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CHARACTERISTICS OF RUMINAL MICROBIAL COMMUNITY: EVOLUTIONARY AND ECOLOGICAL PERSPECTIVES

AMLAN KUMAR PATRA*

Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, Belgachia, K.B. Sarani 37, Kolkata 700037, India

*Corresponding author.

Email address: patra_amlan@yahoo.com (A.K. Patra)

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Abstract

Ruminants perhaps appeared about 50 million years ago (Ma). Five ruminant families had been extinct and about 200 species in 6 ruminant families are living today. The first ruminant family probably was small omnivore without functional ruminal microbiota to digest fiber. Subsequently, other ruminant families evolved around 18-23 Ma along with woodlands and grasslands. Probably, ruminants started to consume selective and highly nutritious plant leaves and grasses similar to concentrates. By 5-11 Ma, grasslands expanded and some ruminants used more grass in their diets with comparatively low nutritive values and high fibers. Historically, humans have domesticated 9 ruminant species that are mostly utilizer of low quality forages for human benefits. Thus, the non-functional rumen microbiota to predominantly concentrate fermenting microbiota, followed by predominantly fiber digesting microbiota had evolved for mutual complementary benefits of holobiont over the million years. The core microbiome of ruminant species seems the resultant of hologenome interaction in an evolutionary unit. The inertia and resilience properties of ruminal ecosystem seem to be due to this core microbiota, which makes the ecosystem most stable in response to perturbations because this core microbiota has evolutionary advantages with logically more generalists (i.e., wide metabolic versatile and redundancy). Also, a part of the ruminal microbiome shows highly plasticity, which is likely useful for evolutionary adaptability of holobiont. This review discusses ecological characteristics of ruminal microbial community in evolutionary perspectives. The updated understanding of ecological traits of ruminal microbiome would be helpful to better modulate the ruminal fermentation favorably for human benefits.

Keywords: Ecology, evolution, hologenome, microbiome, rumen

Introduction

Humans and ruminants have shared a long history over thousands of years. Prehistoric nomadic hunters perhaps recognized that the photosynthetic potential of grasslands could be exploited by domesticating ruminants to increase their food supply, which would allow them to form stable social communities (Russell and Rychlik, 2001). Humans had, thus, domesticated mostly such ruminant animals that can utilize low-quality fibrous diets (von Engelhardt *et al.,* 1985), not suitable for human consumption and will not compete for their foods. In the last 10,000 years, humans had domesticated nine ruminant species and they naturally prefer some grass in their diets, and except reindeer, all belong to Bovidae family (Hackmann and Spain, 2010).

Ruminant animals, ruminal microorganisms, and their biome (holobiome) had co-evolved for millions of years as an evolutionary unit, and the rumen harbors diverse and interdependent populations of bacteria, protozoa including flagellates, archaea, fungi including yeasts, and virus. Ruminants cannot produce fiber-degrading enzymes, but bacteria, fungi, and protozoa that are inhabited in the rumen of ruminant animals can digest fibrous materials. The host creates an appropriate habitat for growth of the microbiota, and the microbiota, in turn, supplies short-chain fatty acids (energy source; derived from the fibrous carbohydrates), protein, and vitamins to the animals, which is a complementary mutual approach for a holobiont (host and its microbiota). In the past 60-70 years, humans, however, have significantly changed the forage-based diets of ruminants to grain-based diets (Russell and Rychlik, 2001). Much of the ruminant production system is recently based on grain feeding, especially in technologically developed countries, to improve the productivity of ruminants (Russell and Rychlik, 2001) to fulfill the needs of burgeoning human population, which is against the evolutionary forces. Microorganisms in the rumen can also ferment soluble sugars and starch quickly, and these soluble materials increase fermentation rate. However, ruminants fed on fiber-deficient diets may not have proper homeostatic mechanisms of digesta flow, pH regulation, and gas removal, and the health and production of ruminants can be adversely affected (Russell and Rychlik, 2001). Furthermore, different nutritional interventions have been explored to modulate ruminal microbiome for enhancing the advantages and minimizing the drawbacks of ruminal fermentation such as reduction of methanogenesis, improvement of fiber digestions, alteration of fatty acid biohydrogenation, and enhancing nitrogen utilization efficiency (Belanche *et al.,* 2020). The modulation of microbiome perhaps has some limitations due to inherent characteristics of the microbiome and hologenome. In this review, different ecological properties of the ruminal microbiota are discussed in an evolutionary viewpoint which will be useful to modulate the ruminal fermentation for obtaining human and animal benefits.

Ruminants in evolutionary perspectives

The ecology and evolution of ruminants has been reviewed previously (Hackmann and Spain, 2010). Ruminants include any artiodactyl (mammalian in order Artiodactyla) possessing 3-4 chambered stomach (i.e., rumen, reticulum, omasum or isthmus, and abomasum). Tylopods were previously classified as ruminants, but they are not considered in ruminant groups (Cornelis et al., 2013). It has been recognized that 5 ruminant families (i.e., Hypertragulidae, Leptomerycidae, Gelocidae, Palaeomerycidae, and Dromomerycidae) had been extinct from the world (Hackmann and Spain, 2010). Hypertragulidae, Gelocidae, and Leptomerycidae were small ruminants without horns and they were perhaps most closely similar to moschids or tragulids that are living today (Webb and Taylor, 1980). Dromomerycidae and Palaeomerycidae were medium to large size ruminants with deer-like limb proportions and giraffe-like horns, and their ecological niche was perhaps similar to subtropical deer (Janis and Scott, 1987).

Six ruminant families living today include Tragulidae, Moschidae, Cervidae, Antilocapridae, Giraffidae, and Bovidae (Hackman and Spain, 2010). Tragulidae (chevrotains) are the most primitive among all living ruminant families without changing morphologically to a large extent over evolutionary history, and are small forest-dwelling deer-like animals (Hackman and Spain, 2010). Moschidae (e.g., musk deer) are small forest deer in the Southern Asia, particularly in the Himalayas. The remaining 4 families, Bovidae (e.g., cattle, sheep, goats, and antelopes), Cervidae (true deer; e.g., red deer, caribou, moose, elk, and white-tailed deer), Giraffidae (giraffe and okapi), and Antilocapridae (pronghorn) are more common (Hackman and Spain, 2010). The median body weight of modern ruminants is 45 kg with body weight ranging from about 2 kg (e.g., royal antelope, Salt's dik-dik, and lesser Malay mouse deer) to 800 kg (e.g., bison, gaur, and Asian water buffalo).

In the evolutionary history of ruminants, Hypertragulidae were possibly the first to be evolved about 50 million years ago in Southeast Asia (Hernández Fernández and Vrba, 2005). This first ruminant family evolved was small (<5 kg) omnivores (Hernández Fernández and Vrba, 2005) without functional rumen microbiota for fiber fermentation and did not ruminate until about 40 million years ago (Jermann *et al.,* 1995). The remaining families appeared approximately 18 to 23 million years ago (Janis, 1982) in Eurasia (Moschidae, Antilocapridae, Dromomerycidae, Palaeomerycidae, Cervidae, and Bovidae) and Africa (Giraffidae). In this time, the climate was drier and cool, and consequently temperate woodlands were the most prevalent flora (Janis, 1982; 1993). These ruminant families started to consume primarily leaves or mixture of grass and leaves (Semprebon and Rivals, 2007; DeMiguel *et al.,* 2008). The body weights of these ruminant species were initially about 20 to 40 kg (Janis, 1982). By around 5 to 11 million years ago, grasslands had expanded (Jacobs *et al.,* 1999), and some ruminant animals continued to include more grass in their diets.

The first domesticated livestock species (ruminants or non-ruminants) was goat at around 8,000 BC in the Near East (Zeder and Hesse, 2000). They were originally domesticated for meat purpose whose population had depleted in the wild due to hunting and predating (Clutton-Brock, 1999; Diamond, 2002). The majority of other 8 ruminant species (European and Zebu cattle, Bali cattle, sheep, water buffaloes, reindeer, yaks, and mithuns) were domesticated by 2,500 BC in either the Southern Asia or Near East (Clutton-Brock, 1999). Some of these species, such as the goat, were initially domesticated for meat, but later for milk, transportation, sacrifice, draft, and trade (Clutton-Brock, 1999).

Feeding habits in evolutionary perspectives

Of approximately 200 ruminant species in 6 families living today (Hackman and Spain, 2010), only about 25% are "grass and roughage eaters" (e.g., sheep, cattle, water buffalo, and banteng) which are well-equipped with digestive systems to process fibrous forages rich in structural plant cell wall carbohydrates (Hofmann, 1989). More than 40% of living ruminant species are ‘concentrate selectors’, which are far less adapted to plant fiber digestion, but are most adapted to processing easily digestible, natural high quality forage diets, rich in soluble cell contents (Hofmann, 1989). Their exceptionally distinct selective ability is based primarily on olfactory signals to choose highly nutritive forage diets. In this category, there are no domesticated ruminant species (i.e., dikdik, roe deer, and moose). Approximately 35% of all ruminant species are morphophysiologically intermediate (e.g., goats, sika deer, and impala) between the two extreme types and they have a greater extent of forage selectivity and prefer a mixed diet but avoid fiber-rich diets as long and as much as possible (Hofmann, 1989). From the evolutionary trend, concentrate selectors have higher selectivity for plant cell contents, hydrochloric acid producing tissue in abomasum, postruminal (caecocolic) fiber digestion ability, ruminal amylolysis capacity with possibly bypass of solubles (reticular groove), food passage rate, and lower fiber digestion (cellulolysis) capabilities in rumen, total intestinal length, and rumino-reticular weight and capacity relative to body mass, whereas they are vice versa for the grazers (Hofmann, 1989).

Ruminal microbiota

Ruminal microbial ecosystem is a complex and diverse comprising of mostly anaerobic prokaryotes (bacteria and archaea), eukaryotes (protozoa and fungi), and virus. This microbial community has highly correlated microbial interaction networks (Patra *et al.,* 2019) and is essential for coordinated digestion of feeds, especially cell wall carbohydrates. Bacterial community (number $>10^{10}$/g of ruminal content) is most diverse and dense with wide ranging metabolic activities. The number of bacterial species present in rumen is not still clear even using next generation sequencing techniques. Kim *et al.* (2011) predicted 5721 bacterial OTU in about 3500 genera and 943 archeal OTU in 670 genera. From metagenomic sequencing of rumen samples of 42 cattle, Stewart *et al.* (2018) reported 913 novel microbial genomes. However, the same authors recently reported over 4941 microbial genomes after assembling metagenomic sequencing from the rumen of 283 cattle (Stewart *et al.,* 2019). Stewart *et al.* (2019) compiled 4941 genomes along with other publicly available genome dataset, which resulted in a total of 5845 rumen microbial genomes. The analysis of this total set of genomes indicated the presence of 2690 unique species and 5574 strains of microorganisms and the authors estimated there are about 3276 species in the rumen of cattle. Seshadri *et al.* (2018) reported 480 bacterial genomes from rumen of different ruminant species in Hungate 1000 collection project. The Hungate collection, as per Stewart *et al.* (2019), represents only a small part of the diversity in their microbial genomes that have been assembled from metagenomic sequencing. Protozoa (number 10^5 to 10^6/g of ruminal content) can contribute up to 50% of the ruminal microbial biomass due to their larger size and most ruminal protozoa are ciliate species, with a few flagellates (Newbold and Ramos-Morales, 2020). The diversity and density of the protozoa are lower, and they have possibly lower metabolic activity compared with the bacterial community in the rumen. The benefits of protozoa in the rumen are controversial. Protozoa contributes digestion of fiber and stabilization of ruminal pH by consuming lactate and engulfment of starch, but it is responsible for methane production (endo- and ecto-symbiotic relationship with methanogens) and decreasing the efficiency of protein utilization in ruminants. Rumen fungi (10^4 to 10^5/g of ruminal content) may contribute up to 10% to 20% of the rumen microbial biomass based on rRNA transcript abundance and chitin measurements (Huws *et al.,* 2018). Rumen fungi are important fiber degraders, especially in poor quality forage-base diets. The close association of ruminal fungi with methanogens has been shown (Edwards *et al.,* 2017), which may increase the activity of fungi and contribute to ruminal methane production (Newbold and Ramos-Morales, 2020). Several genomes and transcriptomes of rumen fungi have been reported recently compared to what was known earlier (Edwards *et al.,* 2017). Yeasts are also present in the rumen (Fernandes *et al.,* 2019), but their roles remain poorly elucidated. Methanogenic archaea inhabit in the gut of both ruminant and non-ruminant animals (Misiukiewicz *et al.,* 2020). Methanogenic archaea (10^8 to 10^9/g of ruminal content) is responsible for methane production, which is a greenhouse gas. Thus, several strategies have been explored to mitigate methane emissions from ruminants (Patra, 2016). The archaeal diversity is lower than that of the bacterial population and 21 archaeal genomes from rumen have recently been isolated and characterized via the Hungate 1000 (Seshadri *et al.,* 2018). In the majority of the studies, *Methanobrevibacter* genus is the most the most abundant methanogens in the rumen, which are hydrogenotrophic producing methane from H_2, CO_2 and formate produced by bacteria, protozoa, and fungi (Newbold and Ramos-Morales, 2020). Other important hydrogenotrophic methanogenic genera include *Methanosphaera, Methanimicrococcus* and *Methanobacterium* (Newbold and Ramos-Morales, 2020). Methylotrophic methanogens, e.g., *Methanosarcinales, Methanosphaera,* and

Methanomassiliicoccaceae, which produce methane from methanol and methylamines, and aceticlastic methanogens, e.g., *Methanosarcinales*, which generate methane from acetate (Patra *et al.,* 2017; Newbold and Ramos-Morales, 2020) are less abundant in the rumen. The function of rumen virome is poorly known. Recently, genome sequence of ruminal lytic phages has been published (Gilbert *et al.,* 2017), metagenomic analysis of ruminal virome has been performed (Namonyo *et al.,* 2018), and the presence of RNA viruses infecting fungi (mycoviruses) has been reported (Hitch *et al.,* 2019). However, their effects on ruminal fermentation, microbiota and feed degradation require further investigation. The ruminal microbiome possesses a few distinct ecological characteristics, which have been discussed below including evolutionary perspectives.

Host individuality

Recently, host microbiome individuality concept has received considerable interest, which means gut microbiota contains a set of taxa common in all animals (a "core microbiome"). There are also a large number of taxa, which varies among hosts. The hologenome (microbiome plus host genome) theory suggests that the microbiome and their genome jointly make an evolutionary unit undergoing adaptation in the selection pressure (Shapira, 2016). The microbiota comprises a multilayered community structures, i.e., a host-adapted core microbial pool, an environmentally modulated flexible microbial community, and a range of intermediates in between them (Shapira, 2016).

The rumen of cattle has been shown to contain a core microbiome in different studies (Jami and Mizrahi, 2012; Petri *et al.,* 2013). Sasson *et al.* (2017) indicated that a number of ruminal bacterial operational taxonomic units (OUT, a proxy for species) were greatly heritable in dairy cattle. In a recent study, lambs shifted from a non-grazing concentrate diet to a forage grazing diet had a core microbiota composed of 34 for bacterial genera, which were shared by all sheep, regardless of diets (Belanche *et al.,* 2019). The core bacterial community was formed by

Prevotella, Treponema, Ruminobacter, Succinivibrio, Fibrobacter, Sporobacter, Ruminococcus, Anaeroplasma, and *Selenomonas*, representing 20% of the ruminal bacterial community composition. Moreover, this core community composition was mostly similar to those reported in another study with dairy cattle fed different diets in which 32 genera were common to 16 animals (Jami and Mizrahi, 2012). By analyzing microbial community in 742 samples of rumen content of 32 animal species from 35 countries, seven abundant bacterial groups, *Prevotella*, *Ruminococcus*, and *Butyrivibrio* as well as unclassified *Bacteroidales, Clostridiale, Lachnospiraceae*, and *Ruminococcaceae*, comprising of 67.1% of all bacterial sequences were detected as a "core bacterial microbiome" (Henderson *et al.,* 2015). Stewart *et al.* (2019) suggested that Proteobacteria would also fall into the core rumen microbiome along with above 7 bacterial groups. However, abundances of these core bacterial groups were not equal among all animal species (Henderson *et al.,* 2015). Across different studies, the core ruminal microbiota of cattle generally includes members of Firmicutes family (particularly *Ruminococcus* and *Butyrivibrio* genera) and Bacteroidetes family (especially *Prevotella* genus), together with a number of taxa present in lower abundance. The core microbiota composition is stable independent of diet or host genetic differences (Petri *et al.,* 2013; Henderson *et al.,* 2015).

The host specificity of the ruminal microbiota is not only restricted to Bacterial domain, because it has also been reported for the members of Archaeal and protozoal communities (Zhou *et al.,* 2012). In beef cattle, relative abundances of archaea were consistent indicating that archaeal abundance in the rumen is under host genetic influence (Roehe *et al.,* 2016). In sheep, 9 and 13 genera of methanogens and fungi comprised of the core microbiota (Belanche *et al.,* 2019). It has been suggested that as number of animals in each study was small, the number of genera in the core microbiome may be lower with the inclusion of a global population of hosts (Weimer, 2015). The metabolic functions to the members of this core community are broad performing as 'generalists'.

Much research has now centered on the elucidation of core microbiome in the rumen, which may be a major regulator of overall ruminal fermentation stabilizing other microorganisms in the ruminal ecosystem. Because few ruminal microbial species are highly correlated with other species (Figure 1; Patra *et al.,* 2019), the identification of this species in the core microbiome, if present, would be useful for understanding better rumen function. Also, because this core microbiome is a part of holobiont, correlations between host genome and bacterial genome may identify the drivers of this core microbiome. At this point of research, it is unclear when this compositionally and functionally unique community is assembled, what biotic and abiotic factors determine an early establishment and maintenance of this community, and whether any of its core members have a large influence on the ruminal environment (Weimer, 2015). The use of recent metagenomic and bioinformatic tools may unravel this scientific understanding of the poorly characterized core ruminal microbiome in future.

Redundancy

In a microbial community, redundancy is the overlapping metabolic and physiological functions of multiple microbial taxa. This means a number of microbial specifies can degrade a particular feed substrate (e.g., different polymeric substrates, monomeric units, and linkages between monomeric units) and utilize intermediate metabolites. Although there is great complexity and diversity of feeds consumed by ruminants, the small number of degradation sites of feeds or monomeric units and metabolites is relatively less (Weimer, 2015). Conversely, there are a large number of microbial species in the rumen. For instance, most next generation sequence studies have obtained 100–1000 of OTU in ruminal samples (Weimer, 2015). This indicates that there are usually several species that potentially degrade each substrate and metabolize each intermediate (Figure 2). Moreover, several species can contribute to the degradation of a number of substrates or breakdown many linkages in feeds as ‘generalists’. For example, xylan or xyloglucan can be degraded or pyruvate can be metabolized to acetate by the 8 major genera representing 70% of the total abundances (Figure 2) (Seshadri *et al.,* 2018). Again, *Fibrobacter* can participate in the many points in the breakdown and metabolism of carbohydrate (Figure 2). Besides carbohydrate degradation, *Prevotella* and *Butyrivibrio,* two most abundant bacterial genera with numerous OTU from each of these genera (Pitta *et al.,* 2010; Li *et al.,* 2012; Mohammed *et al.,* 2014) display extremely a wide range of degradative capabilities including proteins and peptide hydrolysis, and fermentation of several amino acids, and intermediates (Kelly *et al.,* 2010). Therefore, it is evident that the microbial community in the rumen is highly redundant in its composition from a metabolic aspect. However, it is unclear which particular species or strain performs a degradative function predominantly in situ when many competitors and symbionts are present in the degradation sites (Weimer, 2015). For instance, *Butyrivibrio fibrisolvens* YE44 showed rapid and dense growth in the presence of wheat starch as the sole energy substrate and carbon source in the in vitro growth medium, this capability was not evident in the in vivo conditions upon introduction to the rumen of cattle fed grain rich diets because its abundance declined rapidly and was not detectable 8 days after introduction (Klieve *et al.,* 2003). Perhaps the role of *Butyrivibrio* as a starch degrader is minimal as other starch degraders outcompete *Butyrivibrio* in vivo. Acetogens that produce acetate using hydrogen and carbon dioxide are present in the rumen at a low number, but no acetate is produced from carbon dioxide in the rumen confirmed using labeling studies (LeVan *et al.,* 1998). The acetogens is not thermodynamically favorable to compete with the methanogens for available hydrogen, as indicated by their poorer affinity for hydrogen, lower free energy yield per mole of hydrogen consumed, and slower growth rates (Ungerfeld, 2013). Still acetogens can present in the rumen in low number due to their versatile capability to survive by catabolizing a broad range of organic compounds (Rieu-Lesme *et al.,* 1996). It has been suggested that acetogens in the rumen may participate in reduction of carbon dioxide with hydrogen to produce acetate when the competitors such as methanogens are inhibited. In general, despite the presence of keystone functional groups of microbiota, such as fibrolytic microbes and methanogens, many species represent each of these functional groups in the rumen. Due to this redundancy, it is doubtful that any one species would have a special role in the rumen contributing predominantly to the ruminal fermentation, which would be designated as keystone species (Weimer, 2015).

It has also been predicted that catabolic redundancy of the ruminal microbiota would be greater for the abundant and degradable substrates and their monomers (Weimer, 2015). Consequently, the number of species metabolizing glucose becomes higher than those can degrade less abundant substrates; for example, oxalate (Weimer, 2015). Thus, the ruminal microbial community contains a mix of ‘generalists’ that utilize many abundant substrates, and ‘specialists’ that utilize a smaller number of less abundant substrates and also face much less competition. Therefore, despite substantial changes in microbial community composition, alterations in fermentation characteristics such as pH, volatile fatty acid concentrations or molar proportions are not reflected as a result of redundancy of the ruminal microbial community (Sandri *et al.,* 2014; Patra *et al.,* 2019). A study with dietary menthol in lambs showed that menthol altered a few microbiota compositions, including a decrease in certain methanogens numbers, an increase in microbiota diversity, and microbial and metabolic networks (Patra *et al.,* 2019). However, these changes did not translate to ruminal microbial fermentation because the ruminal species interacted both positively and negatively in response to menthol (Figure 1), indicating that stable ruminal fermentation is maintained in spite of minor microbial changes due to metabolic redundancy of the ruminal ecosystem (Patra *et al.,* 2019).

Resilience

Ruminal microbiota responds to perturbation by different physical (e.g., temperature), chemical (e.g., diet composition, ruminal pH or plant toxins), or biological (e.g., introduction of non-native microbes) changes like any microbial ecosystem (Weimer, 2015). Intensity, frequency and duration of perturbations may differ, which may determine the nature of the microbial responses. Some perturbations, for example, variations in feed intake patterns or water consumption are common features, whereas other perturbations such as gradual change in forage quality in grazing fields are noticeable in the ruminal environment (Weimer, 2015). The response of the ruminal microbiome to these perturbations can be better explained in terms of *inertia* and its *resilience* (Westman, 1978; Weimer, 2015. From classical ecology theory (Westman, 1978), inertia (i.e., ability to resists a change) reflects the stability of the ruminal ecology, while resilience indicates how the ruminal microbial community returns back once its community has been altered. In ecosystem, the properties of inertia and resilience are simply examined at the individual species level over time or across spatial domains (Westman, 1978). Because of high species diversity and complex interactions among organisms in ruminal habitats, exploring the influencing factors of inertia and resilience would be difficult for individual species and more difficult for entire microbial communities (Weimer, 2015). Nevertheless, these concepts could be useful to theoretically supporting the discussions on how successfully the ruminal microbiota and fermentation might be manipulated (Weimer, 2015).

Few experiments were performed for dosing with fibrolytic microbes in the rumen to improve fiber digestibility. But inoculation of fiber degrading microbes of ruminal origin has not been of much success due to resilience nature of the ruminal communities (Weimer, 2015). Varel *et al.* (1995) isolated a *Clostridium longisporum* strain from the rumen of bison, and a *Clostridium herbivorans* strain from the intestinal tract of pigs, which had greater fiber degrading activity in pure culture than the common ruminal cellulolytic bacteria (*Fibrobacter succinogenes, Ruminococcus albus*, and *Ruminococcus flavefaciens*). These fermenter-grown *Clostridium* cultures were dosed into ruminal cannulated cows whose rumen content was almost completely emptied and then the cows were fed with hay (Varel *et al.,* 1995). These *Clostridium* strains were expected to establish in the rumen of cows fed fiber rich hay diets. However, these strains were not detectable, generally within 24 h of dosing. These strains were isolated from the rumen or hindgut, but they are present at low levels in the ruminal habitat, which indicated that these *Clostridium* strains were not very much competitive in the rumen and the quantitatively modest residual ruminal community was quickly and easily capable to expel the inoculated species. It may suggest that native (*autochthonous*) fiber hydrolyzing microbes in the rumen of each individual animal are adequately adapted in their native habitat to oust non-native (*allochthonous*) species from other habitats, including the rumen of other animals (Weimer, 2015). Besides abiotic stressors, an introduced species or strain needs to face many opponents, i.e., direct competitors along with their co-adapted symbionts with which they have productive mutualistic relationships, and finds few friendly microbes that may get immediate benefits from cooperating with the dosed species (Weimer, 2015).

In contrast to cellulolytic microbes that have many competitors, establishment of dosed strains with non-fibrolytic ‘specialist’ microbes in the rumen has been successful in a few cases. For example, ruminal inoculation of *Synergistes jonesii* to degrade minosine present in *Leucaena leucocephala* shrub conferred resistance to mimosine poisoning in the recipient animals (Jones and Megarrity 1986; Allison *et al.,* 1992; Hammond, 1995). In another study, *Megasphaera elsdenii* YE34 (a lactate utilizer) culture isolated from the rumen of cattle was introduced to cattle, which immediately established in the rumen of inoculated grain-fed cattle, 7-10 days earlier than in uninoculated cattle (Klieve *et al.,* 2003). Similarly, dosing of a recombinant strain of ruminal *Butyrivibrio fibrisolvens* containing dehalogenase gene from a *Pseudomonas* strain successfully established in sheep for several months and provided enhanced tolerance to fluoroacetate (Gregg *et al.,* 1998). These studies suggested that inoculation success depends upon the introduced species or strains that fill an open niche along with highly specialized metabolic activities and substantial substrate concentrations. There are also a few other specialists such as nitrate reducers and sulfate reducers which can act as electron acceptors in the rumen and they are metabolically favorable compared with the methanogens (Figure 2). However, the substrate availability for these ‘specialists’ that have less competitors are low in the rumen. Increasing the substrate availability may increase their abundances and reduce methane production in the rumen (Patra *et al.,* 2017).

Plasticity

Outside the core microbiome, there is perhaps extensive variability and metabolic plasticity of the microbiota composition in the rumen among individual animals, which is altered easily in response to diets, genetics and other abiotic factors. Microbial diversity and density change in response to diets (Henderson *et al.,* 2015; Petri *et al.,* 2013). For an example, when sheep were shifted from a concentrate-hay diet to pasture diet, diet-specific core microbial community was small in size (<2%) but had increased concentrations and diversity, which suggested that degradation of pasture is a complex process which needs a coordinated work by the diverse consortium of microbiota (Belanche *et al.,* 2019). The correlations between the ruminal fermentation variables and abundance of microbial taxa were not consistent across diets, which suggest that microbiota adapted to different substrates and shifted their fermentation products (Belanche *et al.,* 2019). Also, both the overall structure of the methanogenic community and the abundance of 55% of the methanogens taxa were affected by the diets consumed by the ruminant indicating a larger diet adaptation process for the methanogens than for the bacterial community (Popova *et al.,* 2013). This may suggest that most of the adaptation processes to the diets do not replace bacterial genera but increase abundance or diversity of microbiota or modified their metabolic activity (Belanche *et al.,* 2019). Moreover, microbiome composition after feeding changes considerably manifested as substantial alterations in the relative abundance (3–5-folds) of methanogens and major bacterial taxa such as *Prevotella*, which was independent of individual host variation and diet (Shaani *et al.,* 2018). The high plasticity of the ruminal microbial community in response to dietary and ruminal environmental pressure is likely to be useful for evolutionary adaptability of the holobiont.

Co-occurrence

The presence of a microbe in the rumen is highly dependent on other microbes. The classical example is ecoto- and endosymbiotic relations between protozoa and methanogens (Patra *et al.,* 2017). Therefore, defaunation (reduction of protozoal number) causes reduced number of total methanogens in the rumen, at least, some methanogenic species (Patra *et al.,* 2016; 2017). Ruminal fungi and methanoogens may also have a symbiotic relation (Edwards *et al.,* 2017) where both communities seem share mutual complementary metabolic activities. Similarly, the correlated networks of the bacterial species may suggest co-occurrences and interdependence of some bacterial species, but this relation is not well studied (Patra *et al.,* 2019).

Conclusions

Ruminal microbial community has co-evolved as a part of hologenome of an evolutionary unit over 20-30 millions years. Thus, the core microbiota of a ruminant species seems the resultant of the host-microbiota (symbiont) interaction for mutual benefits. The intertia and resilience properties of ruminal ecosystem seems to be due to this core microbiota, which make the ecosystem most stable in response to biotic and abiotic forces in the rumen because this core microbiota has evolutionary advantages. This core microbiota has logically more generalists with wide metabolic versatile and redundancy. Also, a part of the ruminal microbial community shows highly plasticity in response to dietary and ruminal environmental pressure, which seems to be useful for evolutionary adaptability of the holobiont. The updated understanding of host-specificity, redundancy, resilience and plasticity of the ruminal microbiome ecology would be useful to better modulate the ruminal fermentation in a favorable way and obtain benefits from ruminants.

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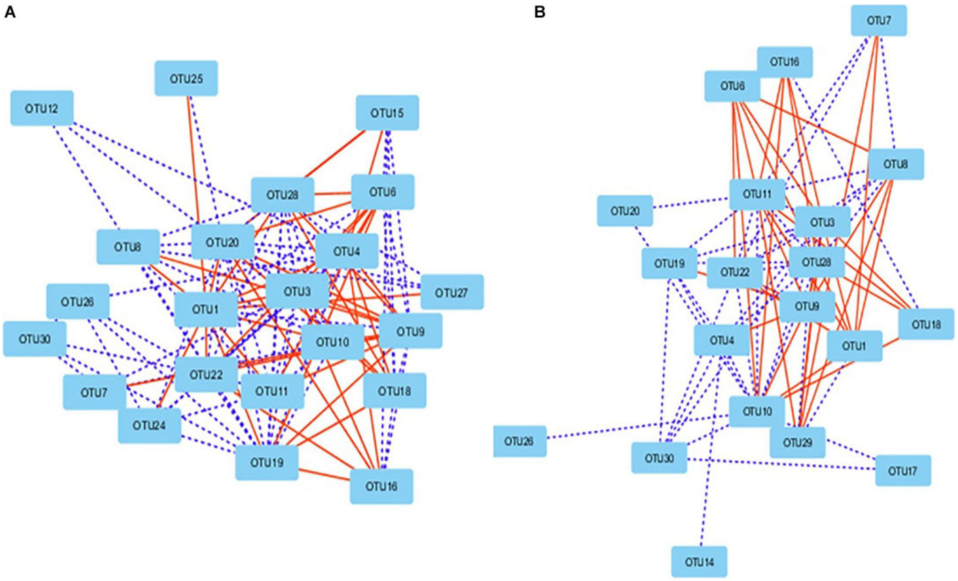

Figure 1. Correlation networks showing correlations between major operational taxonomic unit (OTUs, a proxy for species; each representing abundance of ≥0.5% total sequence abundances) in the rumen of the control sheep (A) and menthol-fed sheep (B). Dotted lines indicate positive correlations, while solid lines represent negative correlations. The shorter the distance between two nodes, the stronger the correlation between them (Patra *et al.,* 2019).

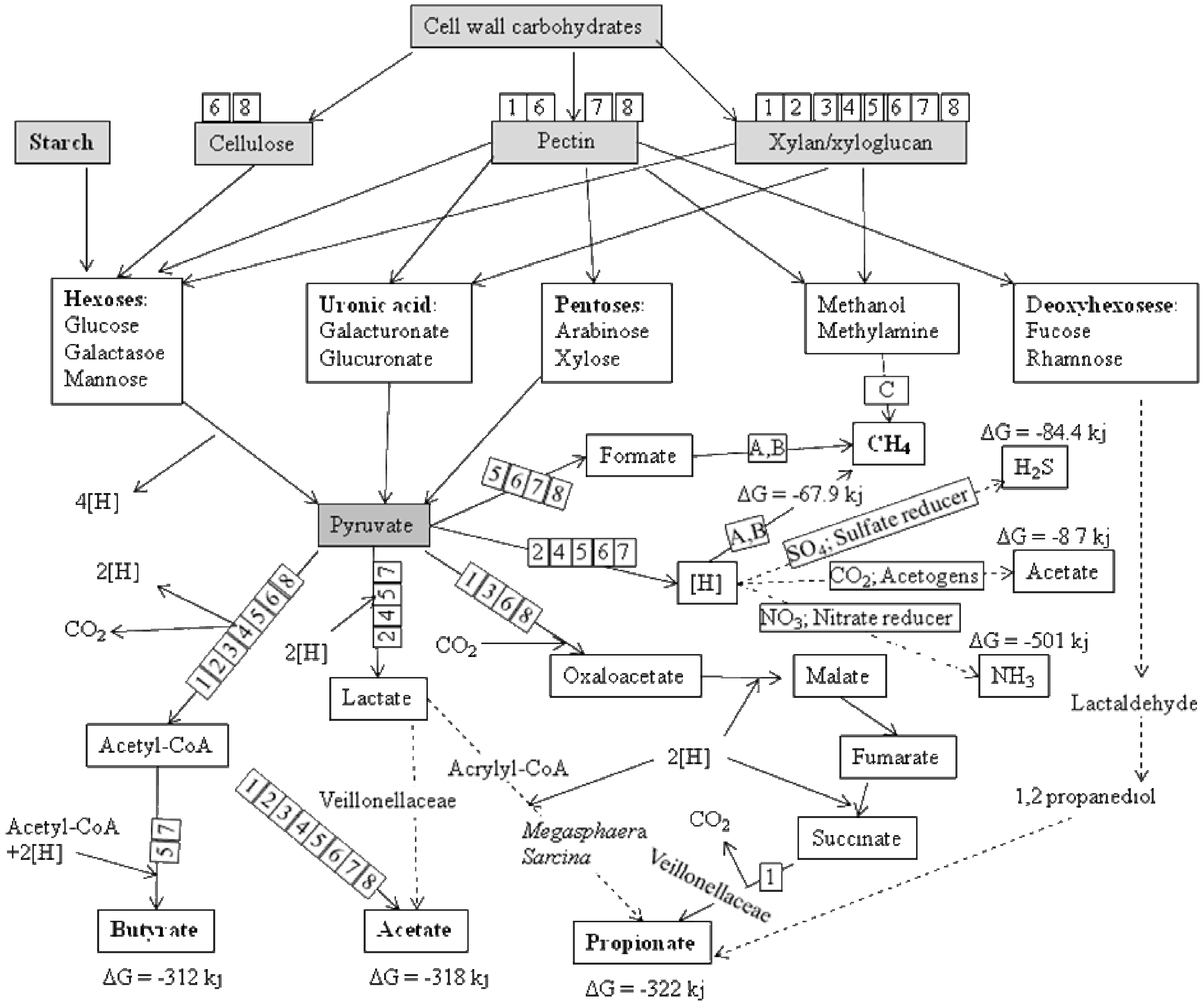

Figure 2. Fermentation of carbohydrate and their products by ruminal microbiota. 1- *Prevotella*, 2 – Clostridiales, 3- Bacteroidales, 4 – Ruminococcaceae, 5 – Lachnospiraceae, 6 – *Ruminococcus*, 7 – *Butyrivibrio*, 8 – *Fibrobacter*. A - *Methanobrevibacter gottschalkii*, B – *Methanobacter ruminantium*, C - Methanomassiliicoccales group 12 sp. Solid lines indicate the fermentation by dominant pathways and the dotted lines indicate the other minor pathways. Adapted with modifications (Seshadri *et al.,* 2018; Beauchemin *et al.,* 2020).