Chapter 4
Rumen Microbiology and Microbial Degradation of Feedstuffs

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Abstract The mixed and complex microbial ecosystem of the rumen comprises numerous interactions between its anaerobic inhabitants, viz., bacteria, protozoa, fungi, archaea, and also the bacteriophage. The rumen is a dynamic system open to the external environment, but the ruminal microbial niche is maintained at a constant milieu well suited for these diverse anaerobic rumen microbial population to grow and multiply. Temperature, pH, buffering capacity, osmotic pressure, and redox potential are the primary factors affecting the growth and activity of these microbes. Efficient microbial fermentation inside the rumen depends on the actions of diverse enzymes secreted by these microorganisms on the complex feed compounds. Cellulase, hemicellulose, esterase, and pectinase from the fiber digester microbes (bacteria, protozoa, and fungi) ferment complex structural carbohydrates. In addition, these microorganisms ferment starch and soluble sugars, protein, and non-protein nitrogen substances. Certain ruminal microbes are also capable of detoxifying potentially toxic substances in feed such as phytotoxins, anti-nutritional factors, and mycotoxins. Ruminal archaea or methanogens create an environment conducive for other ruminal microbial population by capturing hydrogen and converting them to methane. However, methane is a potent greenhouse gas which is a major global environmental concern, and efforts are going on to find effective mitigation strategies. Anaerobic rumen fungi perform important function in fiber degradation by splitting feed particles through mycelial development, by firmly attaching to these lignified feed particles through their rhizoid, and by exposing them for the action of fibrolytic enzymes of the ruminal microbes. Ruminal bacteriophages are the least explored among all the rumen microbes, but they provide

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enormous scope for their wider use such as controlling and eliminating certain ruminal microbes and their applications in treatment through the novel branch of phage therapy.

Keywords Rumen microbes · Fiber degradation · Ruminal fermentation · Methanogens · Ruminant digestion

4.1 Introduction

Rumen is the first and the largest of the four chambers of ruminant foregut. Pre-gastric fermentation of fibrous feedstuffs ingested by animals takes place in this chamber by the complex microbial interactions between a range of anaerobic microorganisms comprising bacteria, protozoa, fungi, and primitive archaea and also the bacteriophage (Forsberg and Cheng 1992). This mixed and complex microbial ecosystem not only facilitates growth and multiplication of the microbes involved but also facilitates nutrient derivation and energy for host animals. The primary source of energy and other nutrients on a forage-based diets of ruminant animals consists mainly of the structural polysaccharides of the plant cell walls such as cellulose, hemicellulose, and pectin, in addition to other non-structural polysaccharide such as starch. The structural complexity and insolubility of recalcitrant cell wall components of the forage-based feedstuff limit their degradation and ruminal fermentation (Nagaraja et al. 1997). However, the degradation and metabolism of these structural carbohydrates are achieved in the complex ruminal microbial ecosystems through the synchronous activities of the diverse microbial enzymes secreted by the ruminal microbes interacting mostly in a synergistic relationship (Burns 2008). Microbial fermentation of ingested structural carbohydrates is vital for digestion and metabolism of feedstuffs by the host animal yielding volatile fatty acids such as acetate, butyrate, propionate, formic acid, methane, H_2, and CO_2 (Krause et al. 2003). These short-chain fatty acids mainly acetate, propionate, and butyrate are rapidly absorbed by the rumen epithelium and make up to 80% of the energy requirements of the ruminant host (Bergman 1990; Gäbel and Sehested 1997).

4.2 Ruminal Environment and Microbial Niche

The ruminal microbial niche is almost a constant environment well suited for large and diverse anaerobic ruminal microbial population to grow and multiply, making it a highly efficient continuous culture system for these microbes. Food and water supply, pH and buffering capacity, temperature and osmotic pressure, type and strength of microbial culture, outflow of end products of fermentation, undigested residues and microorganisms, all are fairly constant in the ruminal milieu (Wang and McAllister 2002). The digestive tract of ruminant animal constitutes quite larger proportion of the total body weight (about 40%), and volume of the reticulo-rumen

Table 4.1 Summary of physical and chemical characteristics of the rumen ecosystem

Physical criteria	Range characteristics
pH	5.5–6.9 (mean 6.4)
Redox potential	−350 to −400 mV
Temperature	38–41 °C
Osmolality	250–350 milliosmole/kg^{-1}
Dry matter	10–18%
Chemical criteria	Range characteristics
Gas phase (%)	CO_2, 65; CH_4 27; N_2 7; O_2 0.6; H_2 0.2
Volatile fatty acids (mmol L^{-1})	Acetate 60–90; propionate 15–30; butyrate 10–25; branched chain and higher 2–5
Nonvolatile acids (mmol L^{-1})	Lactate <10
Amino acids and oligopeptides	<1 mmol L^{-1} present 2–3 h post feeding
Ammonia	2–12 mmol L^{-1}
Soluble carbohydrates	<1 mmol L^{-1} present 2–3 h post feeding
Insoluble polysaccharides	
Dietary (cellulose, hemicelluloses, pectin)	Always present
Endogenous (mucopolysaccharides)	Always present
Lignin	Always present
Minerals	High Na; generally good supply
Trace elements/vitamins	Always present; good supply of B vitamins
Growth factors	Good supply; branched-chain fatty acids, long-chain fatty acids, purines, pyrimidines, other unknown

Source: adapted from Mackie et al. (1999)
Note: mmol L = millimole per liter; mV = millivolts

makes up about 85% of the total capacity of the digestive tract. Temperature, pH, buffering capacity, osmotic pressure, and redox potential are the primary factors affecting the growth and activity of ruminal microbial populations (Russell and Rychlik 2001) (Table 4.1). The rumen temperature is typically regulated in the range of 38–41 °C and has an oxidation potential of −350 mV (Choudhury et al. 2015). Strict anaerobic conditions (−150 to −350 mv) required for the efficient microbial fermentation in the rumen are preserved by the facultative anaerobic bacteria of the ruminal wall which quickly utilizes the traces of oxygen entering into the rumen through feed or water or through diffusion across the ruminal wall (Clarke 1977). In spite of the large volume of liquid consumed through feed, drinking water, or saliva, the dry matter content of the rumen is maintained at a fairly constant range of 10–18%. This not only provides a liquid environment but also ensures a sustained optimal supply of substrates necessary for efficient and continuous microbial fermentation. Further, frequent and regular rumination (i.e., regurgitation, re-mastication, and re-swallowing of coarse foregut digesta by the

ruminant animals) enhances the process of fermentation by reduction in particle size of the coarse fibers and increases surface area of feed particles for microbial enzymatic exposure and action. Although the physiological pH is among the most variable physicochemical factors of the ruminal environment (Russell and Strobel 1989), the rumen content has a high buffering capacity, and pH of the ruminal content is maintained within a fairly constant range of 5.5–7.0. (Dehority 2003; Krause and Oetzel 2006). The pH and buffering capacity in the reticulo-ruminal environment are determined by the amount of saliva and its bicarbonate and phosphate content, quantity and the nature of the feedstuffs ingested by the host ruminant, production and absorption of the short-chain fatty acids, and neutralization and absorption of the bicarbonates and phosphates in the rumen (Krause and Oetzel 2006). Exo-enzymes secreted by the ruminal microorganisms which degrade the feedstuffs and perform microbial fermentation are sensitive to the changes of ruminal pH. Similarly, the normal neutral intracellular pH of rumen microorganism is affected greatly by the H^+ ion imbalance caused by the drop in pH due to high VFA and LA formation on high starch-based diets (Russell and Wilson 1996) which inhibits the microbial function and growth.

The osmolality of the ruminal fluid is about 250 mOsm/kg, and it depends on the concentrations of ions and molecules which generate gaseous tension and influences pH due to VFAs formation (Lodemann and Martens 2006). Further, the osmotic pressure inside the rumen is affected by the nature of diets and other environmental factors inside the rumen which influence the microbial fermentation. Immediately after feed intake, the osmotic pressure increased from 350 to 400 mOsm and then decreases gradually over the next 8–10 h (Lodemann and Martens 2006).

The microbial fermentation inside the rumen depends on the actions of enzymes secreted by the anaerobic microorganisms interacting in synergistic, synchronized, and systematic way on the complex food compounds. Actions of cellulase and hemicellulase degrade complex structural polysaccharides of plant cell wall to produce VFAs to be further utilized as source of energy by the host animals (Burns 2008; Russell and Mantovani 2002). Further, several microbial proteases and deaminases act on low-quality protein of the roughage-based feedstuffs and non-protein nitrogenous substances to produce ammonia and their further uptake and utilization for de novo synthesis of microbial proteins. The microbial proteins are digested further by the host animals inside the abomasum—the true stomach—and small intestine from which all of the essential amino acids required by the host animals are derived (Cole et al. 1982). Additionally, certain ruminal microbes are also capable of detoxifying potentially toxic substances in feed such as phytotoxins, anti-nutritional factors, and mycotoxins to harmless intermediates that have no adverse effect on the animals' health. Nevertheless, ruminal fermentation processes cannot be totally efficient as it generates some by-products such as methane gas and excess ammonia. Thus, in a mutualistic and symbiotic relationship where microorganisms provide all the essential nutrients to the host animals, the latter provide all the substrates and the suitable environment for establishment of the microbial niche in which proper growth and multiplication of microorganisms and efficient microbial fermentation take place (Russell and Rychlik 2001).

4.3 Rumen Microorganisms and Degradation of Feedstuffs

Much of our understanding about the anaerobic microbial ecology of the rumen is credited to Hungate—the father of anaerobic microbiology and the father of rumen microbiology, who invented the roll tube technique for isolation and culture of anaerobic rumen microorganisms during the 1950s (Hungate 1947, 1950; Hungate 1957). This technique provided much of the insight about the anaerobic ruminal microbes and especially about rumen bacteria. The ruminal microflora consists of a wide range of obligate anaerobic microorganisms occurring in a symbiotic and mutualistic relationship with host ruminant to perform microbial fermentation. After separating the ruminal methanogens from bacteria (Woese et al. 1990), all these ruminal microorganisms can be classified into the following five groups: bacteria, ciliate protozoa, anaerobic fungi, methanogens (archaea), and bacteriophages. These microorganisms are present in the rumen in different concentrations and perform various functions (Table 4.2), all of which contribute toward efficient anaerobic fermentation, making the rumen an ideal fermentation vat.

The rumen is a dynamic system, and the inhabiting microorganisms continuously adapt themselves to the form and physical structure of the ingested feedstuffs, quantity and frequency of consumption, changes in the ingredients composition of the diet, and its chemical and nutritional composition. Just after ingestion of feedstuffs, microorganisms quickly associate themselves with different components of the rumen content and ingested feed particles (Craig et al. 1987; Bonhomme 1990). The slow-working fiber-digester microbes attach themselves to the fibrous materials and digestible dry matter on the fiber mat of the dorsal rumen contents, and the fast-working microbes which ferment sugars and starches associate themselves in the rumen fluid in ventral contents. Bacteria in the ventral ruminal fluid has the mean

Table 4.2 Anaerobic microbes of the rumen, their concentrations, proportions, and important functions

Microbes	Concentration	% of cell number[a]	% of microbial weight	Functions
Bacteria	10^{10}–10^{11} g^{-1} (> 200 species)	~98%	50–60%	Ferment fiber, starch, sugars, protein, and other substances
Archaea	10^{7}–10^{8} g^{-1} (25 genera)	1%	1–3%	Produce methane gas
Ciliate protozoa	10^{4}–10^{6} g^{-1} (25 genera)	1%	40–50%	Ferment starch, fiber, and feed upon bacteria
Fungi	10^{3}–10^{5} g^{-1} (5 genera)	<1%	5–8%	Break down fiber, facilitates action of microbial enzymes on fibers
Bacteriophage	10^{7}–10^{9} g^{-1}	–	< 0.1%	Scavenge rumen bacteria and archaea

Source: Castillo-González et al. (2014), Agarwal et al. (2015)
[a]Excluding bacteriophage concentration

generation time of 7 h, while bacteria present on dorsal ruminal mat have comparatively slow passage rate and longer generation time (Bryant 1970). As an integral part of the rumen ecosystem, microorganisms associated with the ruminal fluid survive on soluble feed components and colonize and initiate digestion of newly ingested feed particles. Further, loosely associated bacteria and firmly attached microbes which account for 70–80% of the microbial population in the rumen are responsible for 70–80% of the amylase, protease, and endoglucanase activity (Minato et al. 1966; Brock et al. 1982) and comparatively higher hemicellulase and cellulase activities in the ruminal fibrous mat (Williams and Strachan 1984). Fungal zoospores chemotactically attach rapidly to plant-based fibrous materials of the dorsal mat and break these fibrous materials through their penetrating branching mycelia which grow afterward. This increases the area of exposed substrate combined by the joint effect of slow passage rate and prolong residency of the insoluble fibrous feed particles, which increases the reaction time between substrates and fungal and other enzymes from some other fiber fermenter bacteria and protozoa (Orpin 1975; Williams and Orpin 1987).

4.4 Rumen Bacteria

4.4.1 Fiber and Starch Degrading Bacteria

Efficient digestion of complex fibrous substrates in the rumen requires the combined and coordinated actions of all the ruminal microorganism and their enzymes. However, the collective activities of bacteria, fungi, and protozoa in fiber degradation are particularly important as 80% of degradation is performed by bacteria and 20% by protozoa (Dijkstra and Tamminga 1995). *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* are the three most important bacteria which perform fiber degradation in the rumen (Cheng et al. 1991; Forsberg and Cheng 1992). Efficient fiber degradation depends on the optimum ruminal environmental condition, nature of the fodder, its stage of maturity, rate of passage of digesta, microbial communities involved, their attachment with the fibrous feed, and the nature and extent of cellulolytic, hemicellulolytic, and pectinolytic enzymes secreted (Fondevila and Dehority 1996; Mitsumori and Minato 1997). However, fiber degradation is also affected by other diverse microorganisms which utilizes starch, sugars, lipids, proteins, phytates, and other anti-nutritional compounds present in feed and fodders (Table 4.3). Also important are bacteria which thrive on and utilize fermentation end products of other microorganisms such as lactate-degrading bacteria, acetogens, acid utilizers, etc. (Castillo-González et al. 2014). The action of amylolytic and saccharolytic bacteria in the rumen is also important as high milk yielding cattle are fed on concentrates containing large proportions of grains. Starch and sugars are the readily fermentable source of energy for ruminants, and the major bacteria which thrive on them are *Streptococcus bovis, Bacteroides ruminicola*, *Ruminobacter amylophilus*, *Selenomonas ruminantium*, *and Succinomonas*

Table 4.3 Different types of anaerobic rumen bacteria, the enzymes involved, and the end products of fermentation

Type of rumen bacteria	Important bacterial species	Enzymes involved	Fermentative products
Fiber degraders			
Cellulolytic	*Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*, *R. albus*, *Clostridium cellobioparum*, *C. longisporum*, *C. lochheadii*, *Eubacterium cellulosolvens*	Endo-β-1,4-glucanase Exo-β-1,4-glucanase β-1,4-Glucosidase Cellulodextrinase O-Acetyl xylan esterase Ferulic acid esterase p-Coumaric acid esterase	Acetate, formate, lactate, butyrate, succinate, H_2, CO_2
Hemicellulolytic	*Prevotella ruminicola*, *Eubacterium xylanophilum*, *Eubacterium uniformis*	Xylocellulase Endo-β-1,4-xylanase β-1,4-Xylosidase α-L-Arabinofuranosidase α-Glucuronidase O-Acetyl xylan esterase Ferulic acid esterase p-Coumaric acid esterase	Acetate, formate, lactate, butyrate, succinate, H_2, CO_2
Pectinolytic	*Treponema saccharophilum*, *Lachnospira multiparus*	Pectin lyase Polygalacturonase Pectin methylesterase	Low-methoxyl pectin, polygalacturonic acid, disaccharides
Soluble carbohydrate (starch and sugar) fermenters	*Streptococcus bovis*, *Ruminobacter amylophilus*, *Prevotella ruminicola*, *Bacteriodes ruminicola*, *Succinomonas amylolitica*, *Succinivibrio dextrinosolvens*, *Selenomonas ruminantium*, *Lactobacillus acidophilus*, *L. casei*, *L. fermentum*, *L. plantarum*, *L. brevis*, *L. helveticus*, *Bifidobacterium globosum*, *B. longum*, *B. thermophilum*, *B. ruminale*, *B. ruminantium*	α-Amylase, maltase, invertase, sucrase, etc.	Formate, acetate, succinate, lactate, propionate, alcohols, H_2, CO_2
Proteolytic	*Prevotella ruminicola*, *Ruminobacter amylophilus*, *Clostridium bifermentans*	Proteases, peptidases, deaminases	Ammonia, amino acids, VFAs
Lipolytic	*Anaerovibrio lipolytica*	Lipases, biohydrogenases	Acetate, propionate, acetate

(continued)

Table 4.3 (continued)

Type of rumen bacteria	Important bacterial species	Enzymes involved	Fermentative products
Acid utilizers	*Megasphaera elsdeni*, *Wolinella succinogenes*, *Veillonella gazogene*, *Micrococcus lactolytica*, *Oxalobacter formigenes*, *Desulfovibrio desulfuricans*, *Desulfotomaculum ruminis*, *Succiniclasticum ruminis*	Lipases	Acetate, propionate, butyrate, succinate, Valerate, H_2, CO_2
Tanninolytic	*Streptococcus gallolyticus (Streptococcus caprinus)*, *Selenomonas ruminantium*, *Lonepinella koalarum*	Tannin acyl hydrolase (tannase)	Gallic acid, pyrogallol

Sources: Wang and McAllister (2002), Choudhury et al. (2015), Agarwal et al. (2015), Bhat et al. (1998)

amylolytica. These microorganisms ferment starch and sugars into VFAs such as acetate, propionate, butyrate, formate, and succinate. However, when large amounts of starch and sugars are introduced suddenly through a high grain-based diet, the ruminal pH drops below 5.5 and favors explosive growth of *Streptococcus bovis* which yields lactic acid as end product as against VFAs and alcohol on normal fermentation. This causes a further sudden drop of pH in the rumen and a potential lethal metabolic disorder of rumen called ruminal acidosis or lactic acidosis (Gressley et al. 2011). The condition may be avoided by gradual introduction of starch-based diet to facilitate growth of other amylolytic and saccharolytic bacteria and also lactic acid-degrading bacteria like *Megasphaera elsdenii*.

4.4.2 Proteolytic and Lipolytic Bacteria

There are bacteria which hydrolyze protein and non-protein nitrogenous substances of feedstuffs. About 60% of the proteins are degraded by ruminal proteolytic species *Bacteroides amylophilus, Bacteroides ruminicola, Butyrivibrio fibrisolvens*, *Streptococcus bovis*, and *Prevotella albensis* (Cotta and Hespell 1986; Sales-Duval et al. 2002). The degradation of proteins and NPN substances such as polypeptides, oligopeptides, amino acids, and urea causes production of ammonia, VFAs, and H_2S. Microorganisms utilize these and cause de novo synthesis of microbial protein. About 60% of protein needed by the host ruminant comes from the subsequent digestion of ruminal microorganisms in omasum and small intestine. Lipolytic microorganisms such as *Anaerovibrio lipolytica* causes lipolysis, and *Butyrivibrio fibrisolvens* causes saturation of unsaturated fatty acids by hydrogenation. Acidic and highly reductive environment of rumen, with surplus of H_2 coupled with the actions of microbial lipases and hydrogenase from these microorganism, favors saturation of polyunsaturated fatty acids (Maia et al. 2010).

4.4.3 Other Important Ruminal Bacteria

Lactate-degrading bacteria have important role in utilizing lactic acid, which is an intermediate product of fermentation in ruminants fed on high grain-based diets (Agarwal et al. 2015). As mentioned earlier, *Megasphaera elsdenii* is the main lactate degrader and is helpful in preventing ruminal acidosis when high grain diets are introduced gradually over an extended duration for adaptation on such diets. Further, there are important bacterial species, which causes degradation of pectin by pectinolytic enzymes, viz., pectin lyases, polygalacturonase, and pectin methylesterase (Dušková and Marounek 2001; Gordon and Phillips 1992). The important pectin-degrading bacteria are *Butyrivibrio fibrisolvens*, *Bacteroides ruminicola*, *Prevotella ruminicola*, and *Lachnospira multiparus*. There are some important bacteria which scavenge reducing potential through alternate hydrogen sink. These include acetogenic bacteria such as *Acetitomaculum ruminis* and *Eubacterium limosum* which reduce CO_2 to form acetate, a process which is called as reductive acetogenesis. Other alternate sinks are sulfate-reducing bacteria and bacteria which reduce fumarate to succinate (Asanuma and Hino 2000). Tannin-degrading bacteria such as *Selenomonas ruminantium* (Odenyo and Osuji 1998) and *Streptococcus gallolyticus* (Singh et al. 2011) with significant tannase activity and utilizing tannin as energy source have been identified. Similarly, mimosine-degrading bacteria *Synergistes jonesii*, isolated from rumen of Hawaiian goats, hydrolyze mimosine—a toxic phytochemical present in *Leucaena leucocephala* leaves—into a nontoxic compound, viz., 2,3-DHP (Allison et al. 1992).

4.5 Archaea

Archaea or methanogens represent about 3–4% of the total rumen microbial population (Sharp et al. 1998; Ziemer et al. 2000). Most of the methanogens remain free floating in the rumen fluid or stick to the feed particles, whereas some methanogens are ecto- or endosymbionts to other rumen microbes particularly bacteria and protozoa (Belanche et al. 2014; Valle et al. 2015). Although ruminal archaea constitute a minor proportion of the ruminal microbial population, but by capturing H_2 and converting them to CH_4, they create a ruminal environment conducive for efficient fermentation of the key nutrients by the other ruminal microbial population. The main fermentation end products of fiber, starch, sugars, and proteins from ruminant diets are VFAs, NH_3, CO_2, and H_2. While, VFAs and NH_3 are utilized as energy source and synthesis of microbial proteins, H_2 and CO_2 are taken up by these methanogens for conversion into methane (van Zijderveld et al. 2011). Although in ruminants methane production is the main sink of H^+ (Moss et al. 2000), it is a wasteful process in which 2–12% of gross energy is wasted through methane emission (Johnson and Johnson 1995). In livestock farming, about 80% of methane is produced through microbial fermentation in the rumen, while remaining 20% is

emitted through the decomposition of manure (Vergé et al. 2007). However, methane is a potent greenhouse gas, and its global warming potential has been revised to 27 times that of the CO_2 (IPCC 2007). Methane production from enteric fermentation of ruminants is a major global environmental concern which contributes to 20–25% of the total anthropogenic methane emissions (Thorpe 2009). Methane emission by dairy cattle, beef lot, buffalo, and sheep and goat is 18.9, 55.9, 6.2–8.1, and 9.5 Tg per year, respectively (McMichael et al. 2007).

The methanogens share their ancestral line with bacteria but has been placed into a separated domain Archaea and the phylum Euryarchaeota. As against the bacteria, methanogens lack peptidoglycan and instead may have pseudomurein, heteropolysaccharide, or a protein in their cell wall (Balch et al. 1979). Further, they have coenzyme M_{420} (having absorbance at 420 nm) associated with hydrogenase and formate dehydrogenase, which has blue-green fluorescence at 470 nm (Rouviere and Wolfe 1988). All methanogens also need coenzyme M, which they produce themselves, or they have a nutritional need (Hobson and Stewart 1997). This coenzyme M (or 2-mercaptoethanesulfonic acid) is a methyl group carrier which produces methane. There are about 28 genera of methanogens with about 113 species. The important methanogenic groups are *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, and *Methanopyrales*; among these, *Methanobacteriales* are the predominant archaeal group in the rumen (Nicholson et al. 2007). Archeal species which have been cultured are *Methanobacterium formicicum*, *Methanobacterium bryantii*, *Methanobrevibacter ruminantium*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanomicrobium mobile*, and *Methanoculleus olentangyi*. However, the most important methanogens of the rumen are *Methanobrevibacter*, *Methanomicrobium*, and a cluster of uncultured archaea called *Thermoplasmatales* or rumen cluster C (RCC) (Janssen and Kirs 2008).

4.5.1 Rumen Protozoa

Although Grubby and Delafond described the anaerobic rumen protozoa way back in 1843, much of the knowledge signifying their role and specific functions have originated only during the past few decades. Ruminal protozoa are by far only a small proportion of total microbial population of the rumen, but they make about 40–50% of the microbial biomass inside the rumen. Protozoan population in the rumen consist mainly of the two types of ciliate anaerobes: entodinomorphids (with firm pellicles and discreet cilia generally only on peristomes) and holotrichs (flexible pellicles, covered all over with cilia). Important holotrichs are *Isotricha, Dasytricha, Buetschlia*, and *Charonina*, and some important entodinomorphs are *Entodinium*, *Diplodinium*, *Epidinium*, *Eudiplodinium*, *Metadinium*, *Polyplastron*, *Eremoplastron*, *Elytroplastron*, and *Ostracodinium*.

Depending on the enzyme profiles, protozoa may be sugar and starch utilizers (amylolytic), cellulolytic, hemicellulolytic, pectinolytic, proteolytic, or lipolytic

(Williams and Coleman 1992). Holotrichs are generally soluble sugar utilizers and stores them as starch granules for use when starch and sugar supply are limited. This ability of protozoa for engulfing and storing starch grains and other soluble sugars prevents sudden decrease in ruminal pH and saves the host animals from lactic acidosis (Williams and Coleman 1992; van Zwieten et al. 2008). Entodinomorphs remain attached to fiber and digest cellulose, hemicellulose, pectin, and starch. Fiber-degrading entodinomorphs contribute about 19–28% of the total cellulase activity of the rumen. End products of fiber and soluble carbohydrate fermentation by ciliate protozoa are the same as that of ruminal bacteria, i.e., VFAs, H_2, and CO_2. Immediately after quick engulfing of starch and soluble sugars, protozoa migrate to the fibrous mat which are retained longer in the rumen, giving sufficient time to these slow multiplying ciliates (Hook et al. 2012). Thus, both holotrichs and entodinomorphs have comparatively slow passage rate and high retention time in the rumen, the former by migrating to the ruminal mat after feeding upon soluble sugars and starch granules and the latter by attaching to the fiber particles. Fibrolytic protozoa (entodinomorphs) can feed upon ruminal bacteria and degrade microbial protein and thus affect microbial protein utilization by host animal (Belanche et al. 2012). Defaunation (elimination of protozoa from rumen) has shown to decrease ruminal methane production and improve microbial protein supply (Ivan 2009).

4.6 Rumen Fungi

The ruminal anaerobic fungi were first discovered in 1910 and were initially assumed to be flagellate protozoa (Liebetanz 1910; Braune 1913). However, during the 1970s, Orpin's study helped them be recognized as anaerobic fungi (Orpin 1975, 1976, 1977). Anaerobic rumen fungi have been classified into six genera, viz., *Neocallimastix*, *Piromyces (Piromonas)*, and *Caecomyces (Sphaeromonas)*—all with monocentric sporangia—and *Orpinomyces*, *Anaeromyces (Ruminomyces)*, and *Cyllamyces*, with polycentric sporangia. These fungi represent a small proportion of the ruminal microbes (10^3–10^5 g^{-1} rumen content in number and 3–8% of the microbial biomass) but perform important function in fiber degradation through their cellulolytic and hemicellulolytic enzymatic profiles and breaking lignocellulosic bonds. Motile fungal zoospores get attracted to the fibrous feed particles through chemotaxis, colonize the feed particles followed by development of hyphae and mycelium (splitting feed particles mechanically), and remain attached firmly to the feed particles through their rhizoids (Denman et al. 2008). This adhesion is enhanced especially by lignified substrates, and thus fungi are especially important in breaking the lignocellulosic bonds of the recalcitrant structural plant cell walls. Fungal esterase enzymes such as feruloyl esterase, p-coumaryl esterase, and acetyl esterase break lignocellulosic and ligno-hemicellulosic ester bonds and release cellulose and hemicellulose for further microbial degradation (Yue et al. 2009). The rhizoid penetrates further into the feed particles through their polysaccharide-degrading enzyme system. Akin (1989) reported that non-lignified tissues such as mesophyll,

parenchyma, and phloem and the softer sclerenchyma tissue of leaf blades are degraded more extensively and completely; however, lignified xylem and sclerenchyma ring tissues in the stem are degraded partially by the fungi. Nevertheless, these breakdowns of the fibrous feed particles by the rumen fungi further enhance the site exposure and fiber degradation by fibrolytic enzymes from bacteria and protozoa.

4.7 Rumen Bacteriophage

Diversity of ruminal bacteriophages presents ample opportunity for exploring them to understand their genetic nature and interactions, lateral gene transfer, modulation of rumen fermentation, and controlling and eliminating certain ruminal microbes such as methanogenic archaea (Gilbert and Klieve 2015). Further, these are also explored for development of novel enzymes, modulation of host enzyme interactions, and application in treatment through novel branch of phage therapy. Rumen phages were first reported from bovine rumen during the 1960s (Adams et al. 1966), and soon it was established that these are common resident of the rumen affecting mostly the rumen bacteria. Rumen phages are present in significant number 10^8–10^9 particles mL^{-1} of the ruminal fluid; however, these are the least studied microbes of the rumen. Tailed phages belonging to the families of *Myoviridae*, *Podoviridae*, and *Siphoviridae* (Ritchie et al. 1970) and phage particles from tail-less *Tectiviridae* family are some important phages observed at the ruminal fluid. In spite of their diversity in type and number in the rumen fluid, the biological properties or genetic makeup of phages are poorly understood (Gilbert and Klieve 2015). Although majority of the ruminal phages are lytic phage, only a small portion are lysogenic (temperate) phage; pseudolysogenic phages which are present simultaneously with bacteria in the same culture, in which phages multiply only on a part of the bacterial population, have also been reported (Weinbauer 2004). It has been suggested that the specificity of these bacteriophages may be utilized for control and elimination of certain harmful bacteria such as *Streptococcus bovis* and methanogenic archaea (Klieve et al. 1999; Bach et al. 2002). Diversity of ruminal phages presents ample opportunity for exploring phages to understand their genetic nature and interactions, lateral gene transfer, modulation of rumen fermentation, and controlling and eliminating certain ruminal microbes (Gilbert and Klieve 2015). Further, these are also explored for development of novel enzymes, modulation of host enzyme interactions, and application in treatment through novel branch of phage therapy.

4.8 Conclusions

The dynamic and complex ecosystem of the rumen is maintained at a constant milieu well suited for the diverse inhabitant anaerobic microbial population to grow and multiply. Temperature, pH, buffering capacity, osmotic pressure, and redox potential are the primary factors affecting the growth and activity of these microbes. Efficient microbial fermentation ensures degradation of complex structural carbohydrates and other components of feed stuffs in addition to detoxifying certain potentially toxic substances. However, methane, which is an end product of microbial fermentation inside the rumen, is a potent greenhouse gas which is a major global environmental concern. Ruminal bacteriophages are the least explored among all the rumen microbes, but they provide enormous scope for their wider applications in controlling and eliminating certain ruminal microbes and their use in treatment through the novel branch of phage therapy.

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