

Why Don't Ruminal Bacteria Digest Cellulose Faster?

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ABSTRACT

The bacteria *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* generally are regarded as the predominant cellulolytic microbes in the rumen. Comparison of available data from the literature reveals that these bacteria are the most actively cellulolytic of all mesophilic organisms described to date from any habitat. In light of numerous proposals to improve microbial cellulose digestion in ruminants, it is instructive to examine the characteristics of these species that contribute to their superior cellulolytic capabilities and to identify the factors that prevent them from digesting cellulose even more rapidly. As a group, these species have extreme nutritional specialization. They are able to utilize cellulose (or in some cases xylan) and its hydrolytic products as their nearly sole energy sources for growth. Moreover, each species apparently has evolved to similar maximum rates of cellulose digestion (first-order rate constants of 0.05 to 0.08 h⁻¹). Active cellulose digestion involves adherence of cells to the fibers via a glycoprotein glycocalyx, which protects cells from protozoal grazing and cellulolytic enzymes from degradation by ruminal proteases while it retains—at least temporarily—the cellodextrin products for use by the cellulolytic bacteria. These properties result in different ecological roles for the adherent and nonadherent populations of each species, but overall provide an enormous selective advantage to these cellulolytic bacteria in the ruminal environment. However, major constraints to cellulose digestion are caused by cell-wall structure of the plant (matrix interactions among wall biopolymers and low substrate surface area) and by limited penetration of the nonmotile cellulolytic microbes into the cell lumen. Because of these constraints and the highly adapted nature of cellulose digestion by the predominant cellulolytic bacteria in the rumen, trans-

fer of cellulolytic capabilities to noncellulolytic ruminal bacteria (e.g., by genetic engineering) that display other desirable properties offers limited opportunities to improve ruminal digestion of cellulose.

(**Key words:** rumen, digestion, cellulolytic microbes, fiber)

Abbreviation key: RCB = ruminal cellulolytic bacteria.

INTRODUCTION

Because cellulose is the most abundant component of plant cell walls, ruminal cellulolytic microorganisms play a central role in the nutrition of ruminant animals fed diets based on forage. Recognition of this fact has stimulated decades of investigation into the physiology and biochemistry of these microbes. However, only within the last few years has a sufficient amount of quantitative data been gathered to allow placement of the metabolic capabilities of cellulolytic microbes into the larger perspective of fiber digestion in the ruminant. This paper summarizes recent advances in knowledge of the predominant cellulolytic microbes of the rumen, including evidence that these species are among the most intensely cellulolytic organisms described to date. In light of numerous proposals to improve microbial cellulose digestion in the ruminant—particularly by the use of genetic engineering—it is instructive to examine the characteristics of these species that contribute to their superior cellulolytic capabilities and to identify factors that prevent them from digesting cellulose even more rapidly.

THE PREDOMINANT RUMINAL CELLULOLYTIC BACTERIA

The ability to digest cellulose has been ascribed to a large number of bacterial, fungal, and protozoal species isolated from the rumen (3, 12, 37). However, scientists generally agree that cellulolysis in the rumen is primarily due to the activities of the ruminal cellulolytic bacteria (RCB), in particular three predominant species: *Fibrobacter* (formerly *Bacteroides*) *succinogenes*, *Ruminococcus flavefaciens*,

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TABLE 1. Comparison of predominant and secondary species of ruminal cellulolytic bacteria.

Characteristic	Predominant	Secondary
Exemplary species	<i>Fibrobacter succinogenes</i> <i>Ruminococcus flavefaciens</i> <i>Ruminococcus albus</i>	<i>Butyrivibrio fibrisolvens</i> <i>Clostridium longisporium</i> <i>Clostridium locheadii</i>
Adherence to fiber	Extensive, via glycocalyx	Minimal
Chief localization of cellulases	Cell surface	Extracellular
Nutrition	Specialist, based on cellulose (and sometimes xylan)	Generalist, based on sugars
Motility	Nonmotile	Motile or nonmotile

and *Ruminococcus albus* (3, 37). These three species have common characteristics that set them apart from other ruminal bacteria [including secondary cellulolytic species, such as *Butyrivibrio fibrisolvens*, *Clostridium longisporium*, and *Clostridium locheadii* (Table 1)] and from cellulolytic bacteria from habitats other than the intestine.

One of the more obvious characteristics of the predominant RCB is their nutritional specialization. As shown in Table 2, most ruminal bacteria that ferment carbohydrates are capable of using numerous monosaccharides and disaccharides as growth substrates, and even those species with limited capability for digesting cellulose can utilize at least a few of these sugars. By contrast, *F. succinogenes* and the ruminococci are nearly restricted to cellulose and its hydrolytic products as growth substrates (12). The consequence of this nutritional specialization is that

the primary means by which these species gain selective advantage in the rumen is by optimizing only two catabolic activities: cellulose hydrolysis (depolymerization) and efficient utilization of the hydrolytic products (cellodextrins). However, these tasks are made challenging by the nature of the substrate (an insoluble, well-ordered biopolymer woven into a matrix of other biopolymers) and the nature of the ruminal environment (a continuously flowing system with a dense and diverse microflora that includes grazing protozoa, proteolytic bacteria, and cellodextrin-utilizing, noncellulolytic opportunists).

How have the predominant RCB responded to these challenges? As shown in Table 3, they have evolved to digest cellulose relatively rapidly; in fact, the only organism that has been shown to digest cellulose more rapidly is the thermophilic, anaerobic bacterium *Clostridium thermocellum*, an organism

TABLE 2. Carbohydrate utilization patterns of cellulolytic and noncellulolytic ruminal bacteria.

Species	Polysaccharides ¹	Cellodextrins ²	Monosaccharides and disaccharides ³
Predominant cellulolytics			
<i>Fibrobacter succinogenes</i>	Cellulose	++	G, C
<i>Ruminococcus flavefaciens</i>	Cellulose, xylan, pectin	++	C
<i>Ruminococcus albus</i>	Cellulose, xylan	++	C, G, ⁴ X, ⁴ A ⁴
Secondary cellulolytics			
<i>Butyrivibrio fibrisolvens</i>	Cellulose, xylan, dextrin, pectin	++	G, Ga, Mn, F, M, ⁴ X, ⁴ L, ⁴ C ⁴
<i>Clostridium longisporium</i>	Cellulose	NT	G, Ga, F, C, M, L, S
<i>Clostridium locheadii</i>	Cellulose, dextrin	NT	G, M, S
Noncellulolytics			
<i>Prevotella ruminicola</i>	Pectin, starch, ⁴ dextrin ⁴	++	G, Ga, F, L, C, X, ⁴ A, ⁴ R, ⁴ M ⁴
<i>Ruminobacter amylophilus</i>	Starch	NT	M
<i>Selenomonas ruminantium</i>	Starch, dextrin	++	G, Ga, F, X, A, C, M, L, S ⁴
<i>Streptococcus bovis</i>	Starch	+	G, Ga, F, Mn, C, M, L, S
<i>Succinomonas amylolytica</i>	Starch, dextrin	NT	G, M
<i>Succinivibrio dextrinosolvens</i>	Dextrin, pectin	NT	G, Ga, Mn, X, M, A, ⁴ F, ⁴ S ⁴

¹Data of Hungate (12).

²Data of Russell (25). Absorbance at 600 nm in batch culture after 24 h: ++ = >1.0; + = <1.0. NT = Not tested.

³Data of Hungate (12), except data for *R. albus*, which are from Thurston et al. (33). Abbreviations: A = arabinose, C = cellobiose, F = fructose, G = glucose, Ga = galactose, L = lactose, M = maltose, Mn = mannose, R = rhamnose, S = sucrose, and X = xylose.

⁴Positive for some strains.

TABLE 3. Comparison of rate constants for digestion of crystalline cellulose by various ruminal and nonruminal microorganisms.

Organism	Substrate ¹	Rate constant (h ⁻¹)	Basis	Reference
<i>Clostridium thermocellum</i> ATCC27405	AV	0.16 ²	Weight loss, chemostat	Table 1 of (16)
<i>Ruminococcus albus</i> 8	AV	0.05	Weight loss, chemostat	(23)
<i>Ruminococcus flavefaciens</i> FD-1	SC	0.08	Weight loss, chemostat	(29)
<i>Fibrobacter succinogenes</i> S85	SC	0.07	Weight loss, chemostat	(38)
<i>Cellulomonas uda</i> ATCC 21399	AV	0.027 ²	Heat production, batch	Figure 1 of (4)
<i>Cellulomonas flavigena</i> JC3	AV	0.006 ²	Weight loss, batch	Figure 1 of (2)
White-rot fungi (5 species)	Cot	<0.004 ²	Weight loss, soil block	Table 4 of (11)
Brown-rot fungi (8 species)	Cot	<0.002 ²	Weight loss, soil block	Table 3 of (11)

¹AV = Avicel microcrystalline cellulose PH 101, SC = Sigmacell 20 microcrystalline cellulose, and Cot = cotton cellulose.

²First-order rate constants were not supplied directly, but were calculated from data supplied by authors of the indicated references.

that displays many nutritional and adaptive similarities to the predominant RCB but that also benefits from enhanced catalytic rates at its 60°C growth optimum (16). Interestingly, the first-order rate constant for the digestion of highly ordered cellulose by the three ruminal species under optimal growth conditions appear to be fixed within a rather narrow range of 0.05 to 0.08 h⁻¹ (23, 29, 38). This finding suggests that these species have adapted to some sort of upper limit of cellulose digestion within the constraints of digesting a structurally ordered, insoluble polymer.

BACTERIAL STRATEGIES TO ENHANCE RUMINAL CELLULOLYSIS

One potential strategy to support rapid rates of cellulose hydrolysis is the synthesis of large amounts of cellulase enzyme. This route has been adopted by many nonruminal microbes, such as the aerobic fungus *Trichoderma reesei*, and the ease of assaying for extracellular cellulases produced by these organisms has been exploited by industrial microbiologists to generate hypercellulolytic mutant strains for commercial enzyme production (21). A second strategy, used by the ruminal fungi, is to produce modest amounts of enzymes that have very high specific activities (45). The predominant RCB use a third strategy, in which the cellulolytic enzymes are located primarily at the cell surface (20, 32), probably in multienzyme complexes within organelles resembling cellulosomes, which were originally described in the nonruminal anaerobic bacterium *C. thermocellum* (20). The cellulosome is a surface-bound complex of 18 to 20 proteins arranged in a supramolecular orientation that facilitates binding to and degradation of the cellulose microfibril (5). Several of the proteins in the cellulosome have been shown to contain binding domains that facilitate a physical contact between cell and

cellulose substrate (5). With the predominant RCB, this contact is stabilized by the synthesis of a glycoprotein-containing structure (the glycocalyx) (1, 14). This strategy of strong adherence to cellulose has several apparent advantages. First, the cellulolytic enzymes are concentrated on the substrate. Moreover, this strategy excludes other microbes and their enzymes from the site of hydrolysis, which allows the RCB to have first access to the products of cellulose hydrolysis and protects the cellulolytic enzymes themselves from ruminally abundant proteases. In addition, the strong cell-substrate contact probably protects the adherent RCB from grazing by ruminal protozoa.

The ability of these well-adapted, predominant RCB to outperform in competition with nonadherent cellulolytic species is striking. The superiority recently was illustrated by Varel et al. (35) with a ruminal isolate of the secondary RCB, *C. longisporum*. Despite its ruminal origin and its respectable cellulolytic activity in pure culture, this strain was almost eliminated from the rumen 24 h after 6 L of fermentor-grown culture and 20 L of buffer were inoculated into three emptied rumens. Varel et al. (35) have suggested that the inability of *C. longisporum* to persist in the rumen in the presence of competition may be due to the failure of this species to synthesize a cellulosome-like structure. Regardless of the reasons that this species does not compete effectively, the rapid recovery of the native predominant RCB should give pause to microbiologists who indiscriminantly promote genetic engineering of ruminal bacteria as a bromide for improving ruminal fiber digestion.

The adherence of cells to fiber presents ecological constraints on the colonization of forage particles as they enter the rumen. Colonization of fresh forage most likely proceeds via daughter cells released from forage particles that have become fully covered

through growth and binary fission of adherent cells (Figure 1). Because even nonadherent cells of the predominant RCB are not actively motile through the bulk liquid phase (12), the initial contact between nonadherent cells and freshly introduced forage particles would seem to be a random event hastened by animal activity (ruminal contraction, rumination, or mastication) and the high microbe density in the rumen.

SUBSTRATE CONSTRAINTS ON CELLULOSE DIGESTION

As noted, the maximum rate constant for the digestion of crystalline cellulose by RCB is -0.08 h^{-1} (23, 29, 38). This apparent upper limit in the rate of digestion may be dictated by some inherent property of cellulose itself. One such property may be the physical separation of individual chains of the cellulose crystalline lattice that occurs as digestion proceeds. Experiments with different allomorphs of cellulose have revealed that those forms having nearly identical unit cell dimensions but increased stability because of both interchain and intersheet hydrogen

bonding display slower rates of digestion (39). Celluloses treated by ball milling to destroy their crystalline structure often have rate constants that are somewhat higher, but this increased degradation rate may be due largely to the considerable reduction in particle size and a consequent increase in available surface area of the cellulose (40).

Experiments conducted both *in vitro* and *in sacco* have revealed that the digestion of the cellulose component of forages normally follows first-order kinetics with respect to substrate (i.e., the rate of digestion is a function of the fraction of digestible substrate remaining) (34, 36), and this kinetics model has been embraced by some researchers (6, 8, 18) as a model of ruminal fiber digestion. However, the rates of cellulose digestion in forages by mixed ruminal microflora rarely approach the rates for crystalline cellulose. The slower rates of cellulose digestion of forages have largely been blamed on effects of the plant cell-wall matrix (i.e., the physical and chemical interactions among cellulose and the various cell-wall biopolymers, primarily hemicelluloses and lignins) (10, 13). Certainly these effects play a major role, but other factors may be involved. The architecture of the

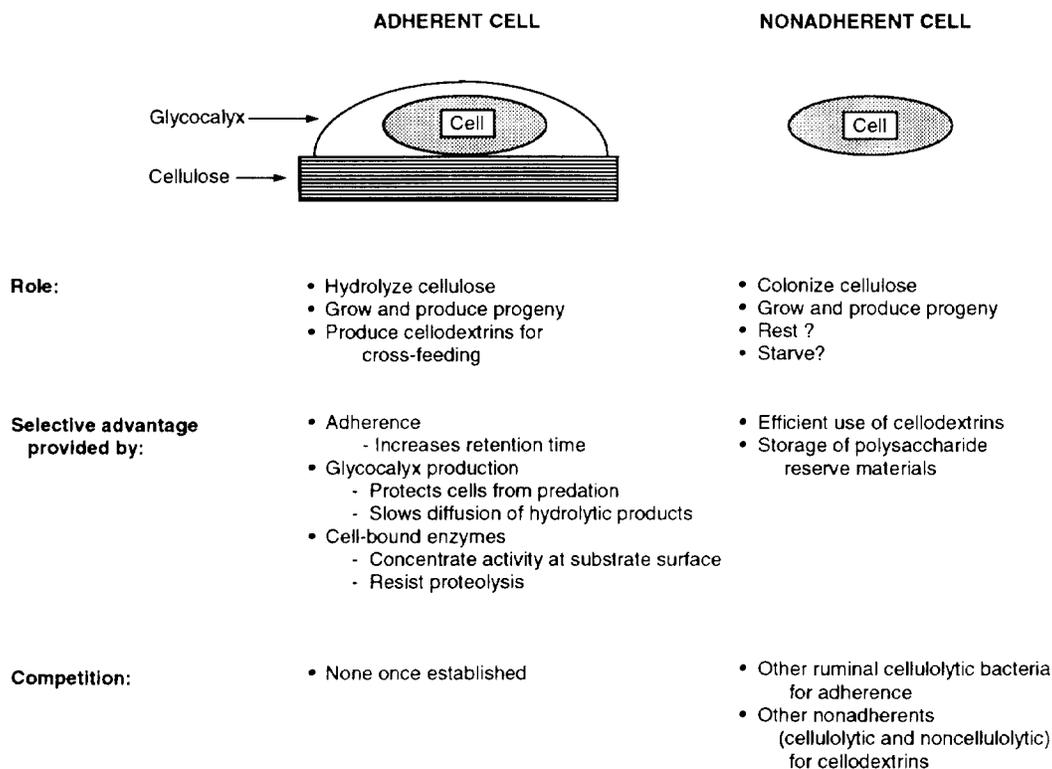


Figure 1. Comparison of the characteristics of adherent and nonadherent forms of the predominant species of ruminal cellulolytic bacteria.

plant cell may be just as important as its chemistry. Unlike the cellulose powders that are often used for *in vitro* studies, the walls of some plant cell types are digested from the inner lumen toward the primary wall and middle lamella; thus, digestion of those cell walls requires that the bacterial cells, which in the case of the predominant RCB are not actively motile through the rumen liquor, enter the lumina, apparently by purely passive means (*viz.*, diffusion). In noting this, Wilson and Mertens (44) calculated expected rates of diffusion of microbial cells and determined that fiber digestion is greatly limited in some plant cell types (e.g., sclerenchyma) by the slow entrance of the bacterial cells into, and their slow diffusion down, the lumina.

ENVIRONMENTAL CONSTRAINTS ON CELLULOSE DIGESTION

Most ruminal bacteria prefer pH near neutrality for growth, although some species (e.g., *Streptococcus bovis* and *Prevotella ruminicola*) can grow in the pH 5 to 6 range. The predominant RCB are particularly sensitive to low pH. None of the three predominant cellulolytic species grow at pH <6.0 (26, 29, 38). The adverse effects of low pH on cellulose digestion by ruminal bacteria have been discussed in detail by Russell and Wilson (27, 28). This paper discusses the effect of a second environmental factor, microbial interactions, on cellulolysis.

Individual species of RCB encounter significant competition for nutrients with other ruminal species. The competition among *F. succinogenes* S85, *R. flavefaciens* FD-1, and *R. albus* 8 recently has been examined *in vitro* in batch cultures supplied either with pure cellulose or with wheat straw treated with alkaline hydrogen peroxide (22). These studies indicate that the ruminococci are quite effective in competing with *F. succinogenes*, despite the reputation of *F. succinogenes* (9, 32) as the most intensely cellulolytic of the ruminal bacteria. In cellulose-limited continuous culture, *R. flavefaciens* FD-1 easily outcompetes *F. succinogenes* S85 at all dilution rates and pH values tested within the normal growth range of these organisms (30). The *R. flavefaciens* apparently succeeds in the competition because of both a more rapid binding to cellulose (24) and a stronger affinity for cellodextrins released by cellulose hydrolysis (31). An additional complication is that different species of RCB, despite similarities in their rate of digestion of pure cellulose, show distinct preferences for individual types of plant cells (15).

Because most saccharolytic, but noncellulolytic, ruminal bacteria also are capable of utilizing cellodextrins (25), those organisms probably compete with the RCB for products of cellulose hydrolysis. The adherence of the RCB to the fiber at the site of cellulose digestion may provide a strong selective advantage in the competition for hydrolytic products, but, as noted here, nonadherent daughter cells must compete directly with the planktonic sugar fermenters until the daughter cells can attach to forage fiber. Whether the nonadherent daughters can effectively compete or subsist by utilizing stored energy reserves [as is likely with *F. succinogenes* (41)] remains unanswered. The fact that the RCB can efflux a portion of the cellodextrins taken up as cellodextrins of longer chain length suggests a possible crossfeeding mechanism that may have implications in promoting survival of the nonadherent cells—both cellulolytic and noncellulolytic—in *vivo* (42). This hypothesis is strengthened by the observation that a significant fraction (11 to 37%) of the nonadherent cells in cellulose-limited continuous cultures of *F. succinogenes* S85 underwent cell division that was observable under phase-contrast microscopy (42).

POTENTIAL FOR IMPROVEMENT OF RUMINAL CELLULOSE DIGESTION

Genetic engineering of ruminal bacteria for improved cellulose digestion has been touted as one route to improve animal productivity (27). Two strategies for genetic engineering may be readily envisioned. The first involves improving the existing RCB. The adherent lifestyle developed by the predominant RCB clearly provides considerable selective advantage to these species but greatly complicates the engineering of strains with enhanced cellulolytic capabilities. Adherence of many cells to a single fiber complicates the isolation of individual clones. Moreover, because the enzymes are cell-bound and act synergistically with other proteins in the complex, their activities cannot be assayed easily, and, thus, hypercellulolytic mutants cannot readily be screened. If the predominant RCB have indeed reached an upper limit in the rate of cellulose digestion, attempts to engineer improved strains should focus not on altering the inherent properties of the cellulolytic enzyme system, but should focus instead on removing other character defects. Removing these defects would include improvements in acid tolerance (to maintain rates of fiber digestion at the low pH values encountered in modern ruminant diets) and introduction of motility (to permit more rapid penetration of cells into plant cell-wall lumina).

Because these complex functions cannot at this point be genetically transferred (let alone expressed) into RCB, there has been some interest in a second strategy: introducing cellulolytic functions into other, more robust ruminal bacteria. *Prevotella ruminicola* has been suggested as a potential recipient of genes encoding cellulolytic functions (27), because this species already possesses acid resistance and produces a carboxymethylcellulase enzyme containing a catalytic domain but lacking a cellulose-binding domain. However, this species does not display strong adherence to cellulose (19), and the cellulolytic capacity of a single enzyme—even one containing both binding and catalytic domains—likely will not be as strong as those of a multienzyme, cellulosome-like complex of the predominant RCB. Thus, the cellulolytic capabilities of such engineered strains might be expected to be rather modest, although they may be useful in rumens having chronic pH <6.0, which is the level needed to sustain growth of the typical RCB.

The secondary RCB, *B. fibrisolvens*, also might be a useful candidate for genetic manipulation. This species displays somewhat more acid tolerance than do the predominant RCB (26), and some strains are moderately cellulolytic. Enhancement of cellulolytic capabilities in this actively motile species may permit more rapid digestion of certain thick-walled cell types, the digestibility of which may be limited by slow diffusion of nonmotile RCB into the lumina to the secondary wall (44). This species does not appear to possess a cellulosome-like structure but apparently secretes its enzymes into the culture medium. As has been noted, this strategy appears to be inferior to that of the predominant RCB for growth on most plant cell types. However, this mode of cellulose digestion may facilitate the isolation of hypercellulolytic mutants that may be of some use in the digestion of forages of very low quality. Hungate (12) doubted that *Butyrivibrio* species were of importance in ruminal cellulose digestion under most circumstances, but cited reports that those species were the most abundant fibrolytic isolates from the rumens of animals fed very poor quality forage (7, 17), in which thick-walled plant cell types are particularly abundant. Also, *B. fibrisolvens* is one of the few ruminal microbes for which gene transfer technology has been successful (43).

CONCLUSIONS

The predominant RCB have developed an extreme nutritional specialization into one of the most aggressive capacities for cellulose digestion found in nature.

The specialized machinery for cellulose digestion developed by these organisms appears to have reached a practical upper limit in the rate of cellulose digestion within the constraints of an adherent cellulolytic lifestyle. Consequently, significant increases in the rate of forage fiber digestion by these species will be difficult to obtain. More success might be obtained by improving the cellulolytic capabilities of other ruminal bacteria that display other desirable features (e.g., motility or acid tolerance). Alternatively, improving the extent of digestion (e.g., through removal of matrix interactions among forage cell wall biopolymers) or use of feeding management strategies that minimize undesirable environmental conditions (e.g., low pH) may provide a productive route to enhancing animal performance.

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