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ROLE OF FIBER AND NON FIBROLYTIC BACTERIA IN FEED DIGESTION IN BOVINE RUMEN - AN OVERVIEW

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ABSTRACT

In developing countries like India, ruminants fed with lignocellulosic feed. It contains cellulose, hemicelluloses and lignin. Ruminants can utilize these particles with the help of microbial population present in rumen. Among these, bacterial population play key role in feed digestion. The fibrolytic bacteria degrades insoluble fiber into soluble sugars and non fibrolytic bacteria associates with them converts soluble sugars into metabolic products, thereby helps to control feedback inhibition of fiber degrading bacteria. Hence, it is important to understand the association between fiber and non fiber degrading bacteria. In this review, we discussed about predominant fiber degrading and non fiber degrading bacterial species and their association.

KEY WORDS

Fiber degrading bacteria; ruminant; Fibrobacter succinogenes; volatile fatty acids.

INTRODUCTION

In lignocellulosic feed, cellulose is a main component, most common carbohydrate on earth and its production is estimated to be 100 billion tons per year (17). The ruminants are able to utilize cellulose with symbiotic association with microbes in their rumen. In ruminants, microbes are comprises of 10^{10} to 10^{11} bacteria, 10^{3} to 10^{6} fungi, 10^{4} to 10^{6} protozoan populations per ml of rumen fluid (12) (34). The contribution of Protozoa in fiber degradation is less significant in terms of the proportion of total neutral detergent fiber (NDF) degrading activity (8). Fungi are easily able to penetrate and degrade lignin effectively. Hence, the population is less in rumen (26). Rumen bacteria are important in feed degradation because of their superior biomass and higher activity. In bovine rumen, bacterial species *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Butyrivibrio fibrisolvens*, *Prevotella ruminicola*, *Eubacterium cellulosolvens* and *Eubacterium ruminantium* are recognized as fibrolytic bacterial species (32), degrade lignocellulosic material into glucose and their fermentation products including volatile fatty acids (VFA), acetate, propionic and butyric acids, these nutrients are utilized by ruminant and, is determined by the type of microorganism in the rumen. The species of *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* are more dominant species, in addition the ability of cellulose degradation is much higher in these species than other cellulolytic bacteria. Therefore, *Fibrobacter succinogenes*, *Ruminococcus flavefaciens,* and *Ruminococcus albus* are considered as a major representative species of fiber degradation in rumen.

The maintenance of fibrolytic activity of bacteria depends on the nutritive interactions, cross feeding of fermentation products (10). This maintenance is correlated by positive interaction between fiber and non fiber degrading bacteria (14). (30) first reported that growth of non cellulosic bacteria association with

cellulolytic bacteria supports the enhancement of the fiber degradation. The cross feeding of non fibrolytic bacteria on fibrolytic products also enhances the fiber degradation. Therefore, the positive interaction is very important to maintain the rumen ecosystem and host health.

Detection of rumen microbes on culture based methods showed diffident changes in culturable bacteria, meanwhile changes in the total bacterial community of culturable and un-culturable species, are mainly uncharacterized (13). Recently, studies on rumen microbial communities and population shifted from culture-based methods to molecular method such as polymerase chain reaction (PCR)-based assays to quantify rumen bacteria accurately within a span of time.

Ruminant animals can utilize plant fiber as energy source, depending on a symbiotic relation with microbes present in rumen. Of these, bacteria are more preside microbes. Though *Fibrobacter succinogenes* and *Ruminococcus* species play a key role in fiber degradation, association with non- fibrolytic bacteria is important to enhance the efficiency of enzyme activity either by cross feeding or utilization of fermentation products (30) (16).

Mechanism of fiber degradation

Based on the environmental existence, ruminal bacteria are classified into five groups known as 1. Free living bacteria in liquid phase, 2. Bacteria loosely associated with feed particles, 3. Bacteria strongly associated with feed stuff, 4. Bacteria associated with epithelial cells of rumen, and 5. Bacteria attached to surface of fungi and protozoa (21). The bacteria associated with feed particle, have endoglucanase, xylanase, amylase and protease activity in rumen (35), (21).

Bacterial attachment to fiber feed particles

The adhesion of bacteria to feed particle can be understood in different steps. Once the feed (substrate) is entered into the rumen, the bacteria transport to substrate and starts its action by attaching to feed particle, it is a difficult process for rumen bacteria as they are non-motile (33). The adhesion between bacteria and feed particle depends on the free suspended cellulolytic population to bind new particles suspended in the rumen (23). After the bacteria arrive to substrate in a range of 2 to 5 nm, a non-specific interaction is initiated (28), the gycocalyx is a glycoprotein or protein containing structure present in bacterial cell membrane, participate initially in non specific interaction with substrate. The adhesion of bacteria with substrate is differ from each bacterial species. The *Ruminococci* species adhesion to substrate occurs within 5mins after their addition to the medium, whereas *Fibrobacter succinogenes* attaches after 15 to 30mins after contact with cellulose (28). Now the ligands on the bacterial cell recognize receptors on the substrate surface. During initial cell wall digestion, bacteria receives signals for assemble of inducible linkages between the glycocalyx layer of bacteria and substrate to initiate cellulolytic enzyme (21). The specific and strong interaction of bacteria to the cellulosic substrate provides protection from grazing by ruminal protozoa and protects their cellulolytic enzymes themselves from ruminal proteases (33). If the specific linkage forms between bacteria and substrate, digest the substrate and starts proliferation of new generation of induced bacteria to produce colonies. These fiber degrading bacteria have specificity to bind and colonize on substrate. *Ruminococcus* species digest primarily on grass leaves cell wall, where as *F. Succinogenes* colonizes first on cotton fibers (9). In ruminants, *Fibrobacter succinogenes*, *Ruminococcus* species, *Butyrivibrio fibrisolvens*, *Prevotella* species, *Eubacterium cellulosolvens* and *Eubacterium ruminantium* are recognized as fibrolytic bacterial species (32).

Fibrobacter succinogenes, a potent fibrolytic species in ruminants, was isolated 49 years ago from a bovine rumen and has been used since then as a model for extensive studies. *Fibrobacter succinogenes* degrades plant cell wall by adhesion to surface of plant material tightly (20). In *F. succinogenes* produces at least 7 types of glucanases, a cellodextrinase, a cellobiosidase, and both a cellobiase and a cellobiose-phosphorylase (4) (18). This organism contains, along with cellodextrinase gene, 9 glucanase genes and 4 xylanase genes were identified (5).

The species *Ruminococcus flavefaciens,* first isolated by Sijpelsteinj in 1951, are Gram positive, yellow in pigment and grow on cellobiose in chains. These species adhere immediately to feed particles and start degrade faster than all other fibrolytic bacteria (22). *Ruminococcus flavefaciens* contains two enzyme complexes, a large 3000 KDa complex A, and a small 89 KDa B complex, A complex contains 13 different where as B complex 5 unique endoglucanase activities (11). The adhesion of bacteria with cellulose is regulated by

the addition of 0.1% carboxymethylcellulase, but with the cellobiose at 1% concentration also, from these finding it was conformed that the recognition site of cellulose binding factors is larger than a repeating cellobiose moiety (6). Hence, in *Ruminococcus flavefaciens*, cellulosome-like complexes and carbohydrate epitopes of the glycocalyx layer mechanisms are involved to degrade fibrous feed particles.

The bacteria *Ruminococcus albus*, first isolated by Hungate in 1957, are Gram positive and grow on cellobiose in diplococci (12)**.** These species contains two mechanisms for specific adhesion to cellulose, a cellulosomal like mechanism and a cbpC-protein mechanism. The cellulosome complex isolated from *Ruminococcus albus* F-40 grown on cellulose, contains endoglucanase V, VI and VII components, additionally 5 endoglucanase components, 3 xylanase and 4 non enzymatic proteins (25). The cellulose-binding protein type C (*cbp* C) is not like cellulose binding domains (CBD) families. The *cbp* C contains well characterized proteins, motifs of Pil-family protein, repeating units of these proteins are building blocks for fimbriae creation (24), which helps in adhesion to substrate. The metabolic functions are also similar, but *Ruminococcus flavefaciens produces* succinate as major fermentation end product (15).

The genus rumen *Prevotella* classified into *Prevotella ruminicola* (formerly known as *Bacteroides ruminicola*), *Prevotella bryantii*, *Prevotella albensis* and *Prevotella brevis* (1). These four species shows higher degree of diverse genetically (27), and have different capabilities for metabolic function such as polysaccharide degradation (19). The *Prevotella* is a major member of the rumen bacterial community, and uncultured *Prevotella* constitute a large proportion of ruminal *Prevotella*. The diet-specific association of *Prevotella* clones observed suggests significant functional diversity of members of this genus in the rumen (2).

Association between fibrolytic and non-fibrolytic bacteria

The *In vitro* studies have shown efficient cellulolytic activity of *Fibrobacter succinogenes* when co-cultured with *Treponema bryantii* than mono-culture. These bacteria depend on the metabolic products of fibrolytic bacteria present in the rumen to obtain CO_2, vitamins, and volatile fatty acids, which are essential for their growth in rumen. So, *Treponema Bryantii* cannot grow in pure culture but grows only in co-culture with fiber degrading bacteria (31). The species of spirochetes, Treponema are capable of degrade plant soluble polysaccharide (36). The real-time PCR quantification indicated that the relative abundance of the *Treponema* group was 1.05%, while the known species *Treponema bryantii* accounted for only 0.02% (3). The fibrolytic bacteria such as *F. succinogenes*, *R. flavefaciens* or *R. albus* enhances the cellulose digestion when co-cultured with non-fibrolytic *Treponema* or *Butyrivibrio* species (7). The growth of *Selenomonas ruminantium* was observed first time when *Fibrobacter succinogenes* cultured on cellulose containing medium (30). This was confirmed by (29), the growth of *S. ruminantium* and *P. ruminicola* was supported by cellodextrins. This is due the crossfeeding of these bacteria on succinate, it is produced during fiber digestion by *Fibrobacter succinogenes* and *Ruminicoccus flavefaciens*. However, the produced succinate was not accumulated, hence it is converted into propionate. For this conversion, succinate-decarboxylating bacteria such as *Selenomonas ruminantium* are considered to play a central role in the rumen.

CONFLICT OF INTEREST:

All authors declared no conflict of interest.

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