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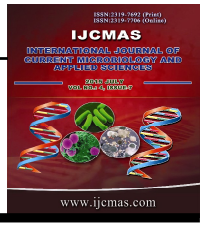

Review Article

Advancements in Bovine Rumen Microbial Ecology: A Review

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A B S T R A C T

Ruminants have served their important role in agricultural systems as well as in wellbeing of mankind as they utilize vast renewable sources (pasture, roughages) and convert them in to food edible for human. Ruminants are known to harbour a vast microbial community that functions in utilizing cellulosic feedstuffs, converting them to volatile fatty acids, providing animal nutrition. However, their farming is of considerable economic value in developing nations. The awareness of ruminant farming and its impact on the environment is to be taken under consideration. Few decades ago, the niche like rumen, researchers surprised with its tendency in harbouring a variety of microbiota, the functional abilities of which remained puzzled in those days. With the advancement in molecular techniques and introduction of high throughput sequencing technologies, the rumen microbial ecology have been assessed with greater ease and depth that can be helpful for developing the feeding strategies to minimize the methane production by animal as well as for providing the information on volatile fatty acid (VFA) production and thus, livestock nutrition.

Keywords

Methanogenesis, Next generation sequencing, Roughage, Rumen, Volatile fatty acids

Introduction

Historical background

Ruminants, cloven-hoofed mammals of the order *Artiodactyla*, obtain their food by grazing on plant material. The ruminants are distinguished from other mammals as they possess peculiarities for cud chewing, the process which is called rumination; hence ruminants. After the description given by Aristotle about the four compartments of the ruminant stomach, several experiments were performed to test the rate of passage of material through the alimentary tract (Spallanzani, 1776). In 1831, Tiedemann and Gmelin concluded that fermentation occurred in the rumen that leads to production of acetic and butyric acid in rumen contents. Another important product of rumen fermentation, propionic acid, was not identified until Elsden's research (1945)

(Hungate, 1966). Rumen fistulas were first mentioned by Fluorens in 1833. In 1854, cellulose had been distinguished by its solubility in strong acid, and insolubility in weak acid and alkali, and established as an important constituent of plants. Later, in 1855, Haubner showed that large amounts of cellulose disappeared as food passed the rumen. The role of bacteria in the fermentation of plant materials became well known as an effort of Pasteur (1863). Methane and carbon dioxide was described by Reiset in 1863 (Putnam, 1991). Zuntz (1879) was first to explain the utilization of forage by ruminants. Von Tappeiner (1884), a student of Zuntz, provided experimental support where he incubated cellulose with the juices of ruminants, in absence of antiseptics, found to be disappeared and gas and acids were formed. Von Tappeiner's experiments stimulated an interest in the cultivation of rumen micro-organisms (Hungate, 1966).

Ruminant digestive system

The four divisions of the ruminant stomach are the rumen, the reticulum, the omasum and the abomasum (Figure 1).

Rumen

The rumen is a fermentation vat which provides an anaerobic environment, constant temperature, pH and good mixing for the ingested cellulosic food, where after mastication and microbial enzymatic action, the fermentation products are either absorbed in the rumen itself or flow out for further digestion (Bowen, 2003). The environment in rumen kept agreeable to the microorganisms where fermentation of the ingested feed produces volatile fatty acids (VFAs), which are the primary sources of energy for an animal, and hence absorbed by thousands of "finger-like" projections lining the bottom and sides of the rumen wall (Umphrey and Staples, 1992).

Reticulum

The reticulum has a distinctive "honeycomb" appearance and aid in bringing the boluses of feed back up to the mouth for rechewing. It also acts as a receptacle for heavy objects that an animal eats. If metal object such as wire or nail is swallowed by an animal, it may puncture the reticulum wall, a condition known as "Hardware Disease". This condition may prove lethal to an animal for two reasons. First, the bacteria and protozoa can contaminate the body cavity resulting in peritonitis and second, the heart and diaphragm may be punctured by the object causing failure of these tissues (Umphrey and Staples, 1992).

Omasum

The feed once passes the rumen, it reduced in size due to microbial enzymatic action and then enters to the third compartment called the omasum. It appears like an open book with three sides bound where the tissues within are linked to the pages of a book and are called leaves. The leaves are having small papillae on them which absorb a large portion of the volatile fatty acids that were not absorbed in the rumen (Umphrey and Staples, 1992).

Abomasum

The fourth compartment is the abomasum which is also called "true" stomach. The wall of the abomasum secretes enzymes and hydrochloric acid. The pH of the digesta coming into the abomasum is 6.0 but it quickly lowered to about 2.5 by the action of acid. Proteins from the feed and the microorganisms broken down by the action

of pepsin enzyme and convert it into peptides.

Livestock nutrition

The correlation of the management of the plants with the management of the animals that harvest the plants is crucial for successful conservation and efficient use of grazing lands. The plant origin feed that an animal uptake, is only used for maintaining body functions (respiration, blood flow, nervous system), for gain of tissue in growing animals and for animal products (wool or milk). Animal feed is basically classified as concentrates and roughages depending on their composition. *Concentrates* are the feed having a high density of digestible nutrients with usually low fibre content (less than 18% of dry matter). *Roughages* are the feed with crude fibre content over 18% of dry matter with low density of nutrients (http://www.fao.org). Sometimes, animals are also offered a mixed ration of roughage and concentrate to provide them balanced nutrition. Concentrate includes cereal grains (corn, milo or sorghum grain, wheat, oats and barley), oil meals (soybean meal, cottonseed meal, and linseed meal), molasses and dried milk products whereas roughages includes corn (silage, grain, fodder, stover), alfalfa (hay and early bloom) and soybean (seeds).Effect of different proportion of roughage and concentrate ration on ruminants has been observed. Putnam and Loosli (1959) perceived an apparent decrease in digestibility of crude fibre as the proportion of concentrate in the ration increased. Several methods have been devised to estimate the forage digestibility in ruminants. Among them mostly used technique is i*n situ* technique (Ørskov, 2000), which was first intended to provide a dynamic assessment of the degradation of protein. Nutrition to an animal offers a means of making rapid change in milk composition i.e., concentration of milk fat, where the amount of roughage, forage: concentrate ratio, carbohydrate composition are the key factors to be taken care of (Sutton, 1989). However, it has been suggested that high-level concentrate feeding usually increases milk production due to greater intake of energy, unlimited grain feeding may force the animal into a fattening type of metabolism which may be antagonistic to a metabolism geared to produce milk efficiently and also tend to depress milk fat percentage, increase milk protein, depress digestion of dietary fiber, and alter the proportions of rumen volatile fatty acids (Kesler and Spahr, 1964). In smaller ruminant like goat, influence of forage: concentrate ratio on intake, digestibility, chewing and milk production of an animal is observed where intake of dry matter and digestibility increased with a decrease in forage: concentrate ratio (Kawas *et al.*, 1991).

Roughages are further classified into green and dry roughages depending on their quality. Green roughage provides sugars and starches that are fermented by bacteria to VFA (Volatile Fatty Acids). It is relatively soluble and digestible, whereas, dry roughage contains cellulose and hemi-cellulose that are bonded by lignin. This makes it less soluble and less digestible. Whatever the food material ingested by an animal, is enzymatically digested and converted in to VFAs that plays a pivotal role in providing an animal their basic nutrition (http://www.fao.org). Dietary carbohydrates i.e., cellulose, hemicellulose, pectin, starch and soluble sugars, are degraded to their constituent hexoses and pentoses. Pentoses are converted to hexose and triose phosphate by the transketolase and transaldolase reactions of the pentose

cycle so that the majority of the reactions proceed via hexose, which is metabolized to pyruvate by Embden-Meyerhof glycolytic pathway (Figure 2). Acetyl-coA is an intermediate in the formation of both acetate and pyruvate, whilst propionate production occurs mainly via succinate (France and Dijkstra, 2005). The principle VFAs produced in the rumen are acetate, propionate and butyrate that produced in a ratio varying from approximately 75:15:10 to 40:40:20 (Bergman, 1990). It has been claimed that no absorption of VFAs occurs from the rumen when the pH is more than 7 (Gray, 1948). Acetic acid is 50%–60% of the total produced VFAs and it predominates on a high roughage diet. 12–18% of VFAs produced is propanoic acid. It predominates on a high concentrate diet and provides energy via the conversion of blood glucose in the liver. It is used in lactose (milk sugar) synthesis. Butyric acid is 18–20% of the total VFAs and it is used in milk fat synthesis and also for body fat, when excess energy is present in the diet.

The amount of VFAs varies based on the diet given to an animal. It has been noted in dairy cows that the percentage of butyric and higher acids increased with the increase in protein rich diet whereas values for acetic and propanoic acid varied inversely. Moreover, the ratio of acetic to propanoic acid decreased with the decrease in the ratio of fibrous to starchy concentrate (Balch and Rowland, 1957). Moreover, one study on fistulated Holstein steers reported that the concentration of VFA in the rumen is increased after 4-6 hrs of feeding where the rates of VFA production were greatest within first 2 hours after feeding (Stewart *et al.,* 1958). Apart from animal nutrition, volatile fatty acids have been found to be an important mid-product in the production of methane. It has been reported in one study that the acetic and butyric acids do not have significant inhibitory effect on the activity of methanogenic bacteria whereas, the inhibitory activity of propanoic acid was observed on methanogenic bacteria (Wang *et al.,* 2009).

The other end products of the fermentation include CO_2, H_2, microbial protein and methane. Methane is produced by the symbiotic relationship of bacteria that produce H_2as an end product and the methanogens that link the $H_2$with CO_2or format. As a result of this, the methanogens gain energy for their own growth and metabolic $H_2$is removed from the ruminal environment (Johnson *et al.,* 1993; McAllister *et al.,* 1996).

It is well established that methanogens are the only group of archaea that are capable of methane production, the diversity of which depends upon the diet given to the host and its geographical location (Hook *et al.,* 2010). The recent study based on rumen and fecal methanogen diversity *Altay* sheep has reported that the genus *Methanobrevibacter* and unidentified methanogenic-like archaeons in the rumen significantly induced by the high roughage diet (Liu *et al.,* 2012).

A previous report by (Kurihara *et al.,* 1999) indicated that methane production was higher in cattle fed on tropical forages than in those fed on temperate forages, due to comparatively high fiber content in tropical forages. It has been also found that the increasing proportion of concentrate (starchy concentrate as compared to fibrous concentrate) in the animal feeding decreased the methane production from ruminants (Benchaar *et al.,* 2001). Seven studies on dairy cows fed on thirty seven diets highlights that methane production decreased when animals were fed on more dietary ether extract content (Giger-Reverdin *et al.,* 2003).

Methane is emitted by several natural sources (termites, wetland, and oceans) and anthropogenic sources (Agriculture, waste-water treatment, landfills etc) (Figure 3a). The anthropogenic sources contribute around 58% of the global methane emission (EPA, 2010). The international Global Methane Initiative (GMI), launched in November 2004, involved the United States and other 13 countries with large source of methane accounting ~ 60–70% of the global methane emissions from the targeted sources viz., Agricultural methane emission (Figure 3b). If we look at our national scenario, the percentage increase in enteric methane emission (EME) by Indian livestock was greater than world livestock (70.6% vs. 54.3%) between the years 1961 to 2010, and annual growth rate (AGR) was highest for goat (1.91%), followed by buffalo (1.55%), swine (1.28%), sheep (1.25%) and cattle (0.70%) (Patra, 2014).The projected estimates of livestock population indicates that lactating dairy cattle and buffalo are expected to increase by 3.5 and 5.6 million resulting to an expected increase of ~36% and 17% methane emissions, respectively by the year 2021 (Chhabra *et al.,* 2007).

Decreasing enteric methane emissions from ruminants without altering animal production is anticipated as a strategy to decrease global methane emission and also as a means of improving feed conversion efficiency. Numerous techniques have been previously described and also are currently being explored to mitigate methane emission. The use of ionophores in ruminant diets have been found to reduce CH_4emissions by 25% and decrease feed intake by 4% without affecting animal performance (Tedeschi *et al.,* 2003). However, the treatment of ionophoreshas been resulted for short term reduction in methane emission due to microbial adaptation to the ionophore rich diets (Guan *et al.,* 2006). Moreover, there are other approaches that include addition of probiotics, acetogens, bacteriocins, archaeal viruses, organic acids, plant extracts (e.g., essential oils) to the diet (Boadi *et al.,* 2004) as well as elimination of rumen protozoa (Hegarty, 1999) but most of these approaches remain as short-term. It is more reliable to target the enzymes involved in methanogenesis pathway carried out by methanogens rather to target the community itself. So, preliminary focus should be to explore the ruminal microbiota and their interaction and to decipher the metabolic functions carried out by them.

Bovine

The subfamily Bovinae includes a diverse group of 10 genera like cattle, bison, African and water buffalo, the yak and four horned and spiral horned Antelopes. The general characteristic of this group is their cloven hoofs.

Bubalusbubalis

The water buffalo (*Bubalusbubalis*) are divided in to two extant types based on morphological and behavioural criteria – the river buffalo (Chromosome no., 2n=50) and the swamp buffalo (Chromosome no., 2n=48). The origin of swamp type buffalo is expected to be in china around 4000 years ago while the river type may have originated from India around 5000 years ago (Yang *et al.,* 2008).

The milk of water buffalo is richer in fat and protein as compared to dairy cattle. More than 95% of the world population of water buffaloes is found in Asia. India is endowed with 109M head in 2013 representing 56.4% of the world water buffalo population (Figure 4). The second largest population lived in Pakistan with 33.6M heads followed by China 22.3M heads calculated in 2013 (Figure 4).

In India, buffalo breeds are classified on the basis of the region they belong to. Among the mentioned breeds (Table 1), Mehsani breed of buffalo is known as a persistent milker and regular breeder which are evident from the lactation length and short dry period [Avg. age at first parturition in months= 42.83, Avg. Parturition interval in months= 15.64] (Pundir *et al.,* 2000). Mehsani breed is considered to be a cross between Surti and Murrah. It is found to yield 1800-2000 kg of milk per lactation with an average of 7–7.5% of fat (Source: National Bureau of Animal Genetic Resources, http://www.nbagr.res.in/).

Bostaurus

Cattle are a prominent modern member of the subfamily *Bovinae*, genus *Bos* and are collectively classified as *Bostaurus*. Cattle are raised for meat and as dairy animals for milk.

According to archaeological and genetic evidence, the distinct domestication events of *B. Taurus* is near east, and *B. indicus* near the Indus valley of the Indian subcontinent (McTavish *et al.,* 2013).Cattle represent 12.8% of the total world population in India with 189M heads in 2013 (Figure 5), followed by China (7.7%) and Pakistan (2.6%) (http://faostat3.fao.org).

India has 37 pure cattle breeds that include Sahiwal, Gir, Red Sindhi, Tharparkar and Rathi breeds known for its milking prowess. Other cattle such as Kankrej, Ongole and Hariana have both milch and drought qualities (Secretary).

Among the mentioned breeds (Table 2), the highest milk yielding breed is Gir with an average milk yield of 2000-6000kg per lactation. Kankrej, another breed of Gujarat, is used both for milk production and agricultural purposes. This particular breed possesses immense draught power and isresilient to stress conditions and is known for yielding a good quantity of milk (average milk yield 1500–4000 kg per lactation) and good fat content even in stress conditions.

Advancement in rumen microbial ecology

As the emergence of microbiology field during the 19th century, the research began to explore the relationship between the rumen fluid, bacteria and fibre digestion. After Van Tappeiner's effort to uncover the anaerobic type of fermentation by the microbiota in the rumen, (Hungate, 1944) finally discovered a combination of anaerobic techniques and reducing agents for growing cellulose digesting bacteria from the rumen and became leader in ruminal microbiology, earning the title of the father of rumen microbiology. Dr.Hungate modified the traditional Delft University approach by including ruminal fluid as an essential nutritional supplement and a CO_2 atmosphere with bicarbonate to simulate the natural rumen habitat with salivary buffering system to isolate cellulolytic bacteria (Hungate, 1966). During that time, the Hungate technique was modified in several ways to isolate different strains of microbiota characterized eight strains of bacteria isolated from rumen contents using medium containing xylan as a sole source of carbohydrate (Dehority, 1966). Inoculating other carbohydrate sources like lactate, pectin and xylose in semi continuous cultures, the greatest enrichment of microbiota was observed with ammonia production rate eight fold higher than that of the ruminal fluid control (Russell *et al.,* 1988). It was also observed by (Russell *et al.,* 1988) that The *Peptostreptococcus* species was unable to grow on any of 25 carbohydrate or

carbohydrate derivatives tested; but the *Clostridium* species was able to use glucose, maltose, fructose, cellobiose, trehalose, sorbitol, and salicin as energy sources. The bacterial activities on petri dish was checked by Congo red dye, which react with intact beta-D-glucans and can be used to check beta- (1,4), (1,3)-D-glucanohydrolase, beta-(1,4)-D-glucanohydrolase, and beta- (1,3)-D-glucanohydrolase activities (Teather and Wood, 1982). With culture-dependent attempts whatever knowledge of rumen microbiology obtained was only about 10-20% of the total rumen microbial population. The rumen microbiota that provides ruminant with genetic and metabolic capabilities which the host has not evolved on its own, including capabilities to hydrolyse and ferment inaccessible nutrients, is estimated to harbour 100 times more genes than the host animal. However, culture dependent methods discovered the rumen microbial activities, it was unable to capture the total ruminal microbial diversity, due to the lack of growth of uncultivable microbes. With the advent in microbial technologies, the development of unique biomarker to monitor microbial community in environmental samples arose. A variety of biomarkers including cell wall components, proteins, lipids, DNA and RNA evolved. In that, the application of small subunit ribosomal RNA (rRNA) has proven an irreplaceable tool to study the microbial ecology. Two decades ago, molecular approaches to identify microbial community from niche environments were based on cloning of 16S rDNA either by amplification of from extracted DNA or by reverse transcription of rRNA (Ward *et al.,* 1990). These studies only resulted in exploration of microbial diversity instead of giving information related to the microbial dynamics (effect of environmental and diet perturbations). To study the environmental effects on microbial diversity, large number of sample size would require, for that, cloning approach would remain impractical as it is time consuming and labour intensive. Another promising molecular approach to analyse complex mixture of microorganisms was developed that included special kind of electrophoretic technique i.e. denaturing gradient gel electrophoresis (DGGE), where DNA fragments of the same length but with different base-pairs can be separated (Muyzer *et al.,* 1993).The separation is based on the electrophoretic mobility of PCR-amplified DNA fragments in polyacrylamide gels containing a linearly increasing gradient of denaturants. The phylogenetic affiliation of the detected bacteria was inferred after sequencing of individual bands of the DGGE (Muyzer and de Waal, 1994). Afterwards, Single-Strand Conformation Polymorphism (SSCP) technique was introduced where the environmental PCR products are denatured followed by electrophoretic separation of single-stranded DNA fragments on a non-denaturing polyacrylamide gel (Schwieger and Tebbe, 1998). During mean time, (Franklin *et al.,* 1999) presented Random amplified polymorphic DNA (RAPD) and DNA amplification fingerprinting (DAF) techniques which incorporate the use of short primers for PCR amplification, which anneals randomly at multiple sites on the genomic DNA under low annealing temperature, typically $\leq$ 35°C. The PCR amplicons of various lengths can be separated on agarose or polyacrylamide gel where the separation depends on the genetic complexity of the microbial communities. (Smit *et al.,* 1997) used amplified ribosomal DNA restriction analysis (ARDRA) based on DNA sequence variations present in PCR-amplified 16S rRNA genes, where the amplified product from environmental DNA is generally digested with tetra-cutter restriction endonucleases (e.g., AluI, and HaeIII), and restricted fragments are

resolved on agarose or polyacrylamide gels. However, ARDRA is providing rapid monitoring of microbial communities over time, or to compare microbial diversity in response to changing environmental conditions, its limitation lies in resolving the restriction profiles generated from a complex environment. The similar kind of method Terminal restriction fragment length polymorphism (T-RFLP) was also used where the resulting PCR products generated with 5' fluorescently labelled primers, are digested with restriction enzymes and terminal restriction fragments (T-RFs) are separated on an automated DNA sequencer (Thies, 2007). Only the terminally fluorescent labelled restriction fragments are detected, where community diversity is estimated by analysing the size, numbers, and peak heights of resulting T-RFs. Each T-RF is assumed to represent a single OTU or ribotype.

The drawback of this technology is only limited number of ~100 bands per gel get resolved. Other techniques that follow the separation based on probing the amplicons includes Length Heterogeneity PCR (Mills *et al.,* 2007), Automated Ribosomal Intergenic Spacer Analysis (ARISA) (Fisher and Triplett, 1999) and DNA microarrays that are classified in 16S rRNA gene microarrays and functional gene arrays (FGA) (Gentry *et al.*, 2006).Then after, the traditional PCR technology was replaced by Quantitative PCR (Q-PCR), or real-time PCR (Smith and Osborn, 2009)which even nowadays is also used to accurately measure the abundance and expression of taxonomic and functional gene marker. But, all these approaches remained pitfall in exploring total microbiota from a particular niche at a greater depth, as well as in explaining the environmental effect on microbiota and their interaction with other microbiota.

Era of omic technologies

The era of microbial ecology was revolutionized with the commencement of genomics technologies. It started with the introduction of the word "Metagenomics" by Jo Handelsman, Jon Clardy, Robert M. Goodman, Sean F. Brady, and others, in 1998 (Handelsman *et al.,* 1998) and amalgamates the approach with the high throughput sequencing. The term "Metagenome" referred to the concept of high throughput sequencing a collection of genes from the environment in a way analogous to the study of a single genome. The recent developments in sequencing technologies have allowed the researchers to reach the deeper layer of the microbial community. The first next generation sequencing (NGS) platform, pyrosequencer (GS-FLX) reached to the market (Ronaghi *et al.,* 1998) previously generating 20Mb data with 100bp read length, and now upgrading to ~500Mb data longer length (400-500bp). Another platform, Illumina sequencer produce more reads with cheaper price and more accurate (>99%) than 454 GS-FLX (98.93%). Ion Torrent Personal Genome Machine (PGM) from life technologies also available that can be used for metagenomics purpose and now additionally also provides an optimized protocol for 16S rRNA gene-based profiling (Milani *et al.,* 2013; Whiteley *et al.*, 2012). More recently, Pacific Biosciences has released a new sequencing technology, PacBio RS, and Oxford Nanopore Technologies introduced GridION/MinION devices, both of which allow single-molecule sequencing with a much longer read length (Teeling and Glöckner, 2012). With the boon of this approach, the microbial ecology field has boomed the bioinformatics field for discovering the methodologies and pipeline for the

qualitative and quantitative microbial community analysis.

Recent bioinformatic approaches in metagenomics

Assembly

It is a non-trivial question, whether to assemble a metagenome. Generally, by assembling the reads, we can get larger gene fragments, which can be annotated for functional genes and if not assigned, can be predicted for full-length coding sequences (CDS) for further characterization. Although, assembly does yield longer sequences, it also bears the risk of creating chimeric contig, either from closely related species or from highly conserved sequences that occur across species. There are some dedicated metagenome assemblers that try to solve these problems.

MetaVelvet, an extension of Velvet assembler is widely used for metagenomic assembly. It overcomes the limitation of a single genome assembler by minimizing misidentification of the highly abundant species as repeats (Namiki *et al.,* 2012). MetAMOS is a metagenomic assembly and analysis pipeline. It can aid in reducing assembly errors, commonly encountered when assembling metagenomic samples, and improves taxonomic assignment accuracy while also reducing computational cost (Treangen *et al.,* 2013). Another software called IDBA-UD assembler is employed for both single genome and metagenome assembly. The advantage of using this assembler is that it works on the logic that the sequencing depth of different regions of a genome or genomes from different species are highly uneven (as observed in the genome and metagenome sequencing). Comparison of the performances of IDBA-UD and existing assemblers (Velvet, Velvet-SC, SOAP denovo and Meta-IDBA) for different datasets, shows that IDBA-UD can reconstruct longer contigs with higher accuracy (Peng *et al.,* 2012). Meta-IDBA, a de Novo assembler for metagenomic data, first tries to partition the de Bruijin graph into isolated components of different species based on an important observation, then for each component, it captures the slight variants of the genomes of subspecies from the same species by multiple alignments and represents the genome of one species, using a consensus sequence (Peng *et al.,* 2011). Ray Meta assembler profiles microbiomes based on uniquely-colored k-mers, assemble accurately with profiling 3 billion metagenomic read representing 1000 bacterial genome of uneven proportions (Boisvert *et al.,* 2012).

Gene prediction

Many gene finders require longer stretch of sequence to assign that sequence as coding sequence and discriminate it from non-coding sequence. Moreover, many gene predictors predict the genes based on the training sequences from a single species which usually builds a species specific prediction model. There are different tools available for gene prediction that employ Hidden Markov Model (HMM) based approach like MetaGene (Noguchi *et al.,* 2006), MetaGeneAnnotator (Noguchi *et al.,* 2008), FragGeneScan (Rho *et al.,* 2010) and Orphelia (Hoff *et al.,* 2009).

Taxonomic classification and binning

Binning is an approach for sorting DNA sequences into groups that might represent an individual genome or genomes from closely related organisms. The compositional binning is based on the fact that the genomes have conserved nucleotide composition i.e. GC content, particular abundance distribution of k-mers.

Table.1 Breeds of buffalo in India

Murrah group	Gujarat group	Uttar Pradesh group	Central India group	South India group
Murrah	Surti	Bhadawari	Nagpuri	Toda
Nilli Ravi	Jaffarabadi	Tarai	Pandhepuri	South Kanara
Kundi	Mehsana		Manda	
Godavari			Jerangi	
			Kalhandi	
			Sambalpur	

Source: (Dr. Henna Hamadani *et al.*, 2012)

Table.2 Breeds of cattle in India

Tamilnadu	Gujarat	Uttar Pradesh, Bihar, Hariyana	Rajasthan	Maharashtra	Andhra pradesh , Kerala	Madhya pradesh	Karnataka
Kangayam	Gir	Sahiwal	Tharparkar	Deoni	Ongole	Nimari	Khillari cattle
Baraguru	Kankrej	Hariana	Rathi	Red Kandhari	Krishna Valley	Kenkata	Amritmahal
Umblachery		Bachaur	Malvi	Dangi	Vechur		Hallikar
		Kherigarh	Nagori		Kasaragod		MalenaduGidda
		Ponwar	Mewati				

Source: Department of Animal Husbandry, Dairying and Fisheries (http://www.dahd.nic.in)

Figure.1 The digestive tract of ruminants

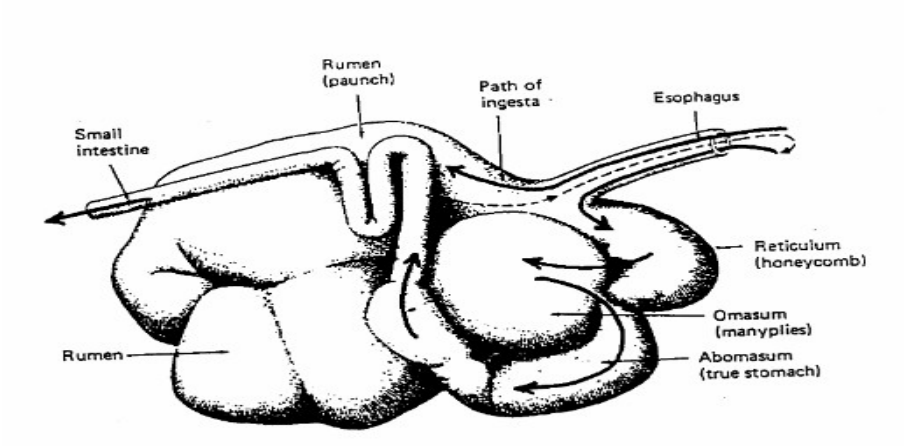


Figure.2A schematic representation of the major pathways of carbohydrate metabolism in the rumen. Source: (France and Dijkstra, 2005)

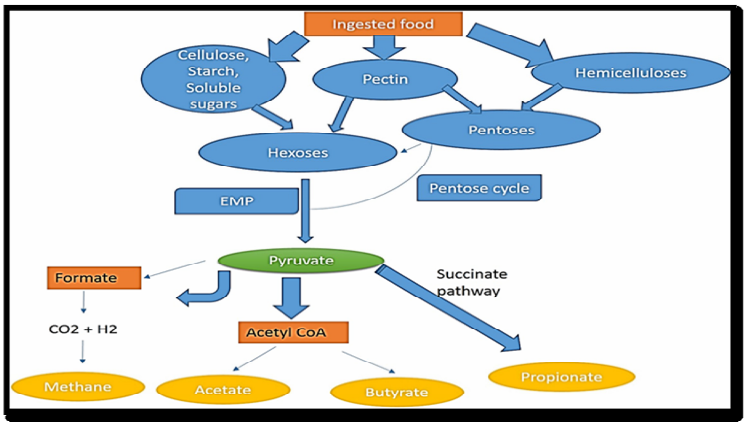


Figure.3 a) Global methane emission sources, b) Agricultural global methane emission. Sources: a) EPA-2010, b) The World Bank (IBRD-IDA)

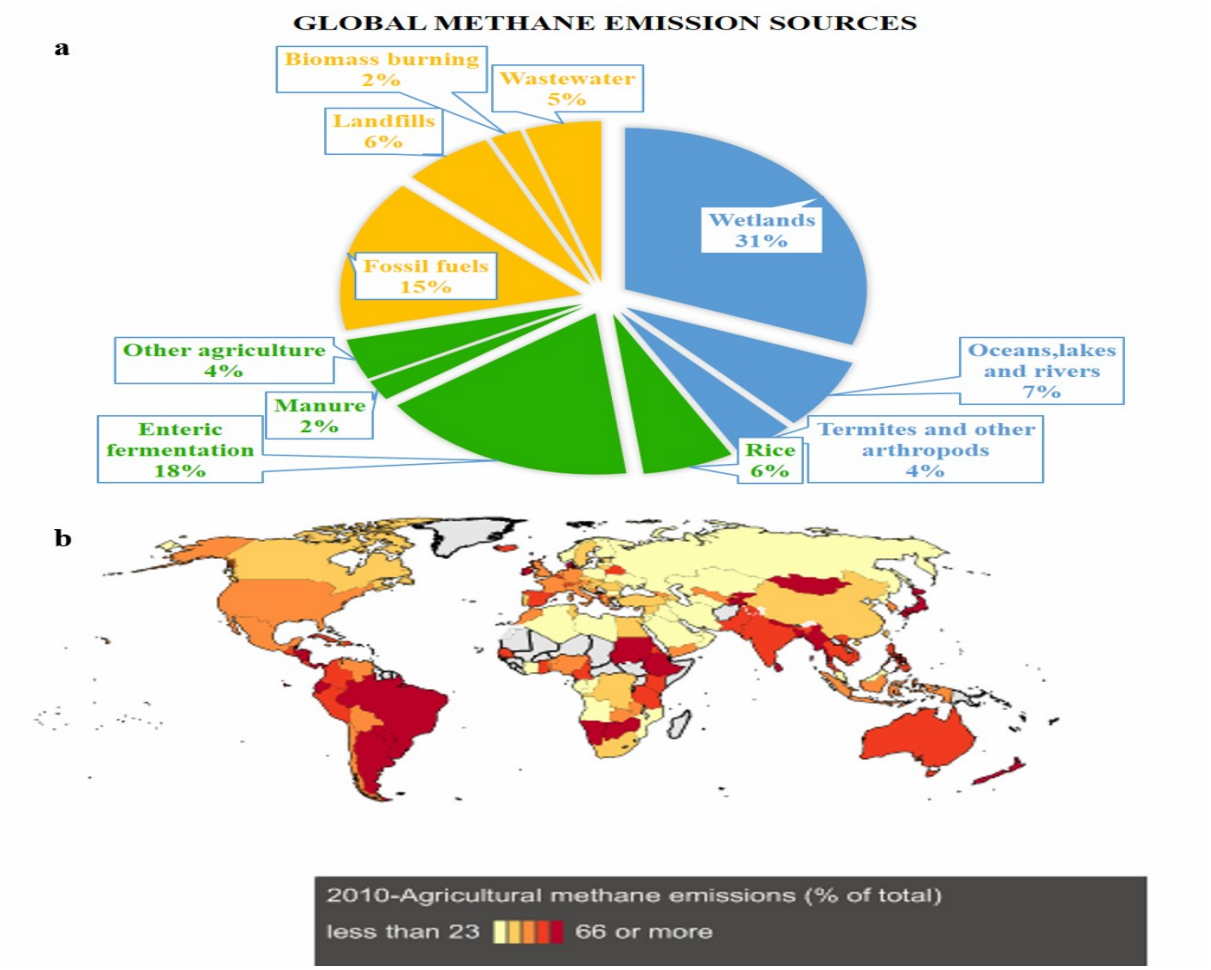


Figure.4 Buffalo population of last fifteen years in major countries of Asia

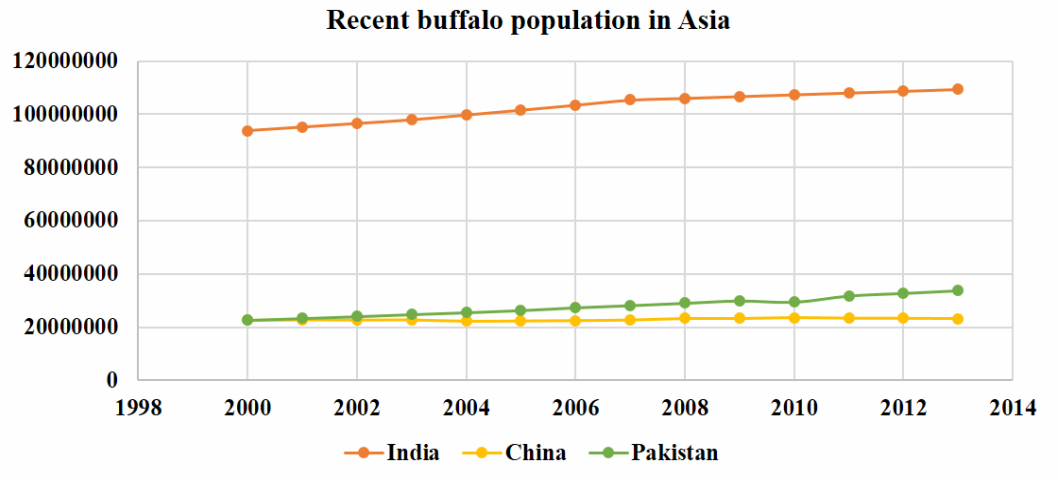
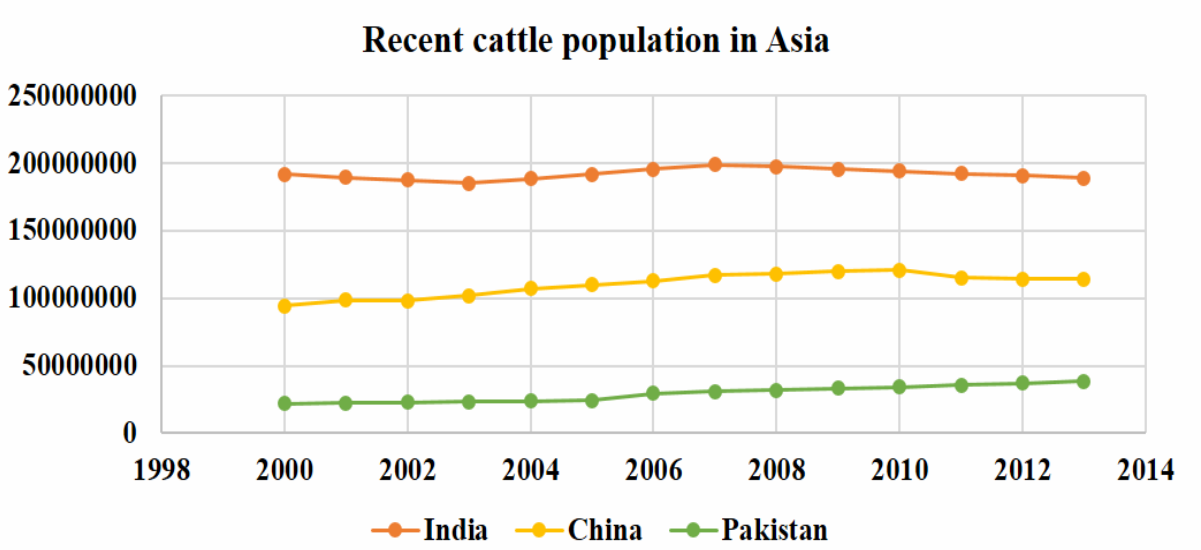


Figure.5 Cattle population of last fifteen years in major countries of Asia



Compositional-based binning algorithm include Phylopythia (McHardy *et al.*, 2007), S-GSOM (Chan *et al.*, 2008) and PCAHIER (Zheng and Wu, 2010). The similarity based approach identifies and classifies the unknown DNA fragment based on matching it with the known genes in reference. The similarity based binning software include IMG/M (Markowitz *et al.*, 2008), MG-RAST (Meyer *et al.*, 2008), MEGAN (Huson *et al.*, 2007), WEB-CARMA (Gerlach *et al.*, 2009) and MetaPhyler (Liu *et al.*, 2010).

Rumen microbial ecology in the era of metagenomics and future prospective

The complex microbiome of rumen has been explored with great ease due to metagenomics technique. One study on bovine rumen metagenome revealed that the initial colonization is from those rumen microbiota that produce enzymes acting on the easily available side chains of complex plant polysaccharides not cellulose (Brulc *et al.*, 2009). Another study based on characterization of biomass degrading genes

into the rumen of a cow identified 27,555 putative carbohydrate active genes, 57% of which were enzymatically active against cellulose (Hess *et al.,* 2011). One study on yak rumen showed that 10,070 ORFs were identified among them 150 were annotated as Glycosyl hydrolase (GH) genes most of them came from Bacteroidetes contigs (Dai *et al.,* 2012). Metagenomic analysis of dairy cows fed on pasture or total mixed ration diets showed that the bacterial and archaeal communities were significantly affected by diet as well as the difference was also observed between the communities of solid and liquid fractions (de Menezes *et al.,* 2011).The complex microbiome from the rumen of Surti buffalo have previously been explored at different diet treatments (Singh *et al.,* 2012).The diet treatments have shown to lead to the fluctuations in carbohydrate acting enzymes (CAZymes), the applications which may help to food processing industries and enzyme industries (Sathya and Khan, 2014). Thus, metagenomics have evolved as an approach to study the activity of ruminal microbes which were impossible few decades ago. Rumen is a niche harbouring a microbial community which is stable as well as dynamic. Stable in the sense, the microbial community remain functional, however it can be denoted as dynamic as it is found to be varied in its abundance at different diet treatments, in different animals, in different geographical locations as well as at different age of an animal. Thus, exploring the rumen microbial community with the metagenomics tools may provide ease to detect several underexplored or yet to be explored microbial communities and their functions.

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