Chapter 1: Introduction

1.1. Classification of an indispensable animal in fragile desert eco-system

In the early 20th century camel was classified basically as ride and pack camel. Since this large even toed ungulate (Artiodactyla) is a most significant part of rural farming in Africa and Asia, it is further classified into dairy, meat, dual purpose and racing camel. The family Camelidae consists of two groups: Old World Camels (OWCs) comprising of a genus Camelus with two species included: Camelus dromedarius (Linnaeus, 1758), the dromedary, one humped or the Arabian camel; and Camelus bactrianus (Linnaeus, 1758), the bactrian, two humped or the Central Asian camel. The second group being of New World Camels (NWCs) comprising of two genera: Llama and Vicugna, and four species: the Llama (Lama glama; Linnaeus, 1758), the Alpaca (Vicugnapacossyn. Lama pacos; Linnaeus, 1758) which are domesticated, the Guanaco (Lama guanaco; Müller, 1776) and the Vicuna (Vicugna vicugna; Molina, 1782) which are wild (Mason, 1979; Novoa, 1989; Stanley et al., 1994). The New World Camels are also known as South American Camelids (SACs) owing to their origin and present distribution.

The clear origin of dromedaries is unclear but domesticated form originated some thousands of years ago in North Africa and Middle East (Groves et al., 2005) and most of them are now present in Horn of Africa,
North African countries, the Arab peninsula and some countries of Asia (Al-Tarazi, 2001). The Australia is a home to the largest introduced feral herd of dromedaries in the world today. Nearly 3, 80,000 of the total world’s camel population reside in India (FAOSTAT, 2014). The Indian camel population is mostly confined to the north western dry part of the country. According to the 19th livestock census (2012), about 0.4 million camels form a very important part of fragile desert ecosystem of Rajasthan, a part of the great Thar desert (Fig. 1). The dromedary camel (Camelus dromedarius) is a unique and interesting animal regarded as a “Gift of God”. They are vitally important in desert ecosystem due to their ability to withstand extreme temperatures and are bred as working animals. They are used for transportation and for production of meat, milk, wool and leather. Even their droppings are used for fertilizer and dried for cooking fuel, making them an important animal in the livestock industry. In India, camels are mostly used for draught and riding purposes, though camel milk is also utilized as a by-product (Bulliet, 1975).

Fig.1: Herd of camels maintained at National Research Centre on Camel, Bikaner (Rajasthan).
1.2. The Stomach complex and mechanism of digestion in camel

Ruminants represent a successful and diverse group of herbivores on earth today spending a large amount of time in chewing cud, a small ball of partially digested plant material that they have consumed but have not completely digested it. They have “eat now, digest later” strategy as their grazing is constantly hindered by attacks of predators and other disturbances.

Camel is classified as “pseudo-ruminant” as its digestive anatomy evolved separately from ruminants in the suborder Pecora (Bohlken, 1960). The camel has three distinct forestomachs (Compartment- C1, C2 and C3) compared to four present in ruminants and also lacks the omasum of true ruminants. Despite having little resemblance to the rumen, reticulum, omasum and abomasum of true ruminants (Arnautovic, 1997) camel still ruminates and shares some of the anatomical features and digestive physiology with true ruminants.

There are remarkable differences in anatomy and physiology of digestion in camel that makes it pseudo-ruminant and justify the separate classification of Tylopoda from Ruminantia. The C1 compartment is the largest of three, an anaerobic fermentative chamber holding nearly 80% of the total gastric volume. The huge amount of plant feed and forage eaten by animal is broken into absorbable nutrients by the help of diverse microbial community that includes bacteria, archaea, protozoa, fungi and bacteriophages. They degrade cellulose into carbohydrates which is unless broken down is impossible to utilize by the animal. The ingesta remain in
somewhat drier and fine form in the caudal and cranial portion of C1 compartment, respectively. The cud is regurgitated from C1. The compartment C1 ends into smaller C2 which holds 6% of the total gastric volume and is a reservoir for liquid digesta that enters from C2 and empties itself into C3 compartment through a short muscular tube. The C3 compartment occupies 11% of total gastric volume lying in the lower right abdominal wall. The ability of highly efficient digestive system of camel lies in its anatomical feature where two microbial compartments both in beginning and end are available for digestion. The symbiotic bacteria in rumen also warrants the complete breakdown of plant material through anaerobic fermentation into short chain volatile fatty acids (VFAs) such as acetate, butyrate, propionate, valerate, lactate along with vitamins and microbial proteins- the absorbable nutrients of bacterial fermentation and gases like carbon dioxide, methane and ammonia are also released.

The unique digestive system in camel and its ability to extract out the nutrition efficiently from the plant feed and forage enables it to utilize the scanty feed resources available and survive the extreme temperatures in dry areas.

1.3. 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 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. 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 (Hobson and Stewart, 1997). The bacteria can further be cellulolytic (fibre 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.

1.4. Methane production in ruminants and its contribution to green house 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 methanogenic 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 (Johnson and Johnson, 1995; Kumar et al., 2009). Among agricultural sources, enteric fermentation along with natural and man-made wetlands, animal wastes; paddy fields contribute to the release and accumulation of major amount of methane in environment. Methane gas has a
major global warming impact (Broucek, 2014). According to the fifth assessment report of Intergovermental Panel on Climate Change (IPCC) published in 2014, global release of green house gases from enteric fermentation grew from 1.4 to 2.1 GtCO\(_2\)eq/ 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 (FAOSTAT, 2013).

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

The methanogens are oxygen intolerant group of archaea that produce methane as a final outcome of their anoxic respiration (Stewart et al., 1997). Forming nearly 3% of total microbes, they live symbiotically with bacteria and some live endosymbiotically with anaerobic protozoa. Ninth edition of Bergey’s manual of Determinative Bacteriology classifies bacteria into 35 groups under 4 major categories- Gram –ve Eubacteria with cell walls, Gram +ve Eubacteria with cell walls, Eubacteria lacking cell walls and Archaeabacteria (Methanogens). Methanogens belong to the 3rd domain of life- Archaea, other two being- Eukarya and Bacteria. Archaea is further divided into phylums Crenarchaeota and Euryarchaeota (Woese et al., 1990). Methanogens belong to the phylum Euryarchaeota and are subdivided into 7 orders- Methanobacteriales, Methanomicrobiales, Methanococcales, Methanopyrales, Methanocellales, Methanosarcinales and Methanomassiliicoccales. The orders are further divided into 10 families and 31 genera (Liu and Whitman, 2008; Sakai et al., 2008; Paul et al., 2012).
1.6. Importance of studying the methanogenic archaeal diversity in camel

The camel plays a significant role in developing social and economic infrastructure of countries with large dry areas by surviving on scarce and limited resources. The dromedary camel has adapted to the harshness of desert areas for comparatively prolonged periods and effectively uses less forage available there. Interestingly, camel is thought to emit lower quantities of methane than true ruminants (Dittmann et al., 2014). Many studies have been published that revealed the gut microbiology of camel but these studies are limited in characterizing its bacterial diversity only (Ghali et al., 2004; Bouraoui et al., 2011; Samsudin et al., 2011, 2012; Gharechachi et al., 2015). The diversity and structure of archaeal methanogens was illustrated in fecal samples of bactrian camel (Camelus bactrianus) maintained at two zoos (Turnbull et al., 2012).

Very little or inadequate information is available regarding the structure of methanogenic population inside the gastrointestinal tract of this ruminant-like animal. In past decades the methanogenic population inside other livestock species has been studied well specially in cattle and sheep but dromedary camel is not subjected to modern studies and improvement. Camel differs from true ruminants anatomically but it is similar functionally in a way that it is still dependent on symbiotic association of microorganisms to degrade the plant fibers, therefore aptly called as “functional ruminant” (Van Soest, 1994). Nearly ninety-nine per cent of all microorganisms in any environment on earth are uncultured, thereby limiting our understanding of their diversity and
abundance (Amann et al., 1990). Culture-based studies fail to reveal the complete microbial diversity owing to the abundance of methanogenic community present in the environment. The 16S rRNA genes are ubiquitously found distributed in all the three domains of life- Bacteria, Archaea and Eukarya. In nature, they are highly conserved having slow rate of evolution, therefore are “gold standards” for establishing correct diversity and constructing phylogenies of bacteria and archaeal (Barry et al., 1991; Amann et al., 1995). The 16S rRNA genes have a hyper variable region offering a species specific signature sequence enabling researcher to identify and compare bacteria on a species level (Kolbert and Persing, 1999). The gene can also be used to compare evolutionary relatedness with 16S rRNA gene of archaea. The Archaea is one of the primitive life forms on earth. The rumen was the initial environment of these micro organisms which is comprehensively investigated and studied. The knowledge of anaerobic micro organism’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. The anaerobes present in natural water helps to reduce pollution and those present in artificial water are essential for waste water treatment and removal of toxic materials. 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, rumen forms a stable and basic source of knowledge concerning anaerobic micro organism. So, it becomes important to accurately identify and classify methanogenic population inside rumen. The fact that carnivore and wild birds rely on herbivores directly for food and humans are
indirectly dependent on them for food, clothing and transportation makes the understanding of ruminal methanogens all the more valuable. To elucidate the microbial dynamics inside camel rumen and to provide it a better ration in order to control various metabolic disorders, a better understanding of the rumen 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 enteric fermentation in ruminants is a significant cause of methane emission in environment. It is an inevitable outcome of their normal digestive process (Moss et al., 2000), which is not used by them and is lost in environment. Since, methane is a potent green house 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.

The objective of this thesis is to gain an insight into the current knowledge of diversity of methanogens present inside the gastro intestinal tract of camel.