H5 was more in the northern region ($P = 0.017$).

Intra-region Diversity

Comparison of methanogen diversity between the bovine species within the geographical regions indicated a higher abundance of *Methanobrevibacter* ($P = 0.0003$) and *Methanomicrobium* ($P = 0.0002$) in buffaloes in the northern region. However, *Group 8* ($P = 0.003$) and *Group 9* ($P = 0.009$) were comparatively higher in cattle (Fig. 4a and b, Additional file 2). Furthermore, at species level, the distribution of *Methanosphaera sp.* ISO3 F5, *Group 4* MpT1, *Group 8*, *Group 12 sp.* ISOH5 and *Group 9 sp.* was higher in cattle than buffaloes (Fig. 4a and b, Additional file 2). On the contrary, the prominent methanogen species, such as *Mbr. gottschalkii* clade and *Mbr. ruminantium* clade did not differ significantly between the two host species within the geographical regions.

Unlike in the north region, *Group 4* was the only genus that showed significant differential abundance between cattle and buffaloes ($P = 0.013$) in southern region (Fig. 4b, Additional file 2). Furthermore, in the southern region, *Mbr. gottschalkii* clade had a higher abundance ($P = 0.035$) in cattle than in buffaloes, whereas, the abundance of *Group 10 sp.* was two times more in the buffaloes ($P = 0.0047$).

Methanogens, such as *Mbr. wolinii* and *Group 9 sp.* were exclusively present in the cattle; while *Group 12 sp.* ISO4 H5 was exclusive to buffaloes from the southern region. However, all the identified methanogen genera and species in the northern region were found in both cattle and buffaloes. The abundance of methanogens between the two regions and host species has been presented in Additional file 1, Figure S2 and S3, respectively. Additional file 4 and 5, respectively provide the number of sequences classified at genus and species level for the individual samples.

Core Microbiome Analysis

Core microbiome analysis revealed that five genera were shared between cattle and buffaloes at a minimum detection threshold of 0.001%. The prevalence of *Group 4*, *Group 9* and *Methanimicrococcus* was observed only in cattle (Fig. 5a). *Mbr. gottschalkii* clade, *Mbr. ruminantium* clade and *Mb. alkaliphilum* were the most prevalent species in both cattle and buffaloes (Fig. 5a). Furthermore, the core microbiome analysis showed *Methanobrevibacter* and *Methanobacterium* to be prevalent in cattle and buffaloes across northern and southern regions (Fig. 5b-e). Interestingly, *Methanomassiliicoccales* related methanogens were not prevalent in south buffaloes (Fig. 5e), whereas, the prevalence of *Methanosphaera* was not observed specifically in the northern counterpart (Fig. 5d). At species level, *Mb. alkaliphilum* and *Mm. mobile* were core microbiome members in both the bovine species and regions except in south cattle and south buffaloes, respectively.

Diversity Index

Alpha diversity for methanogen abundance assessed through Shannon index/ Pielou's evenness for country, inter-region and intra-region analysis has been presented in Fig. 6. Shannon diversity index (Fig. 6a-c) revealed a significant difference in the methanogen abundance at country level ($P = 0.014$), inter-region ($P = 0.002$) and within the geographical region ($P = 0.001$). Similarly, the Pielou's evenness analysis (Additional file 1, Table S2) established a significant difference (Fig. 6d-f) in the methanogen diversity in bovine species between the northern and southern regions ($P = 0.008$) and across the bovine species between the two regions ($P = 0.0005$).

The beta-diversity analysis showed a distinct pattern for the clustering of samples from northern and southern regions on qualitative (Fig. 7a) and quantitative (Fig. 7b) distance matrices. Furthermore, the samples from the same species (cattle or buffalo) were more closely clustered.

Network Analysis

Network analysis (Fig. 8a-d) revealed a strong correlation across the different genera of the rumen methanogens. In the northern region (Fig. 8a), *Group 9* and *Group 4* were positively correlated ($r = 0.93$, $P = 7.56E-06$) in cattle; whereas, *Group 9* showed a negative correlation with *Methanobrevibacter* ($r = -0.7$, $P = 0.010$). *Methanomicrobium* and *Group 8* were positively correlated ($r = 0.96$, $P = 1.66E-06$) in buffaloes (Fig. 8b); however, *Methanomicrobium* and *Methanobrevibacter* were negatively correlated ($r = -0.93$, $P = 2.02E-05$) within the northern region. A positive correlation between *Methanimicrococcus* and *Group 4* ($r = 0.95$, $P = 4.41E-06$) as well as *Group 4* and *Methanomicrobium* ($r = 0.87$, $P = 5.83E-04$) in cattle was evident in southern region (Fig. 8c and d). Furthermore, a negative correlation between *Methanomicrobium* and *Methanobrevibacter* was reported in buffaloes ($r = -0.94$, $P = 5.68E-05$). Heatmap also established a similar correlation among the methanogens between the two host species and regions (Fig. 8e).

Discussion

Methane emission is one of the metabolic inefficiencies which takes away a considerable fraction of the feed energy. About 39.5 KJ of the energy is wasted for each litre of enteric methane emission from the ruminants [37]. Microbiota-host relationship is complex and varies from synergistic to competitive [22]. The host species can affect the rumen archaeal community structure [23–26]. However, in spite of representing two major methane emitting species, the comparative diversity analysis of methanogens between cattle and buffaloes has not been extensively investigated. There is only one report where methanogen abundances between cattle and buffaloes were compared [38]. Community structure analysis from their study revealed a significantly higher abundance of *Methanobrevibacter* genus in Jersey cows than in water buffaloes. In addition, geographical location is known to influence on the methanogen community structure [9]. An analysis of 742 samples across 32 species and 35 countries confirmed that rumen archaea are less diverse than the bacteria [5]. The archaeal community was dominated by the members of *Mbr. gottschalkii* and *Mbr. ruminantium* clade along with *Methanosphaera* sp. and two *Methanomassiliicoccales*; they aggregately represent ~ 90% of the total archaea in the rumen. The abundances of these five dominant methanogens differed among the species [5]. Although India holds 13 and 53% of the global cattle and buffalo population, respectively [21]; they were not included in the study carried out by [5]. Therefore, the present study was aimed at comparing the rumen archaeal community structure between two host species (cattle & buffaloes) and across geographical regions (north & south) of India.

Our study revealed that the distribution of dominant methanogen genera i.e. *Methanobrevibacter*, *Methanobacterium*, *Methanomicrobium* and *Methanosphaera* was comparable between the two host species at the country level (Fig. 1; Additional file 2). Methanogens affiliated to *Methanobrevibacter* genus dominated and constituted ~ 55–62% of the rumen archaeal community in cattle and buffaloes; however, its abundance was significantly higher ($P = 0.0003$) in buffaloes. These findings reaffirm the previous reports on the dominance of *Methanobrevibacter* methanogens in cattle [39, 40] and buffaloes [41, 42]. A meta-analysis of archaeal 16S rRNA sequences available in GenBank [43] supports our results for the similar distribution of *Methanobrevibacter* in cattle and buffaloes. The higher abundance of *Mbr. gottschalkii* could be attributed to the higher proportion of straw in the diet of cattle. In India, cattle are poorly fed and comparatively inefficient milk producer than the buffaloes [44] which leads to the higher distribution of *Mbr. gottschalkii* through higher H_2 concentration and acetate/propionate (A/P) ratio [38, 45]. The difference in the distribution of *Mbr. gottschalkii* in the rumen of cattle and buffaloes was not equally dispersed in both the geographical regions and was more pronounced in the southern region. This could be due to the difference in climatic conditions as north India generally experiences cool and dry winters and hot summers; while the temperature is higher with humidity throughout the year in southern region. Further, the ruminants in southern region are mostly fed on the dry roughages, which may lead to the higher distribution of *Mbr. gottschalkii*. It has been reported that in addition to the geographical locations, feed restriction/under feeding [46] also leads to the higher abundance of *Mbr. gottschalkii*. In the present study, *Mbr. wolinii* was exclusively distributed in cattle. Earlier studies also confirmed the

presence of *Mbr. wolinii* in the rumen of adult cattle [47–49]; while it remains unreported in the buffalo rumen [42,50, Malik et al., 2020, unpublished observations]. These findings suggest that the host species in conjunction with the geographical region and diet variation can affect the prevalence of some of the *Methanobrevibacter* species.

The abundance of *Mm. mobile* did not differ between the two host species at the country level (Fig. 1; Additional file 2) as well as within the geographical regions (Fig. 3a-3b; Additional file 2). The distribution frequency of *Mm. mobile* in cattle and buffaloes was in consonance with the global data sets [5, 12, 49] but there is a disagreement with previous reports of unprecedented higher distribution of *Methanomicrobium* in cattle [51, 52] and buffaloes [53–55]. As these studies used PCR and cloning based approach; the primer bias could be one of the reason for the disagreement in *Methanomicrobium* abundance. A narrow difference in the distribution of *Mm. mobile* in cattle and buffaloes established that the host species has little impact on its abundance, limited to < 10% of the total rumen archaea.

In the present study, *Methanomassiliicoccales* were significantly different between the host species and geographical regions (Fig. 1-3b; Additional file 2). *Methanomassiliicoccales* were previously reported in the rumen [26,56,57, Malik et al., 2020, unpublished observations] and their abundance frequency in this study are within the reported range of < 10% [24, 57]. Until recently, the *Methanomassiliicoccales* order was placed under the RCC (rumen cluster C) and *Methanoplasmatales* [58–60]. *Methanomassiliicoccales* accomplish the reduction of methanol and methyl amines mediated by H₂, as they lack the genes required for first six steps in hydrogenotrophic methanogenesis [61–63]. This study established that the distribution of *Methanomassiliicoccaceae* members, such as *Group 8* ($P=0.001$) and *Group 12* ($P=0.0002$) was significantly higher in the cattle than in buffaloes. In an another study, *Group 10*, *Group 11* and *Group 12* were identified in New Zealand cattle and sheep [57]; whereas, in the current study, *Group 4*, *Group 8*, *Group 9*, *Group 10* and *Group 12* were identified in both the host species and geographical regions. However, the presence of *Group 11* could not be confirmed in the rumen of cattle and buffaloes in the present study. Our results indicated that in spite of the small representation of *Methanomassiliicoccales* in the rumen archaeal community, they were more diversified in Indian ruminants than that of the New Zealand. A meta-analysis [43] strengthened our results on the higher abundance of *Methanomassiliicoccales* in cattle as compared to buffaloes. Though the methane producing ability of the *Methanomassiliicoccales* members has not yet been explored; their high relative abundance, i.e., up to 50% of the total rumen archaea [24, 57], is a clear indication of their possible involvement in rumen methanogenesis. Thus, the correlation between the abundance of *Methanomassiliicoccales* members and their methane producing ability warrants further investigation. In addition, non-availability of type strain hinders our understanding of the taxonomy, substrate requirement, and growth of *Methanomassiliicoccales* methanogens.

Findings from the current study established that the geographical region in conjunction with the host species has a higher influence on the abundance of *Methanomassiliicoccales* than the hydrogenotrophic archaea. The difference in the abundance of *Methanomassiliicoccales* between cattle and buffaloes could be due to the variable production of methylated substrate compounds in the rumen, which may be

mediated through diet [64]. In addition, the differences in the environmental conditions influence the archaeal community structure [28]. A study from [27] in heifers concluded that the elevated temperatures along with high humidity significantly affects the rumen microbiota composition through the alteration in A/P ratio. High environmental temperatures result in less rumen motility, which is known to increase the dry matter digestibility by prolonging the retention time of feeds in the rumen [65, 66]. The climatic conditions of the two geographical regions; southern region (temp. 31°C, humidity 78%) is generally hotter than the northern counterpart in India (temp. 28.5°C, humidity 59%), may have contributed to the variable abundance of archaeal community structure in the current study. It is noteworthy that large size animals are more sensitive to the elevated temperature due to less dissipation of heat, which influences the abundance of rumen microbiota [27]. Hence, larger body size and weight of both the host species in the northern region may explain the higher abundance of *Methanomassiliicoccales*. However, the impact of increasing temperature and humidity on the distribution of various archaeal groups need further investigation.

Network analysis revealed both negative and positive correlations among different genera of methanogens in both the host and geographical region. Low interaction among the genera in buffaloes from south region was obviously due to the absence or relatively less abundance of many methanogens.

Conclusions

The core methanogens were similar between cattle and buffaloes, however, the overall archaeal community structure was influenced by the host species and geographical locations. Further, the archaeal community was dominated by *Methanobrevibacter* associated methanogens and the distribution of most prevalent *Mbr. gottschalkii* was significantly influenced by the host species in southern region. The distribution of *Methanomassiliicoccales* was significantly affected by the host species and geographical regions. Our study provides insights into the archaeal distribution in Indian cattle and buffaloes and established that the *Methanomassiliicoccales* due to their high variability can be one of the targets for achieving reduction in enteric methane emission. Further studies are warranted to determine the methane producing potential of *Methanomassiliicoccales* and explore its role in rumen methanogenesis.

Abbreviations

A/P- Acetate to propionate ratio

ASV- Amplicon sequence variants

F₄₂₀- 8-hydroxy-5-deazaflavin coenzyme

GOI- Government of India

Mb- Methanobacterium

Mbr- Methanobrevibacter

Mm- Methanomicrobium

NCBI- National Centre for Biotechnology Information

NGS- Next Generation Sequencing

OTU- Operational Taxonomic Unit

PERMANOVA- Permutational multivariate analysis of variance

ppb- parts per billion

RCC- Rumen cluster C

RIMDB- Rumen and Intestinal Methanogen Database

Declarations

Ethical approval

The study was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 25/8/ 2016-CPCSEA part-I) to collect the rumen fluid samples. All the procedures were followed as per the Institute Animal Ethics Committee guidelines while collecting the samples.

Consent for publication

Not applicable

Availability of data and material

The datasets generated and/or analyzed during the current study are available in the NCBI Sequence Read Archive (SRA; <https://www.ncbi.nlm.nih.gov/sra>) repository with accession numbers SAMN15083329-SAMN15083364 under BioProject PRJNA636770.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

PKM, APK, AD and RB designed the study. ST and TG collected the rumen fluid samples from cattle and buffaloes. ST, SV and TG performed DNA extraction, down processing and other laboratory work. ST, APK and VSK performed analysis of sequencing data. PKM supervised the findings of this work. RB arranged the financial support and provided logistics support for the study. All authors read and approved the final manuscript.

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Figures

Figure 1

Species and region-wise rumen fluid collection

Figure 2

Comparative abundance of the rumen methanogens at (a) genus and (b) species level

Figure 3

Inter-region abundance of the rumen methanogens in (a) cattle and (b) buffaloes. SR: Southern region; NR: Northern region.

Figure 4

Relative abundance of rumen methanogens in (a) northern and (b) southern regions. NC: North cattle; NB: North buffalo; SC: South cattle; SB: South buffalo.

Figure 5

Core microbiome analysis at genus (left) and species (right) level.

Figure 6

Alpha diversity indices. Shannon diversity index at country, region and intra-region levels is presented in a, b and c, respectively. Figures d, e and f represent the Pielou's evenness at country, region and intra-region levels, respectively.

Figure 7

Beta diversity matrices. Principle coordinate analysis (PCoA) based on (a) unweighted UniFrac and (b) Bray-Curtis distance matrices. Color indicate the region and shape indicate the host species. red = north, blue = south; buffalo = sphere, cattle = star

Figure 8

Network analysis of the methanogens representing the correlation across different genera. Correlation network of archaeal genera in (a) cattle and (b) buffalo from northern region. Correlation network of archaeal genera in (c) cattle and (d) buffalo from southern region. e, Heatmap of archaeal genera across all bovine samples. Copresence and mutual exclusion between the archaeal genera is represented with green and red edges, respectively. The edge thickness represents increasing correlation coefficient or weight (correlation coefficient threshold = 0.3). Node size indicates abundance of archaeal genera.

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