SUMMARY

The digestive anatomy of camel has evolved differently from ruminants and therefore shares only some of the anatomical features and digestive physiology with them. Interestingly, it is supposed to emit low to unnoticeable amount of methane than true ruminants yet sustained to produce and reproduce while surviving on low and poor quality forage. The structure and diversity of archaeal methanogens has been intensively investigated among ruminants of livestock importance but petite knowledge is available about the archaeal community composition of vital desert animal- camel. Methane is emitted by ruminants as an outcome of the enteric fermentation process performed by methanogenic community. The enteric fermentation in ruminants is a noticeable cause of methane release in environment. Methane emission from ruminants as a part of their standard digestive process is a second largest and significant anthropogenic source of releasing potent greenhouse gas in atmosphere accounting for nearly one quarter of the methane release from human activities and accumulating 100 million tons a year. Thus it has a significant impact on climate change. Despite having 25 times more effect than CO₂ over a 100 year time period, its contribution to global warming is still underrated.

The traditional approach of culturing methanogens to classify them is not adequate enough owing to their high number and considerable diversity. Culture dependent approach also misses many of the strict anaerobic microorganisms. Determining methanogenic diversity through culture less approach is a major tool and has very fast magnified the possibility of detecting the evolutionary relationship among archaea. A genetic marker like 16S rRNA has
widely been used as a standard housekeeping target to study the methanogenic phylogeny and taxonomy in wide environmental areas.

The research objective of this study was to identify and characterize the methanogenic archaeal population residing inside the C1 compartment (foregut) of camels from India by constructing 16S rRNA gene clone library. The methanogenic gene specific primers were used to selectively amplify 16S rRNA gene from rumen samples and gene of interest was cloned into vector and transformed into bacterial cells. The transformed colonies were screened by blue- white screening, colony PCR and restriction digestion. Clones with unique RFLP patterns were sequenced and assigned to OTUs. Phylogenetic and evolutionary analysis was performed. The unique sequences were submitted in GenBank, NCBI database and were assigned accession numbers from KT164813- KT164816, KT164818- KT164826 and KU291382- KU291386. A total of 157 sequences were deduced which comprised into 18 OTUs. The salient findings of this research are listed as follows:

- The largest group was found to be of 91 clones comprising of 10 OTUs with similarity ranging ≥ 97% to *Mbb. millerae* strain SM9, a sp. from order Methanobacteriales accounting for 57.96% of the total clones.
- The second largest group had 26 clones comprising of 3 OTUs which were 93% identical to *Candidatus* Methanoplasma termatum accounting for 16.56% of the total clones.
- The third group belonged to 13 clones falling within 1 OTU showing 93% identity to *Mbb. smithii* (Methanobacteriales) making 8.28% of the total clones.
- The fourth group accounted for 13 clones in 2 OTUs having ≥ 97% sequence similarity to *Mbb. ruminantium* (Methanobacterales) representing 8.28% of the total population.

- The fifth and sixth group accounted for 9 and 5 clones comprising of 1 OTU each showing 97% and 99% identity to *Methanocorpusculum bavaricum* strain DSM 4179 and *Methanocorpusculum labreanum* Z, respectively and contributing 5.73% and 3.18% of the total clones.
CONCLUSION

The phylogenetic analysis reveals that out of 157 clone sequences, 117 belonged to the order Methanobacterales, concluding the species of this order to be most abundant phylotype in camel’s gastro intestinal tract under normal diet and environmental conditions. The members of this order remains to be the most recovered species in other livestock ruminants as well from various regions of the world. *Candidatus* Methanoplasmata termitum is a species of newly discovered order Methanomassiliicoccales previously known as Rumen Cluster C (RCC) which is a group of novel uncultured archaea. It was found to be the second most predominant order in camel rumen and consisted of 26 clones. The third order Methanomicrobiales was comprised of only 14 clones out of 157. Many authors from India have reported the order Methanomicrobiales to be the most abundant in cattle and buffaloes. This shift from Methanomicrobiales to Methanobacterales in dromedary camel particularly in Indian subcontinent might have arisen due to different dietary conditions, less food intake, adaptation of camel in extreme climatic conditions with low forage availability. Also the evolution of digestive system of camel is different from other ruminants and feed retention time in foregut of camels is more than true ruminants. Owing to these differences, the majority of archaeal population inside foregut of camel from India is of *Methanobrevibacter* species and not *Methanomicrobium* species.
FUTURE WORK

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. This work might help to develop and design methane mitigation strategies and to explore possible future advancements in improvement of ruminal fibre digestibility. This study might help to develop a taxonomic frame of methanogenic population that helps 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.

The work will result in steadfast insights into the camel's gastrointestinal microbial structure and activity of the rumen microbes in relation to useful interactions, chronological and spatial relationships among diverse conglomerate of microbes and dietary ingredients.