Meat and Muscle Biology™

The Rumen Epithelial Microbiota: Possible Gatekeepers of the Rumen Epithelium and Its Potential Contributions to Epithelial Barrier Function and Animal Health and Performance

Stephan Schmitz-Esser*

Department of Animal Science, Iowa State University, Ames, IA 50011, USA
*Corresponding author. Email: sse@iastate.edu (Stephan Schmitz-Esser)

Abstract: Ruminants are characterized by their unique mode of digesting cellulose-rich plant material in their forestomach, the rumen, which is densely populated by diverse microorganisms that are crucial for the breakdown of plant material. Among ruminal microbial communities, the microorganisms in the rumen fluid or attached to feed particles have attracted considerable research interest. However, comparatively less is known about the microorganisms attached to the rumen epithelium. Generally, the tissue lining the gastrointestinal tract serves the dual role of absorbing nutrients while preventing the infiltration of unwanted compounds and molecules as well as microorganisms. The rumen epithelium fulfills critical physiological functions for the ruminant host in energy absorption, metabolism, and nutrient transport. Essential host metabolites, such as short-chain fatty acids, ammonia, urea, and minerals, are exchanged across the rumen wall, thereby exposing the rumen epithelial microbiota to these nutrients. The integrity of the gastrointestinal barrier is central to animal health and productivity. The integrity of the rumen epithelium can be compromised by high ruminal microbial fermentation activity resulting in decreased rumen pH or by stress conditions such as heat stress or feed restriction. It is important to keep in mind that feeding strategies in cattle have changed over the last decades in favor of energy- and nutrient-rich concentrates instead of fiber-rich forages. These dietary shifts support high milk yields and growth rates but raised concerns regarding a possibly compromised rumen function. This paper will provide an overview of the composition of rumen epithelial microbial communities under physiological and disease conditions and will provide insights into the knowledge about the function and *in situ* activity of rumen epithelial microorganisms and their relevance for animal health and production. Given that an impaired intestinal barrier will negatively affect economically significant phenotypes, a better understanding of rumen wall microbiota is urgently needed.

Key words: rumen epithelium, rumen wall, microbiota, epimural
Meat and Muscle Biology 4(2): 19, 1–11 (2021) **doi:10.22175/mmb.11672**
Submitted 28 September 2020 Accepted 9 February 2021

This paper was accepted as a contribution to the 2020 International Congress of Meat Science and Technology and the AMSA Reciprocal Meat Conference.

Introduction

Ruminants are important for humans in that they produce milk and meat as major protein sources for human nutrition. The symbiosis between ruminant animals and the microbiota present in their gastrointestinal (GI) tract is of critical importance for animal health and performance (O'Hara et al., 2020). Because ruminants lack the digestive enzymes for cellulose degradation, ruminants rely on their GI-tract microbiota to convert otherwise indigestible plant material into fermentation products that can be utilized by the ruminant host animal. In turn, the ruminant will utilize these microbial fermentation products

such as short-chain fatty acids (SCFA) to produce meat and milk, which can be used directly as food or as a basis for meat- and dairy-based food products for human consumption. A unique feature of ruminants is the presence of a stomach consisting of 4 compartments: the omasum, abomasum, reticulum, and rumen. Among those, the rumen is an enlarged, strictly anaerobic fermentation chamber that is critical for the degradation of dietary plant material. The rumen itself can be divided into subsections including the dorsal, ventral, and cranial sac and the caudodorsal and caudoventral blind sacs. The rumen harbors a high density and diversity of microorganisms consisting of bacteria, archaea, protozoa, fungi, and bacteriophages (Gruninger et al., 2019; Morais and Mizrahi, 2019; Newbold and Ramos-Morales, 2020). These microorganisms can either be attached to plant material present in the rumen, be found planktonic in the liquid fraction of the rumen, or be attached to the rumen epithelium (Cho et al., 2006; Sadet et al., 2007; De Mulder et al., 2017). The identity and function of key rumen content microorganisms and their role in the digestion of plant material has been characterized over the last decades using a variety of approaches, including cultivation, *in situ*, and sequencing approaches (Henderson et al., 2015; Seshadri et al., 2018; Gruninger et al., 2019; Morais and Mizrahi, 2019; Stewart et al., 2019; Li et al., 2020; Newbold and Ramos-Morales, 2020). In contrast, our knowledge about the properties and physiological function and activity of the microorganisms attached to the rumen epithelium is still highly limited. The paucity of research on rumen epithelial microorganisms may—at least partially—be explained by the difficulty of sampling the rumen epithelium. This paucity of research on rumen epithelial microorganisms is somewhat surprising given that the rumen epithelial microorganisms are located at a pivotal position regarding host animal health and nutrient exchange between the rumen content and the rumen epithelial tissue. More generally, in humans and animals there is an increased interest in studying the GI-tract epithelial microbiota because the GI-tract epithelium is important for maintaining the GI-tract barrier function. An increased intestinal permeability has also been referred to as leaky gut (Stewart et al., 2017). Conditions leading to leaky gut symptoms can be manifold, including stress, infection, or inflammation. Because GI-tract epithelial surfaces are covered by microorganisms, these epithelial microbial communities are likely intricately involved in metabolic processes occurring at or across the GI-tract epithelium. Animal agriculture conditions in which the GI-tract barrier function is compromised can include, e.g., heat stress and/or rumen acidosis (Khafipour et al., 2009; Baumgard and Rhoads, 2013; Minuti et al., 2014). It is conceivable that an impaired intestinal barrier function could negatively affect economically important phenotypes in livestock. Indeed, in dairy cattle, a decrease in intestinal barrier function can lead to a reduction in productivity (Kvidera et al., 2017). Thus, more research is warranted to determine possible contributions of the rumen epithelial microbial communities to the rumen barrier function. This paper provides an overview of the composition of rumen wall microbial communities under physiological and disease conditions and insights into the current knowledge about the function and *in situ* activity of rumen wall microorganisms and their possible relevance for animal health and performance.

Importance of the Rumen Epithelium for Animal Health and Performance in Livestock Production

In general, one main function of the GI-tract epithelium is to protect the host animal from microorganisms, toxins, or toxic chemicals present in the lumen and to prevent the unregulated entry of harmful substances or microorganisms into the lymphatic or portal circulation. Thus, the rumen epithelium has the dual function of both serving as a barrier against pathogens and toxic substances and being central for adequate absorption of rumen fermentation products such as SCFA and secreting molecules such as urea into the rumen (Steele et al., 2016; Aschenbach et al., 2019). In dairy cattle, feeding of rapidly fermentable grain-rich diets is routinely applied to high-yielding cows to minimize disturbances in early lactation and to maximize milk production economically over an entire lactation period. One challenge of feeding strategies using rapidly fermentable diets is that they may imbalance the digestive physiology of cattle, particularly in the rumen. This can result in reduced chewing and rumination activities, leading to a decreased rumen buffering capacity. Increased concentrations of microbial fermentation products such as SCFA can decrease the ruminal pH, which can have severe consequences for animal health and productivity (Aschenbach et al., 2011). One of the most prominent of these examples is the so-called subacute ruminal acidosis (SARA). SARA is considered one of the major metabolic

disorders affecting animal health and welfare in intensive ruminant production systems (Plaizier et al., 2008; Oetzel, 2017; Humer et al., 2018). The consequences of SARA are diverse and include feed intake depression, reduced diet digestibility, reduced milk yield, reduced milk fat content, GI damage, liver abscesses, and lameness (Plaizier et al., 2008; Humer et al., 2018). Thus, SARA is associated with reduced feed efficiency and significant production losses, which are likely explained by decreased fermentation efficiency in the rumen. The metabolic alterations caused by SARA can also lead to a release of toxic, proinflammatory metabolites, including lipopolysaccharide (LPS) (Zebeli and Metzler-Zebeli, 2012). LPS is a component of the cell wall of gram-negative bacteria and is a well-characterized endotoxin that can stimulate the immune system. The release of LPS can be a result of lysed bacterial cells or of rapid growth of gram-negative bacteria in the rumen or at the rumen epithelium. These metabolic alterations have the potential to damage the rumen epithelium, thereby decreasing its barrier function (Plaizier et al., 2008; Zebeli and Metzler-Zebeli, 2012; Aschenbach et al., 2019). The decrease of the rumen pH during SARA is associated with a possible increase in osmotic pressure resulting in potential damage of the rumen epithelium owing to swelling and rupture of rumen papillae (Plaizier et al., 2008). These processes can result in a degradation of gap and tight junctions resulting in a decrease of the barrier function of the rumen epithelium (Zebeli and Metzler-Zebeli, 2012; Aschenbach et al., 2019). One fairly common consequence of SARA and the more severe rumen acidosis can be liver abscesses (Plaizier et al., 2008; Oetzel, 2017). In feedlot cattle, liver abscesses are generally regarded to be sequelae to ruminal acidosis in cattle fed diets high in readily fermentable carbohydrates and low in forage. Such acidotic conditions can lead to a reduction of the rumen epithelial barrier function, which can allow pathogenic bacteria to enter the systemic circulation and, when reaching the liver, result in liver abscesses. Many of the bacteria observed in liver abscesses, including *Trueperella pyogenes* and *Fusobacterium necrophorum*, have also been found in the rumen and on the rumen wall (Narayanan et al., 1998), suggesting that these bacteria have translocated from the rumen into the circulation and finally into the liver. The incidence of liver abscesses is highly variable, and liver abscess incidences have been reported to range from 10% to 20% (Amachawadi and Nagaraja, 2016). Liver abscesses have significant economic impacts, particularly in the feedlot cattle industry. The economic impact is highly dependent on the severity of liver abscesses (Amachawadi and Nagaraja, 2016). All liver abnormalities have been estimated to cost the United States beef industry more than $15 million annually in lost liver value alone, with approximately two-thirds of these abnormalities being liver abscesses (Brown and Lawrence, 2010; McCoy et al., 2017). Based on the number and size of liver abscesses, a decrease in carcass returns of US$20 to US$80 has been estimated (Veloso and Drouillard, 2020). These findings on SARA and liver abscesses underpin the importance of the integrity of the rumen epithelium for preventing pathogens from entering the systemic circulation.

Structure of the Rumen Epithelium/ Rumen Epithelial Microbiota

The rumen epithelium is a keratinizing stratified squamous epithelial tissue consisting of several layers of cells consisting of the stratum basale, stratum spinosum, stratum granulosum, and stratum corneum, the latter representing the most apical layer of the rumen (Steele et al., 2016; Aschenbach et al., 2019). In contrast to other GI-tract epithelia, such as those in the lower GI tract, the rumen wall is not covered by a mucus layer (Steele et al., 2016). The surface area of the rumen epithelium is increased by papillae that protrude from the epithelium (Steele et al., 2016); this increased surface area is believed to allow higher absorption of SCFA and minerals. The degree of papillae in the dorsal regions of the rumen epithelium is lower than in the ventral epithelium (Clauss et al., 2009). It is important to note that the rumen epithelial microorganisms colonize the surface of the stratum corneum but do not penetrate into the stratum granulosum. The upper layer of the stratum granulosum forms a network of tight junctions that is critical for rumen epithelial barrier function (Aschenbach et al., 2019).

Composition of Rumen Wall Microbial Communities

Microscopic studies, cultivation-, and polymerase chain reaction-based approaches to determine cell densities of the rumen epithelial microbiota

Using scanning electron microscopy, studies performed on different ruminants in the 1970s and 1980s have shown that a layer of microorganisms densely

covers the rumen epithelium (Bauchop et al., 1975; McCowan et al., 1978; Cheng et al., 1979; Dinsdale et al., 1980; McCowan et al., 1980; Dehority and Grubb, 1981; Mead and Jones, 1981; Mueller et al., 1984; Rieu et al., 1989). The bacteria attached to the rumen epithelium have also been referred to as epimural bacteria (Mead and Jones, 1981); the term epimural bacteria is, however, inconsistently being used in the scientific community since it was first introduced. The number of bacterial cells on the rumen wall has been determined to range from 10^5 in newborn lambs to 10^9 per square centimeter of rumen wall tissue in 3-wk-old lambs (Rieu et al., 1989) and around 10^7 in adult sheep (Dehority and Grubb, 1981). Another study in sheep has reported cell densities ranging from 10^7 to 10^8 per gram wet weight of rumen wall tissue (Wallace et al., 1979). Quantitative polymerase chain reaction (qPCR) assays revealed 16S ribosomal RNA (rRNA) gene copy numbers of approximately 10^9 to 10^{10} per gram rumen papillae in calves, adult dairy cattle, and beef cattle (Chen et al., 2011; Malmuthuge et al., 2012; Wetzels et al., 2016, 2017) and 1×10^8 after birth to 4×10^8 after 70 d in young goats (Jiao et al., 2015). These data show that the rumen epithelial tissue harbors a dense microbial population and that the bacterial densities increase with age. It should be noted, however, that estimations of bacterial densities based on qPCR can overestimate the actual bacterial densities, as many bacteria harbor multiple rRNA operons, which are most commonly used for determining bacterial densities using qPCR. The bacterial communities attached to the rumen epithelium consist of strictly anaerobic bacteria and facultative anaerobes (Cheng et al., 1979; Wallace et al., 1979; Rieu et al., 1989). The higher percentage of facultative anaerobic bacteria on the rumen epithelium compared with the rumen lumen is most likely explained by diffusion of oxygen from the rumen wall tissue.

Cultivation-independent approaches to investigate the composition of the rumen epithelial microbial communities

The composition of rumen epithelial microbial communities has been determined using cultivation-dependent and cultivation-independent analyses over the last decades. These analyses revealed the presence of highly diverse microbial communities on the rumen epithelium. The first cultivation-independent analyses using PCR-denaturing gradient gel electrophoresis or cloning approaches revealed initial insights into the composition of rumen epithelial communities and also showed that rumen epithelial and rumen content microbial communities are distinct (Cho et al., 2006; Sadet et al., 2007; Li et al., 2012; Malmuthuge et al., 2012). Some studies have shown the presence of archaea at the rumen wall (Shin et al., 2004; Pei et al., 2010; De Mulder et al., 2017; Scharen et al., 2017; Petri et al., 2020), although at low overall abundance compared with bacteria. In addition, the presence of fungi (which did not affiliate to the Neocallimastigaceae family of rumen fungi) at the rumen wall has been shown in a metatranscriptome sequencing study (Mann et al., 2018). Some similarities in the composition of rumen wall and rumen content microbial communities have been found, but overall, the rumen content and rumen wall microbial communities are largely distinct (Cho et al., 2006; Sadet et al., 2007; Li et al., 2012; Mao et al., 2015; Liu et al., 2016; De Mulder et al., 2017; Scharen et al., 2017; Abbas et al., 2020; Ren et al., 2020). One likely reason for this could be the diffusion of oxygen from the rumen wall tissue, which would inhibit the growth of strictly anaerobic bacteria—which are common in the rumen content—at the rumen epithelium. Over the last decade, a number of studies have performed different sequencing-based approaches to study the composition of rumen wall microbial communities in different ruminants, including cattle, sheep, goats, yak, and deer (Li et al., 2012; Malmuthuge et al., 2014; Jiao et al., 2015; Liu et al., 2015, 2016; Mao et al., 2015; Wetzels et al., 2015, 2016, 2017; De Mulder et al., 2017; Scharen et al., 2017; Seddik et al., 2018; Petri et al., 2019, 2020; Ricci et al., 2019; Abbas et al., 2020; Ren et al., 2020). Particularly, many Proteobacteria phylotypes—including *Campylobacter*-like operational taxonomic units (OTU), deltaproteobacterial OTU (*Desulfovibrio*, *Desulfobulbus*), and betaproteobacterial OTU (affiliating to the Neisseriaceae family or the Burkholderiales order)—can be abundant, or of higher abundance in the rumen epithelium compared with rumen content (Jiao et al., 2015; Mao et al., 2015; Wetzels et al., 2015, 2016, 2017; Liu et al., 2016; Scharen et al., 2017; Petri et al., 2018; Petri et al., 2019; Abbas et al., 2020; Ren et al., 2020). Similar results were observed in the studies mentioned earlier for other (non-Proteobacteria) phylotypes also, including *Butyrivibrio*, *Treponema*, and *Mogibacterium*. In addition to DNA-based sequencing approaches, *Desulfovibrio* and betaproteobacterial phylotypes (identified as *Comamonas* and *Azoarcus*) have been found to be abundant at the rumen epithelium using RNA-based sequencing (Mann et al., 2018; Li et al., 2019c).

It is important to note that, until now, most studies have sampled the rumen wall in the ventral rumen sac; thus, the results mentioned in this paper mostly apply

to the ventral rumen sac. One recent study has determined the rumen epithelial community composition at 4 different sites (cranial sac, ventral sac, caudodorsal blind sac, and caudoventral blind sac) within the rumen of Holstein dairy cattle (Sbardellati et al., 2020). This study significantly advanced our understanding of the composition of rumen wall microbial communities by showing that shared—but also distinct—rumen epithelial microbial communities exist across different locations in the rumen. The differences in rumen epithelial communities at different locations in the rumen can most likely be explained by morphological, physiological parameters found at different locations of the rumen. Based on the reported differences in microbial communities between various locations of the rumen as well as the stratification of the rumen content—such that the heavier, finer, often more digested feed particles sink toward the bottom of the ventral sac and the more recently ingested, lighter feed particles float on top in a layer called the rumen mat—it is tempting to speculate that the rumen epithelial microbial communities might also display a different composition based on their stratification within the rumen epithelium: i.e., are the microbial communities in the dorsal regions of the rumen wall different from those in the ventral parts of the rumen epithelium? A recent study provided the first evidence for this hypothesis showing that the ventral and dorsal rumen epithelial microbial communities in yaks are indeed significantly different (Ren et al., 2020). In the future, it would be interesting to investigate this finding in more detail to determine whether a possible stratification of rumen wall microbial communities also exists in other ruminants and what the functional basis for such a stratification might be.

Several studies have analyzed the effect of high-grain feeding or of inducing SARA on the composition of microbial communities at the rumen epithelium of different ruminants. Overall, the induction of SARA or a rapid change to a high-grain diet resulted in significant changes in microbial community composition. In 2 studies in goats, a shift to a high-grain diet resulted in major changes in microbial community composition (Liu et al., 2015; Wetzels et al., 2015). Similar results were observed in the sheep rumen epithelial microbiota (Seddik et al., 2018). In beef cattle, a switch to a high-grain diet and an acidotic challenge resulted in a decrease in fiber-degrading microorganisms such as *Fibrobacter* and *Ruminococcus* at the rumen epithelium and an increase in bacteria such as *Prevotella* (Petri et al., 2013). Experiments inducing a transient (1-wk) SARA challenge in dairy cattle resulted in significant shifts in the composition of the rumen epithelial microbiota on the whole-community level (Wetzels et al., 2016; Petri et al., 2020). A long-term (4-wk) SARA challenge resulted in significant changes in rumen epithelial microbial communities, including a significant decrease in diversity (Wetzels et al., 2017). The study conducted by Wetzels et al. (2017) also suggested that longer times of high-grain feeding leads to more substantial changes in rumen epithelial microbial communities compared with shorter, and transient, periods of high-grain feeding or SARA challenges. Similar results have been reported from goats (Liu et al., 2015) and sheep (Seddik et al., 2018). A follow-up study of the experiments described in Wetzels et al. (2016) and in Wetzels et al. (2017) showed that the rumen epithelial community composition recovered to their initial status before the start of the high-grain diet within 8 wk after ending of the high-grain feeding (Petri et al., 2019).

For all of the observations and comparisons reported earlier, it is essential to keep in mind that the results from different microbiome studies are only comparable to a limited degree. A lack of comparability is a general challenge of microbiome studies, particularly in studies performed with livestock, which are faced with even more variability than studies performed with model animals (such as rodents) under controlled laboratory conditions (O'Hara et al., 2020). The comparability of different studies is limited because of variation caused by (i) different animal species, genders, and breeds used; (ii) different management strategies; (iii) differences owing to diet and geographical location; (iv) differences in age of the animals; and (v) different methodologies and their inherent biases used to determine and analyze microbial communities. For amplicon sequencing studies, the usage of amplicon sequencing variants (ASV)—also referred to as exact sequence variants—instead of (97% or 99% similarity) OTU can increase the comparability between amplicon sequencing studies (Callahan et al., 2017; Glassman and Martiny, 2018). Another important aspect to consider when comparing ASV and OTU is that ASV are based—in contrast to OTU—not on clustering of sequences based on similarity but on bioinformatic approaches to determine the probability that a given sequencing read is not due to sequencing error. Additional efforts to increase the comparability of amplicon sequencing microbiome studies include the Microbiome Quality Control project consortium (Sinha et al., 2017).

Functions of Rumen Wall Microbial Communities

In general, knowledge about the function of the rumen epithelial microorganisms is still highly limited.

In addition to some early cultivation-based studies (Abdel Rahman, 1966; Cheng and Wallace, 1979; Wallace et al., 1979), only very few recent studies have provided functional insight into the rumen epithelial microbiota. Some recent studies have performed transcriptome sequencing of rumen epithelial samples—albeit either without analyzing the gene expression of the rumen epithelial microbiota (Kong et al., 2016; Zhao et al., 2017) or by analyzing only the microbial rRNA reads from the transcriptome sequencing data (Li et al., 2019a, 2019b, 2019c). One study has recently performed metagenome shotgun sequencing of rumen wall samples obtained from goats. Although this study provided valuable insights into the functional potential of the rumen epithelial microbiota—such as reporting the presence of propionate, butyrate, and vitamin metabolism genes—the analyses in this study were only performed at a very general pathway level, thus limiting the functional insights that can be gained from the study (Shen et al., 2019). As mentioned earlier, one key host characteristic that is fundamentally different for the rumen epithelial microbial communities and the rumen content microbial communities is the presence of oxygen as a result of oxygen diffusion from the host tissue. This is exemplified by the presence of facultative anaerobic bacteria on the rumen epithelium (Cheng et al., 1979; Wallace et al., 1979; Rieu et al., 1989). Thus, it has been suggested that the rumen wall microorganisms scavenge oxygen to ensure strictly anaerobic conditions for rumen content bacteria (Cheng et al., 1979). An exposure of epithelial microbial communities to oxygen diffusing from tissues has been described for nonruminant mammals (Espey, 2013; Friedman et al., 2018) and likely also occurs in the rumen. In line with this, high *in situ* expression levels of genes involved in oxidative stress response—including thioredoxin reductase, glutathione peroxidase, and superoxide dismutase—have been identified in the rumen epithelial microbiota using metatranscriptome sequencing (Mann et al., 2018). Another suggested function of rumen epithelial bacteria is the digestion of cells of the host epithelial tissue, which might represent an important contribution to tissue recycling (McCowan et al., 1978; Cheng et al., 1979; Dinsdale et al., 1980).

Microbial enzymatic hydrolysis of cellulose is a key step in the degradation of fiber-rich plant material in the rumen. *Fibrobacter succinogenes* and *Ruminococcus* are well-characterized major cellulolytic rumen bacteria. Although these cellulose-degrading bacteria are primarily associated with the particle-attached part of the rumen content (Henderson et al., 2015), phylotypes affiliating to the genera *Fibrobacter* and *Ruminococcus* were also shown to be present at the rumen wall in various studies, although mostly in lower abundance compared with the rumen content (Petri et al., 2013; Liu et al., 2016; Scharen et al., 2017; Seddik et al., 2018). A metatranscriptome sequencing study provided *in situ* evidence for cellulose and cellobiose degradation by rumen wall bacteria based on the expression of endoglucanase (Enzyme Commission [EC] number: 3.2.1.4), cellobiose phosphorylase (EC: 2.4.1.20), and beta-glucosidase (EC: 3.2.1.21) genes (Mann et al., 2018). In addition to cellulose degradation, rumen epithelial bacteria might also be involved in the breakdown of starch indicated by the expression of glycogen phosphorylase (EC: 2.4.1.1) and of alpha-amylase (EC: 3.2.1.1) genes in the study by Mann et al. (2018). The high expression levels of glycogen phosphorylase and (to a lesser degree) of alpha-amylase suggest that starch degradation is an important metabolic process in rumen epithelial bacteria. In the latter study, the alpha-amylase genes were exclusively transcribed by Bacteroidetes, namely *Bacteroides* and *Prevotella*.

Sulfate-reducing bacteria have been isolated from ruminants (Huisingh et al., 1974; Howard and Hungate, 1976). Putative sulfate-reducing bacteria affiliating to the genera *Desulfobulbus* and *Desulfovibrio* have been found on the rumen wall in many recent 16S rRNA gene-based amplicon sequencing studies (Petri et al., 2013; Mao et al., 2015; Liu et al., 2016; De Mulder et al., 2017; Scharen et al., 2017; Wetzels et al., 2017; Seddik et al., 2018). In our metatranscriptome sequencing study (Mann et al., 2018), we provided evidence for sulfate reduction activity by rumen wall bacteria based on high gene expression of the dissimilatory (bi)sulfite reductase (*dsrAB*) genes, the key genes for sulfate reduction. Whether the production of sulfide by sulfate reducers occurs at a sufficiently high level to have a possible negative impact on the rumen epithelium is currently unclear. We assume that sulfate reduction activity in the rumen wall has a detrimental effect on the rumen epithelium owing to the toxicity of hydrogen sulfide (Drewnoski et al., 2014).

One key function of rumen content microorganisms is the production of SCFA, such as acetate, propionate, and butyrate, which are of high relevance for the host animal as energy sources, and which can meet up to 70% of the host's energy needs (O'Hara et al., 2020). Members of the rumen epithelial microbial community expressed the genes for the succinate pathway for propionate production (Mann et al., 2018) with *mmdA* as the key gene encoding the methylmalonyl-coenzyme A (CoA) decarboxylase (Reichardt

et al., 2014). This pathway was mainly expressed in Proteobacteria, particularly in uncharacterized members of the Neisseriaceae (Mann et al., 2018). Among SCFA, butyrate is particularly relevant for epithelial tissues as it has been shown that butyrate contributes to epithelial tissue development such as papillae development and barrier function in ruminants (Gorka et al., 2018; Lin et al., 2019). Metatranscriptome sequencing of rumen wall samples revealed medium expression levels of genes involved in butyrate production, including the butyryl-CoA:acetate CoA-transferase (*but*) (EC: 2.8.3.8) and butyrate kinase (*buk*) (EC: 2.7.2.7) genes as terminal genes for butyrate production. These transcripts were derived mainly from Firmicutes such as *Clostridium* and *Butyrivibrio*, as well as from Proteobacteria (Mann et al., 2018). The production of butyrate has recently been shown to stimulate the expression of rumen host animal epithelial genes involved in signaling and cell growth (Lin et al., 2019).

One of the central functions of rumen wall bacteria is urease activity, which results in the formation of ammonia and carbon dioxide derived from the hydrolysis of urea by the enzyme urease (EC 3.5.1.5). Urea influx from the rumen tissue across the rumen epithelium into the rumen content can occur by diffusion or by active transport (Abdoun et al., 2006). Another source of urea in the rumen can be the diet, where urea can also be part of the feed. Urease activity of rumen epithelial bacteria has already been described by various studies in the 1960s and 1970s (Abdel Rahman, 1966; Cheng et al., 1979; Cheng and Wallace, 1979; Wallace et al., 1979). Later, the genes of the urease subunit C gene *ureC* were amplified from DNA samples obtained from the rumen wall revealing a high diversity of potentially ureolytic bacteria on the rumen wall, including many yet unknown, novel, *ureC* gene sequences (Jin et al., 2017). A study from our lab has demonstrated gene expression of all 4 urease subunit genes in rumen epithelial bacteria using metatranscriptome sequencing (Mann et al., 2018). Notably, the gene expression levels of urease genes were among the highest of all genes in this study, suggesting a high level of urease activity of the rumen epithelial microbiota, which is in line with earlier observations (Abdel Rahman, 1966; Cheng et al., 1979; Cheng and Wallace, 1979; Wallace et al., 1979). Expressed urease sequences detected by metatranscriptome sequencing belonged mainly to the genera *Flavobacterium*, *Corynebacterium*, *Helicobacter*, *Clostridium*, and *Bacillus* (Mann et al., 2018). As a result of their urease activity, rumen wall bacteria thus may influence the rumen ecosystem by affecting urea exchange across the rumen wall (Abdoun et al., 2006) more efficiently than previously thought, thereby playing an important role in the rumen nitrogen cycle. Similarly, the observed high expression levels of other key enzymes in nitrogen metabolism such as glutamate dehydrogenase (EC: 1.4.1.4), glutamine synthase (EC: 6.3.1.2), and glutamate synthase (EC: 1.4.1.13, EC: 1.4.1.14) in the study by Mann et al. (2018) underscores the importance of rumen wall bacteria in nitrogen metabolism in addition to urease activity. We have also identified the expression of nitrogenase, the enzyme for nitrogen reduction, which converts nitrogen into ammonia in rumen wall microbial communities (Mann et al., 2018). Taken together, the currently available data suggest that nitrogen metabolism is one key function of the rumen epithelial microbiota.

Quorum sensing is a widespread form of bacterial communication, both within and between species. Quorum sensing signaling molecules have been detected in the rumen content in several independent studies, although sometimes without identifying the microorganisms producing these molecules (Erickson et al., 2002; Mitsumori et al., 2003; Ghali et al., 2016; Ran et al., 2016). More recently, it has been shown that many abundant rumen content bacteria such as *Butyrivibrio*, *Prevotella*, and *Ruminococcus* possess quorum sensing genes and that these genes are expressed in the rumen (Won et al., 2020). It is unknown whether quorum sensing genes are also present and expressed in the rumen epithelial microbiota. Because of the biofilm-like growth of the rumen epithelial microbiota, it is intriguing to speculate that quorum sensing is also occurring at the rumen epithelial microbiota, although experimental proof needs to be provided in future studies.

Conclusions and Future Research Perspectives

Although many studies performed over recent years have significantly increased the knowledge about the composition of rumen epithelial microbial communities, knowledge about the functional potential and *in situ* functions and activity of the rumen wall microbiota is still highly limited. In addition, a better understanding of the long-term temporal stability of the rumen epithelial microbial communities is urgently needed. This should include studying, e.g., the effects of seasonal variation. Future studies will therefore be needed to increase our knowledge about the rumen wall microbial communities. Such studies should

include cultivation-based and cultivation-independent approaches, such as shotgun metagenomics or metatranscriptome sequencing as well as metabolomic approaches. In addition, microscopy-based approaches, including histological tissue staining and fluorescence *in situ* hybridization, will be needed to provide better knowledge about the spatial organization and structure of rumen wall microbial communities. Another important aspect to consider is the functional redundancy between different, taxonomically unrelated microorganisms. Thus, the same metabolic pathways can be performed by various microorganisms. This functional redundancy can lead to differences in the taxonomic composition of microbial communities, which can nevertheless fulfill the same metabolic function. The application of such approaches will yield a better understanding of the biological functions underlying changes in the composition of rumen epithelial communities and might allow the development of targeted interventions to increase the health and performance of ruminant livestock.

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