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Chapter

Methanogenic Diversity and Taxonomy in the Gastro Intestinal Tract of Ruminants

Farah Naz Faridi and Saba Khan

Abstract

To elucidate the microbial dynamics inside rumen of animals of livestock importance and to provide a better ration to them in order to control various metabolic disorders, a better understanding of the rumen microbial ecology is pivotal. The fundamental knowledge of methanogenic population inside gut environment and ruminal fermentation is of considerable importance as it has a significant impact on the various metabolic activities of the animal. The major methanogens isolated and characterized from ruminants like cattle, sheep, steers, goats, reindeers are from the order *Methanobacteriales*, *Methanomicrobiales*, *Methanococcales*, *Methanosarcinales* and *Methanomassiliicoccales*. The chapter deals with present knowledge available regarding the methanogenic diversity present in the gastro-intestinal tract of ruminants all over the world primarily through constructing 16S rRNA gene clone libraries and tries to uncover the new genera in ruminant's microbiome and their adaptations in extreme environment. To get a better idea regarding the composition of methanogen community, further studies are required in relation to the effect of diet and animal species to the rumen methanogens.

Keywords: *Archaea*, gut, methanogens, microbiome, *Methanobrevibacter* spp., rumen

1. Introduction

The methanogens are one of the primitive life forms on earth which have evolved to be able to thrive in extreme harsh temperatures (severe hot and cold) and living conditions (salt and pH) uninhabitable for most of other life forms. Although a vast proportion of methanogens are *Archaea* but protists like algae, fungi and protozoa also form a diversity of this group. Besides their anthropogenic existence, methanogens are present in a wide area of ecological niches ranging from peat bogs to deep sea sediments and hydrothermal vents and hot springs [1, 2].

The large number of microbial population in natural anaerobic systems remains unexplored as enumeration techniques like selective enrichment, pure-culture Isolation, most-probable number estimates are time consuming and labor intensive. Culture less approaches has allowed deciphering the diversity of microbial community thriving across wide environmental ranges. Various anaerobic culture

techniques led to the discovery of a third microbial kingdom, the *Archaebacteria*, which includes methanogens [3, 4]. Further the target specific sequence analysis of 16S rRNA gene in 1970's had redefined taxonomy of all living organisms into three main domains. Methanogens belong to the 3rd domain of life-*Archaea*, other two being—*Eucarya* and *Bacteria*. *Archaea* is further divided into phylums *Crenarchaeota* and *Euryarchaeota* [5].

2. Major rumen microbes

At any time there are billions of any species of anaerobic bacteria and facultative anaerobic bacteria residing in rumen along with a mixed population of various anaerobic protozoa, anaerobic fungi and flagellates making it a diverse microbial consortium in nature. The bacteria along with protozoa make most of the microbial mass (nearly 80%) inside rumen. The bacteria present in specialized niches are a very small fraction that cannot be recovered by cultural methods and even among cultivable bacteria true number of diversity is now revealed only by molecular techniques [6]. The bacteria can further be cellulolytic (fiber digesting), amylolytic (starch and sugar digesting) and lactate utilizing bacteria. The role of symbiotic microbial ecosystem consisting of bacteria, protozoa and fungi is of great significance in ruminants. Phylum Euryarchaeota within domain *Archaea* includes 7 orders—*Methanobacteriales*, *Methanomicrobiales*, *Methanococcales*, *Methanopyrales*, *Methanocellales*, *Methanosarcinales* and *Methanomassiliicoccales*. The orders are further divided into 10 families and 31 genera [7–9].

3. Methane production in ruminants and its contribution to greenhouse gases

Methane is a main byproduct of digestion in ruminants produced by the microbial fermentation of plant biomass. Methanogens ferment the ingested feed into short chain fatty acids which consists of 70% of the total metabolizable energy source for ruminants. The methane is specifically produced by methanogens (*Archaea*) that resides symbiotically in the gut of ruminants by using hydrogen produced by bacteria, fungi and protozoa and reducing CO_2 to methane. It is not used by ruminants and is lost in environment through eructation resulting in a loss of 2–12% of metabolic energy intake to the host [10, 11]. Among agricultural sources, enteric fermentation along with natural and man-made wetlands, animal wastes; paddy fields contribute to the release of major amount of methane in environment. Methane gas has a major global warming impact [12]. According to the fifth assessment report of Intergovernmental Panel on Climate Change (IPCC) published in 2014, global release of greenhouse gases from enteric fermentation grew from 1.4 to 2.1 GtCO2eq/yr between 1961 and 2010. The largest methane emission was by cattle (75% of the total) followed by goat, sheep and other ruminants during the year 2000–2010 [13]. The enteric fermentation in ruminants is a significant cause of methane emission in environment. It is an inevitable outcome of their normal digestive process [14], which is not used by them and is lost in environment. Since, methane is a potent greenhouse gas, to reduce the activity and number of methane producing *Archaea*, it is desirable to have knowledge about the community structure of methanogens and their feed conversion energy mechanism. In order to control various ruminal disorders the insight into microbial ecology will help to develop nutrition and feed management strategies.

4. Methanogenic archaeal population in gastro-intestinal tract of ruminants

The rumen was the initial environment of *Archaea* which is comprehensively investigated and studied. Hungate [15] reported that about 23 bacterial species played prominent role in ruminal metabolism whereas in 1996 the number increased up to 200 [16]. The culture based techniques had serious limitations as they failed to differentiate between two phylogenetic diverse species along with the dire need of maintaining anaerobic environment to culture and isolate bacteria. The 16S rRNA sequencing technology has been far and wide used to explore the methanogens residing inside rumen and to characterize and validate their community structure and taxonomic composition in evolutionary timeline. The methanogenic group in gastrointestinal tract of ovine, caprine and bovine using rRNA targeted oligonucleotide probes were identified and *Methanobacteriales* were reported to be the abundant methanogens in bovine and caprine rumen whereas *Methanomicrobiales* was found to be predominant in ovine rumen [17]. In 2000, the population of methanogens among rumen microbial diversity of sheep in Japan was reported using 16S rDNA cloning and fluorescence in situ hybridization (FISH) technique and most of the clones were found associated with *Methanomicrobium mobile, Methanobrevibacter ruminantium* and *Methanobrevibacter smithii*. The total methanogens accounted for 3.6% from the total microorganisms present in rumen and population of *M. mobile* among methanogens was found to be 54% [18]. A year later the archaeal libraries generated from the rumen of dairy Holstein cows from Japan revealed two groups of sequences produced from two different sets of archaeal primers. The library generated from primers-D30 and D33, revealed 21% of clones related to *M. mobile* and 79% of clones were anaerobic digester associated archaeal sequences with close identity to *Thermoplasma*. The second library generated from 0025e and 1492 primers showed 56% of the clones related to *M. mobile*, 20% related to the *Thermoplasma* associated sequences and 16% related to *Methanobrevibacter* spp. and 2 sequences were related to the unidentified rumen *Archaea* [19].

Similarly in bovine rumen, 41 cloned sequences were identified in 3 clusters. The largest cluster contained 24 clones with 2 distinct sub clusters with sequences affiliated with *Mbb. ruminantium*. The sub cluster Mbr I contained nine 16S rDNA sequences that had 98.5–98.8% sequence identity to *Mbb. ruminantium* whereas the sub cluster Mbr II contained 15 cloned sequences that had 97.2–97.7% similarity to *Mbb. ruminantium* whereas the second cluster contained 11 cloned sequences having similarity values of 96.1–97.5% to *Methanosphaera stadtmanae*, an organism first time recognized in rumen. The third cluster was found containing 6 cloned sequences that were 89% similar to *Methanosarcina* sp. str. WH_1 and *Methanosarcina thermophila* indicating it to be comprised of a novel group of rumen methanogens [20]. In Japan, clones were deduced from bovine rumen that was 83.9–88.3% identical to *Mbb. ruminantium* [21]. In 2004, the archaeal populations from three fractions of rumen-rumen fluid, rumen solid and rumen epithelium from Korean Hanwoo cattle was constructed using 16S rDNA gene clone libraries. Species belonging to the family *Methanomicrobiaceae* were found dominant in fractions of fluid and epithelium in rumen while *Methanobacteriaceae* was abundant in solid fraction of rumen [22]. The *Methanomicrobium* phylotype was the most abundant phylotype among methanogenic population in rumen of Murrah buffaloes from India as revealed by constructing 16S rDNA gene library. A total of 15 phylotypes out of 17 were affiliated to *M. mobile*; one sequence was identical to *T. acidophilum* and one sequence with *Methanocorpusculum bavaricum* [23]. *Methanobacteriales* was

a dominant order identified from the rumen of Surti buffaloes in India by cloning and sequencing of *mcr*A gene while in an another study on Murrah buffaloes 100% sequence similarity was reported by two isolates to *Mbb. smithii* and 100% sequence similarity by one isolate to *M. mobile* based on 16S rRNA [24, 25].

5. Effect of diet on diversity of rumen methanogens

The rumen is a dynamic system therefore the microbes must change qualitatively and quantitatively in response to the changes in the chemical composition of diet of animal rather than geographical location in general. Wang et al [26] reported members of the order Rumen Cluster C (RCC) to be most abundant ruminal methanogen present in cattle from China fed agricultural residues like corn stover, rapeseed and cottonseed meals followed by the order *Methanobacteriales*. By constructing a gene clone library of *mcrA* gene, they found that by increasing the agricultural residues in diet of cattle, the methanogen community structure did not change however methane production was increased. The effect of diet on rumen methanogen population has also been studied in Western Australia where sheep were fed different diets. Analysis revealed that archaeal diversity in sheep from grazing pasture was more as compared to sheep fed forage diets-oaten hay or lucerne hay. The maximum numbers of clones identified were from *Methanobrevibacter* strains SM9, M6, and NT7 [27].

A corn and cottonseed diet of cattle from Jinnan region of China also reported members of *Methanobrevibacter, Methanobacterium*, *Methanosphaera, Methanomicrobium* and unidentified Euryarchaeota. Overall, *Methanobrevibacter* spp. appeared to be predominant in all three rumen fractions [28]. Similarly, methanogenic population in dairy cattle from Canada was estimated that were fed diets supplemented with enzyme additive by PCR-DGGE and quantitative real-time PCR (qRT-PCR) analysis. The PCR-DGGE profiles were made up of 26 different bands, with two bands affiliated to Methanogenic archaeon CH1270 and one band to *Mbb. gottschalkii* strain HO. Three bands similar to Methanogenic archaeon CH1270 or *Mbb. smithii* ATCC 35061 appeared after enzyme was supplemented [29]. The diversity of rumen methanogens present in Mediterranean water buffaloes from Brazil which were maintained on three different diets-corn silage (library 1), pasture grazing (library 2) and sugar cane (library 3) revealed all three 16S rRNA clone libraries to be consisted of *Methanobrevibacter*-related sequences. The abundance of *Methanobrevibacter* like sequences in water buffaloes was in contrast to previous reports that showed *M. mobile* like methanogens to be predominant *Archaea* isolated from water buffaloes of Murrah and Surti breeds from India [30]. The taxonomy and structure of methanogens in Swedish dairy cattle fed two different diets through clone library consisted by terminal restriction fragment length polymorphism (T-RFLP) showed the genus *Methanobrevibacter* to be dominant in rumen and that the diet may not be an obvious factor affecting the community composition of methanogenic population inside rumen but may give an insight to the structure of ruminal methanogens [31].

Another study on sheep in Queensland, Australia in 2006 revealed 78 clones of 26 different methanogen related sequences were obtained. Eight sequences consisted of 15 clones were found 95–100% similar to the orders *Methanobacteriales* and *Methanomicrobiales*, and rest 18 sequences consisted of 63 clones were 72–75% affiliated to *Thermoplasma acidophilum* (*T. acidophilum*) and *Thermoplasma volcanium* (*T. volcanium*) [32]. The structure of archaeal diversity in feedlot cattle (starch based diet) from two different provinces of Canada-Ontario and Prince Edward Island, were deduced by constructing a clone library of 241 sequences.

Eleven phylotypes (38 clones) in cattle from Ontario region (corn-based diet) were unique to this group as they were not found in cattle from Prince Edward Island. Similarly, 7 phylotypes (42 clones) from Prince Edward Island cattle (potato by-products) were found only in this group whereas 5 sequences representing 161 clones were found common in both herds. Out of 23 different sequences obtained, 10 sequences consisting of 136 clones were 89.8–100% affiliated to the species of the orders *Methanobacteriales*, *Methanomicrobiales* and *Methanosarcinales* and remaining 13 sequences consisting of 105 clones showed 74.1–75.8% sequence similarity to the species *T. volcanium* and *T. acidophilum* [33]. The dominance of total rumen *Archaea* from different ruminant species around the world in a global data set report surveying nine studies assessed that genus *Methanobrevibacter* (61.6%), *Methanomicrobium* (14.9%) and uncultured species from Rumen Cluster C (15.8%) constituted 92.3% of total rumen *Archaea* [34]. Another study from Venezuela indicated *Methanobrevibacter* phylotype to be the most abundant genera in 14 different 16S rRNA gene sequences or phylotypes from 104 clone library constructed in sheep [35]. The rumen of Sika deer fed oak leaf diet and corn stalk diet from China revealed thirty six OTUs assigned to 146 unique sequences and in both the diet group, genus *Methanobrevibacter* was detected as a predominant methanogen. Among the species, *Mbb. millerae* was most abundant in both groups but accounted for a slightly higher population (69.5%) in corn stalk library than in oak leaf library (51.4%). Clones with similarity to *Mbb. smithii* like clones and *Mbb. ruminantium* like clones were present in corn stalk library but were absent in oak leaf library [36].

The majority of sequences were related to genera *Methanobrevibacter* and *Methanosphaera* and a group of novel uncultured methanogens "uncultured marine bacteria" were identified in Moxoto breed goats from Brazil by constructing 16S rRNA gene clone libraries [37]. Likewise, the archaeal methanogen population inside rumen of lactating Jersey and Holstein cattle fed same diet from America revealed species level similarity to *Mbb. ruminantium* [38]. The community structure of methanogens inside rumen of farmed sheep, cattle and red deer which were fed different diets revealed diet and host based differences in framing community structure, but the presence of dominant archaeal species was uniform in all host animals. The dominant members were from following clades: RO clade-*Mbb. ruminantium* and *Mbb. olleyae*, SGMT clade-*Mbb. gottschalkii, Mbb. millerae and Mbb. thaueri* and species of the genus *Methanosphaera* [39].

6. Methanogen diversity inside rumen and/feces under similar conditions of diet

The sequences obtained from rumen and feces of local sheep from Xinjiang, China were divided into three groups based on their affiliation to the following genera: *Methanobrevibacter*, *Methanocorpusculum* and an unclassified methanogen-like group [40]. Order *Methanobacteriales* was found to be dominant in rumen of faunated and unfaunated Holstein cattle from Japan by constructing clone libraries from 16S rDNA gene and mcrA gene [41]. The methanogenic archaeal population in sheep of Scottish uplands were illustrated by Snelling et al. [42] by different methods-Sanger amplicon sequencing by constructing 16S rRNA gene libraries, 16S rRNA gene amplicon sequencing by Illumina, Illumina metagenome sequencing. All the methods revealed the order *Methanobacteriales* containing genera: *Methanobrevibacter*, *Methanosphaera* and *Methanobacteria* to be the most abundant. Among the *Methanobacteriales* order, *Mbb. millerae* comprised of ≥91% of OTU's and remainder of the OTU's were formed by *Methanosphaera*.

Tymensen and McAllister [43] reported the archaeal spp. linked with ruminal protozoa in cattle and obtained 276 final sequences generated from clone libraries using five diverse universal archaeal primer pairs and found that the three genera/taxa viz. *Methanobrevibacter*, Rumen Cluster C (RCC) and *Methanomicrobium* accounted for 94–100% of the sequences in each library. Metatranscriptomics approach-Illumina deep-sequencing with overlapping read paired-end technology revealed that *Bacteria* and *Eukaryotes* contributed to the majority of ribotags (approximately 50%) whereas *Archaea* contributed only 1% of ribotags mainly comprised of the order *Methanobacteriales* (*Methanobrevibacter* and *Methanosphaera*) and *RCC Thermoplasmata*. The *RCC Thermoplasmata* lowered down considerably on rape seed oil (RSO) supplementation whereas *Methanobacteriales* did not show any decrease. A notable decrease in the mcrA and mcrB transcripts of RCC on change in was noticed suggesting the reduced CH_4 emissions [44].

The abundance of two archeal orders-*Methanobacteriales* and *Methanomassiliicoccales* in rumen of sheep and cattle from New Zealand were studied. From the order *Methanobacteriales*, sequences were assigned to only four species—*Mbb. gottschalkii*, *Mbb. ruminantium*, *Methanosphaera* sp. ISO3-F5 and *Methanosphaera* sp. group5. The members of the order *Methanomassiliicoccales* contributed 10.4% of the total relative abundance of the methanogenic archaeal community, *Methanobacteriales* (89.6%) being dominant [45]. The methanogenic *Archaea* in yak from China grazing on natural pastures exhibited the species of the family *Methanobacteriaceae* to be predominant in yak rumen followed by members from the family *Methanomassiliicoccaceae* and *Methanosarcinaceae* [46].

The archaeal methanogenic community from rumen of two indigenous ruminant species-yak and Tibetan sheep and two introduced species-cattle and crossbred sheep in Qinghai-Tibetan plateau from China under similar diet of oaten hay and barley and environmental conditions revealed the more archaeal diversity in indigenous species than in introduced species. *Methanomassiliicoccaceae* was predominant family representing most of the sequences while *Methanobacteriaceae* was second most dominant archaeal family. Among *Methanobrevibacter* genus, *Mbb. gottschalkii* and *Mbb. ruminantium* were the most observed species. Interestingly, *Mbb. woesei* and *Mbb.* sp. RT were only found associated with yak rumen [47]. Salgado-Flores et al. [48] reported archaeal methanogenic density by quantitative real-time PCR and diversity from rumen and cecum samples of Norwegian reindeer fed on standard pellets and lichens by 454 pyrosequencing of 16S rRNA genes. The population density of archaeal methanogens remained almost constant for both the diets in rumen and cecum samples. In rumen samples, *Methanobrevibacter* was found to be main genus and strain *Mbb. thaueri* CW to be predominant in both groups fed different diets. *Mbb. wolinii* SH was second most abundant species found in group fed pellet based diet whereas constituted only 1.5% of the total sequences in group fed lichens. The second most prevalent species was *Mbb. ruminantium* strain M1 in reindeers fed lichens but accounted only 4.2% of the total sequences in pellet fed group of reindeers. In cecum samples also, genus *Methanobrevibacter* was detected predominantly in both the groups. *Mbb. millerae* strain ZA-10 was found to be most abundant in reindeer group fed with pellet but had less than 97% similarity with this archaeal methanogen whereas strain *Mbb. thaueri* CW was main species in lichen fed group with 98% similarity. Franzolin and Wright reported that the density of archaeal methanogens was very low as compared to bacterial counterparts in grazing and feedlot group of buffaloes from Brazil. The density of methanogens as compared to bacteria in reticulum was more as compared to rumen [49].

The rumen methanogenic structure in three Indian cattle and buffaloes which were fed on wheat straws based diet using RT-PCR revealed most abundant orders

of *Methanomicrobiales* and *Methanobacteriales* along with total bacteria and that it remained constant for two animals using a particular diet [50]. Similarly, the ruminal diversity in Indian Murrah buffaloes by using amplified ribosomal DNA restriction analysis (ARDRA) maintained under standard diet of wheat straws revealed a total of 108 clones that were classified into 16 phylotypes. The 9 phylotypes showed less than 97% sequence similarity to any of the cultivated methanogen strain and represented a novel uncultured group of methanogens. The second group comprised of 4 phylotypes that showed 92–99% sequence similarity with *M. mobile*. The third group consisted of a single phylotype clustered with *M. burtonii*, reported for

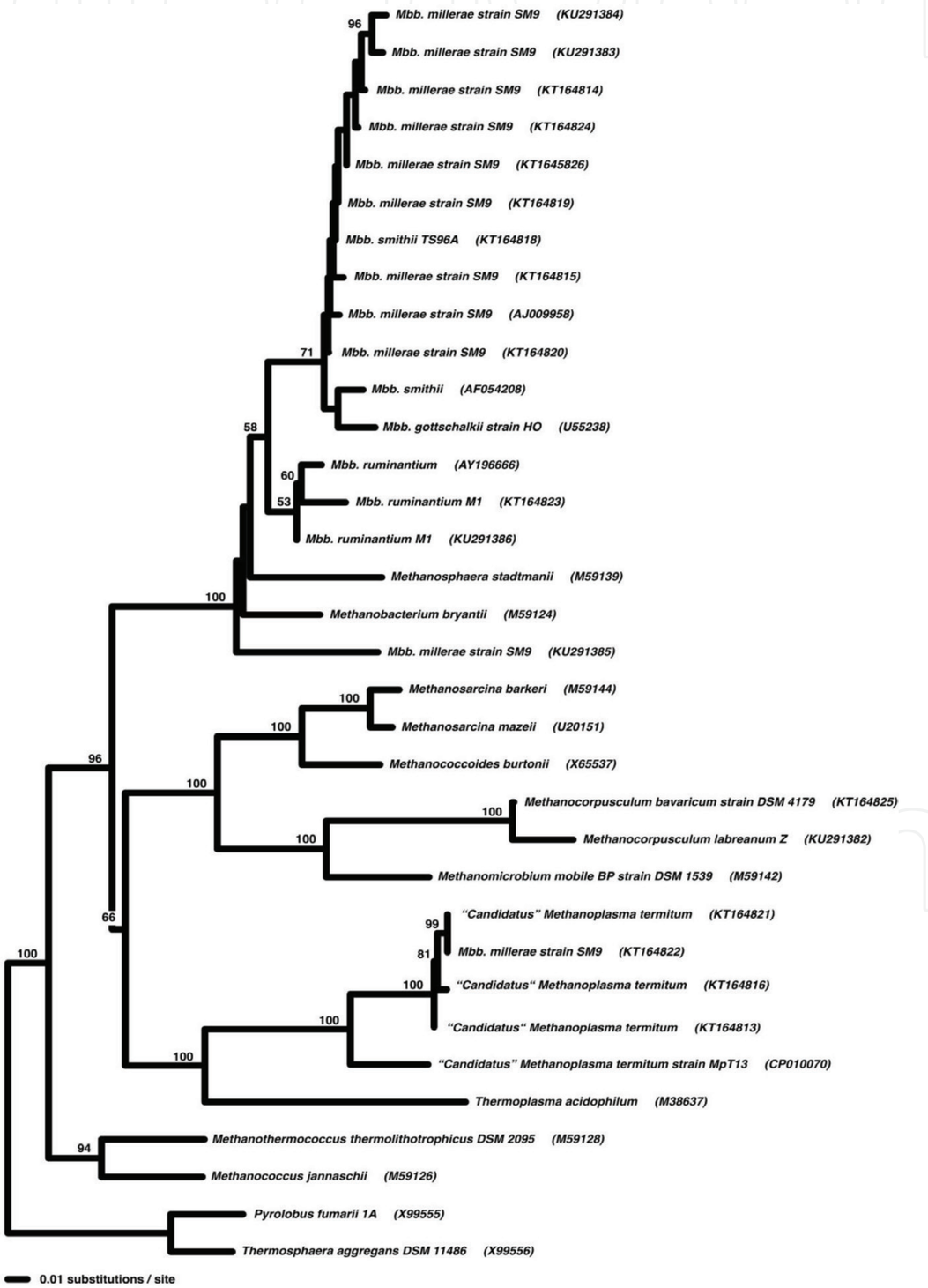

Figure 1.
A phylogenetic tree based on 16S rRNA sequences obtained from camel foregut and reference sequences downloaded from NCBI Genbank database [58].

the first time in rumen. The fourth group was a single phylotype that showed 97% sequence identity with *Mbb. gottschalkii*. The last group of single phylotype showed a sequence similarity to *Mbb. ruminantium* [51].

Likewise, the comparative diversity analysis of methanogens using 16S rRNA and *mcr*A in cattle rumen fed on a high fiber diet reported 13 OTU's consisting of 102 clones from 16S rRNA gene based library. All OTU's were clustered with order *Methanobacteriales* and were further splitted into Cluster I that had 12 OTU's related to *Methanobrevibacter* spp. and Cluster II comprised of one OTU related to *M. stadtmanae* [52]. The Surti buffaloes that were fed wheat straw and compound concentrate mixture diet generated a total of 76 clones representing 21 sequences based on PCR-RFLP patterns. BLAST analysis revealed 13 OTU's (55 clones) that showed sequence identity with *Methanomicrobium* sp., 3 OTU's (15 clones) that showed sequence similarity with *Methanobrevibacter* sp. The remaining 5 OTU's (6 clones) were associated with uncultured *Archaea*. Overall, the methanogenic population inside rumen of buffaloes was from the order of *Methanomicrobiales* (18 OTUs) and *Methanobacteriales* (3 OTUs) [53]. The rumen metagenome of buffalo using q-PCR were compared with MG-RAST based annotation of the metagenomes sequences of 16S rDNA amplicons and high throughput shotgun sequencing and found *Methanomicrobiales* in lower number [54] (**Figure 1**).

7. Methanogenic archaeal population in pseudo ruminants like camelids

Gut methanogens remains largely uncharacterized in camel with no published studies on methanogenic archaeal populations from 16S rRNA gene clone libraries whereas much interest has been paid to domestic ruminants. The community diversity and structure of archaeal methanogens in fecal samples of Bactrian camel (*Camelus bactrianus*) maintained at two zoos from United States of America revealed the genus *Methanobrevibacter* to be the abundant ruminal methanogen however the diversity and structure of methanogens varied significantly between the two libraries with only 2 OTU's in common to both the libraries. Two and seven OTU's were found unique to first and second library, respectively [55]. The methanogenic archaeal population inside rumen of Alpaca (*Vicugna pacos*) from America resulted in a 947 non chimeric gene clone library representing 51 distinct OTU's. Thirty seven OTU's displayed ≥95% genus-level sequence affiliation with the species belonging to *Methanobrevibacter*. Six out of 37 OTU's showed ≥98% species-level sequence identity to *Mbb. millerae*; 2 OTU's showed species-level identity to *Mbb. ruminantium*; 2 OTU's showed >98% identity to *Mbb. smithii*; 27 OTU's showed 95–97.9% sequence similarity to well detected and reported *Methanobrevibacter* species. Of the remaining 14 OTU's, 3 distinct phylogenetic group were made that consisted of 4 OTU's that had 95–97.9% similarity to the species of *Methanobacterium*; other 3 OTU's showed genus level similarity with the species *of Methanosphaera*; 7 OTU's were found to be isolated phylogenetically from order *Methanobacteriales*. Overall, *Methanobrevibacter* was found to be dominant in alpaca rumen like other ruminants but in contrast as described in other ruminants *Mbb. millerae* was found to be in most number of clones showing species level identity [56]. The fecal microbiome of camels maintained at intensive and extensive system of management in Jaisalmer (Rajasthan) was evaluated through non-cultural approach. The both group's fecal metagenomes were compared with available fecal or rumen metagenomes on MG-RAST and *Mbb. smithii* was detected as a predominant archaeal methanogen [57]. A 16S rRNA gene clone library from the content of the C1 compartment (foregut) of Indian camels was constructed by cloning pooled

polymerase chain reaction (PCR)—amplified products. The sequences (n = 151) were clustered into 15 OTU's (operational taxonomic units) based on sequencing of unique RFLP pattern and divided into five species groups: *Methanobrevibacter* (*Mbb.*) *millerae* strain SM9, "*Candidatus*" Methanoplasma termitum, *Mbb. smithii*, *Mbb. ruminantium*, *Methanocorpusculum* (*M.*) *bavaricum* strain DSM 4179. The genus *Methanobrevibacter* (order *Methanobacteriales*) was the most prevalent (76.82%), followed by *Archaea* from the orders *Methanomassiliicoccales* (17.21%) and *Methanomicrobiales* (5.96%) [58] (**Figure 1**).

8. Biotechnological applications of extremophiles

The microbial diversity of extremophiles is of interest particularly for microbiologists and biotechnologists to decipher the enzymes and their functions, their biochemical and metabolic pathways that enable them to survive in harshest conditions. The in depth knowledge will pave the path for creating technologies that can function under extreme conditions. It will improve our current knowledge and perception about the interrelationships between various species and will continue to lead to the classification and assessment of ruminal archaeal species.

For researchers working to explore the microbial ecology of volcanic systems, deep under the earth, oceans, thermal vents, rice fields, waste treatments, bioremediation of soils, the rumen forms a stable and basic source of knowledge concerning anaerobic microorganisms. The knowledge of anaerobic microorganism's reaction going inside rumen flora is of invaluable importance as methanogens are also found in omnivores and humans alike and can be implicated in understanding human and animal diseases. An extensive understanding of methanogens in gastrointestinal tract will contribute to the sustainable farming of animals well into the future. The enteric fermentation in ruminants is a significant cause of methane emission in environment. Since, methane is a potent greenhouse gas, to reduce the activity and number of methane producing *Archaea*, it is desirable to have knowledge about the community structure of methanogens and their feed conversion energy mechanism. In order to control various ruminal disorders the insight into microbial ecology will help to develop nutrition and feed management strategies and also to develop better prospects of altering rumen function to mitigate methane generation while still optimizing digestibility and microbial function. This can be particularly useful for the farmer community who can benefit environment in methane mitigation from livestock at the same time increasing animal efficiency. Reductive acetogenesis is performed by acetogenic bacteria that thrive in non-ruminants and can sometimes replace methanogenesis. A comparative account of dominant methanogens in the ruminants all over the world is depicted in **Figure 2**.

The significance of exploring the archaeal diversity lies in its great potential to identify the genes encoding plant degrading enzymes, thus contributing to an increase in understanding of the mechanisms mediating digestion in ruminants. Moreover, the functional analysis of these genes might uncover strategies for improving feed and fiber digestion in the rumen that could further be applied to manipulate pathways associated with bioreactor processes for biofuels production and to formulate feed with dietary additives that help in reducing methane emissions. A taxonomic frame of methanogens should be developed that would help elucidate the diversity, identification and classification of major rumen archaeal population. Data from antibiotic resistance genes and RATC (resistance to antibiotics and toxic compounds) can be also used to produce antibiotic resistance gene profiles to help in understanding of the microbial community ecology in every environment.

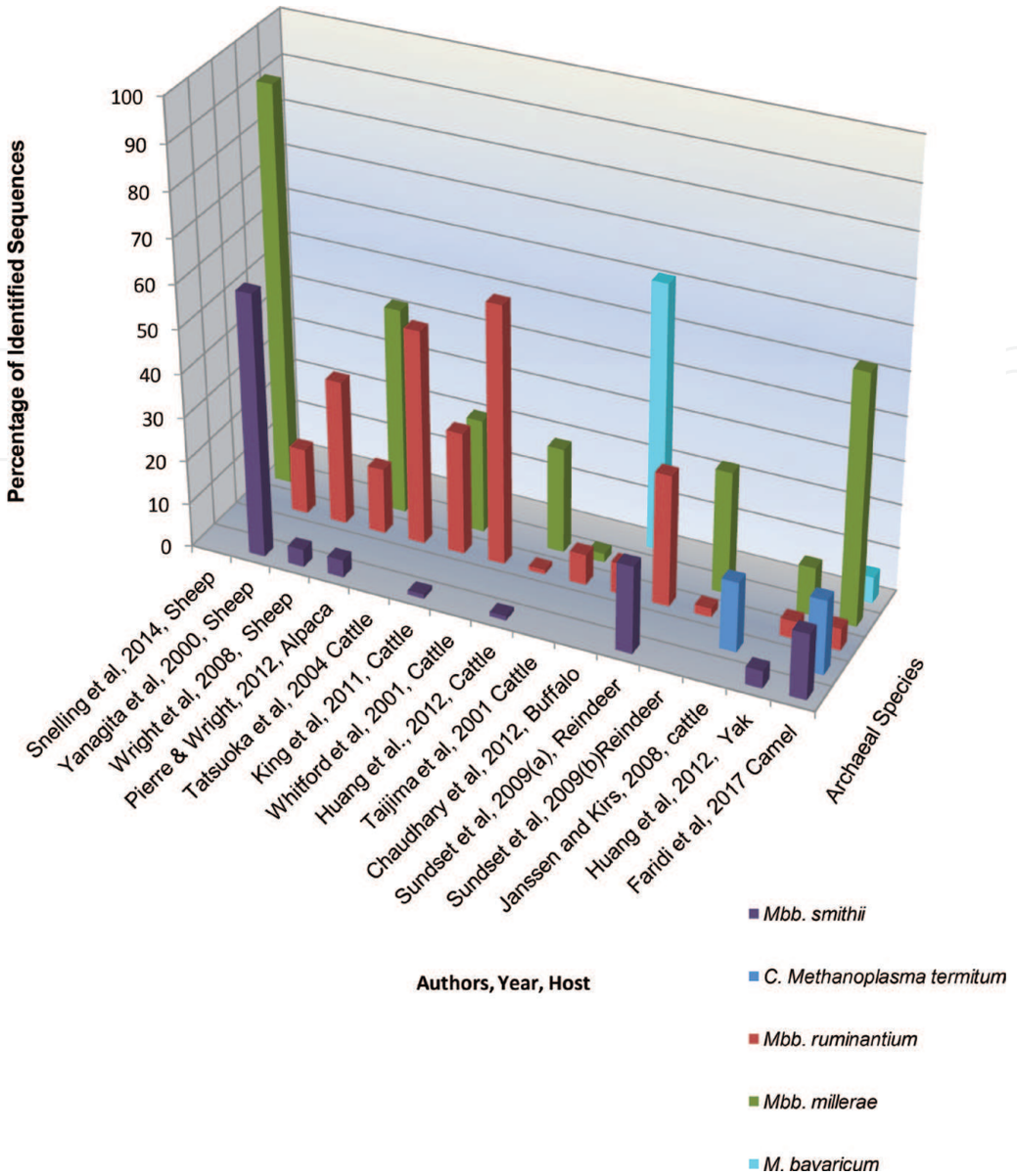

Figure 2.
Methanogenic sequences identified in ruminants around the world.

One can exploit enzymes from extremophile *Archaea* that can endure high temperatures and organic solvents. Acidophiles are used in coal mining to recover metallic minerals and to reduce sulfur levels. Alkaliphiles are used in paper making and spilled oil recovery, besides being used as a common ingredient in dish washing detergent and laundry soap. *Thermus aquaticus* an extremophile that endures high temperature produces an enzyme called *Taq* polymerase that has transformed molecular biology all over the world by aiding in quick DNA replication during polymerase chain reaction (PCR). The extremophiles are immensely used in medical and food microbiology, industrial fermentations to produce acetone, butanol, etc. The understanding of microbial diversity in extreme habitats like wetlands can propose research strategies and priorities to integrate understanding of plant-microbial interactions. Further, studies should provide the break through to link distribution and distinctiveness of various gastrointestinal microbes in their natural environment and to discover their genetic potential for livestock wellbeing and industrial progress by making a significant contribution in understanding ruminant nutrition. Research in microbial genomics will provide the opportunity to make sure that this knowledge is used to enhance ruminant production through an improved understanding of microbial function and ecology.

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