

Ruminal Cellulolytic Bacteria



Physiology, Ecology, and Beyond

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Introduction

The rumen is a complex ecosystem in which feeds consumed by the ruminant animal are digested by an active and diverse microflora. The products of these fermentations are volatile fatty acids and microbial biomass which are in turn used by the host. It is the diversity, adaptability and mutualistic interactions among the ruminal microbes (bacteria, protozoa and fungi) and the host that have given ruminants a competitive edge in their ability to digest and thrive on diets high in fiber but often low in protein.

Modern feeding practices are geared toward high production of milk and meat, and have presented some novel challenges to the rumen microflora. The microbial response to these challenges has not always been satisfactory to the producer, leading to speculation on how the rumen fermentation might be “improved” to enhance both production and animal welfare. Proposals to manipulate the ruminal microflora have generally been narrow in focus and rarely have considered the exquisite complexities of microbial ecology. I will define our work relating to the physiology and ecology of ruminal cellulolytic microbes and how the information from these studies can help us to devise strategies on how such manipulation might be—or might not be—accomplished.

Our current research focuses on three species of bacteria—*Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus*—that are thought to be the primary agents of cellulose digestion in the rumen. These species were first isolated and described over thirty years ago (Hungate 1966). So it is somewhat surprising that until recently we had almost no quantitative information regarding their cellulolytic and physiological

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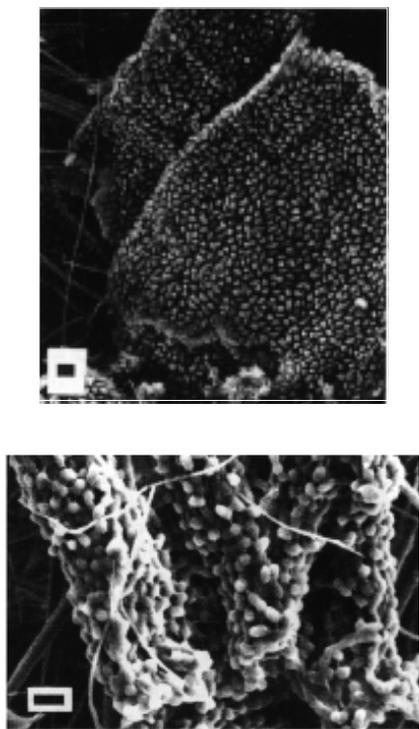


Figure 1. Electron photomicrographs of two predominant ruminal cellulolytic bacteria, *Fibrobacter succinogenes* S85 (top) and *Ruminococcus flavefaciens* FD-1 (bottom) growing on microcrystalline cellulose. Bar represents 2 μ m.

properties. Most of the information that was available had been obtained under very artificial growth conditions (i.e., using high concentrations of glucose or cellobiose as growth substrates). These three cellulolytic species share several common features:

- They are strictly anaerobic and are killed by exposure to oxygen.
- They have a narrow pH range (pH 6-7) for growth.
- They require for growth certain branched-chain volatile fatty acids (usually supplied in the rumen by amino acid fermenting bacteria).
- They form an extracellular glyocalyx coat that allows them to adhere to cellulose (Fig. 1), a property that appears to be essential to ruminal cellulose digestion.
- Most strains are highly specialized nutritionally, being nearly restricted to cellulose and its hydrolytic products as fermentable substrate.

Microbial Physiology of Cellulose Digestion

Although the 1990s is the era of molecular biology, we must not lose sight of the fact that an understanding of an organism's physiology and ecology are prerequisites to any successful strategy to improve an organism's properties through genetic engineering. This is particularly true for the ruminal cellulolytic bacteria, because these highly adapted species have developed some clever strategies for fiber digestion that will be difficult to duplicate or improve upon, along with some glaring weaknesses whose surmounting would be a noble and reasonable goal. Failure to distinguish between the two will result in wasted time, effort, and research money.

Research at this laboratory has recognized the need to study the physiology of cellulose digestion under conditions that more closely resemble those in the rumen environment than others had used in the past. Ideally, these studies should be carried out with forages themselves, and indeed this remains one of our long-term goals. But first we need to understand the degradation of plant cell-wall components before we can elucidate the complexities of cell wall digestion. In particular, we need to reexamine some

of the misconceptions that have resulted from overzealous interpretation of the earlier literature. Cellulose, as the most abundant plant cell wall component, is the most sensible choice for these studies.

Choice of Substrate

Our approach in selecting conditions for quantitative growth studies has differed somewhat from those of previous workers. Firstly, we wanted to use authentic cellulose as a substrate. This would seem logical enough, but it represented a departure from most previous research. In much of the early work, physiological and cellulolytic characteristics of ruminal microbes were determined using either soluble, commercially-available building blocks of cellulose (such as glucose or the disaccharide cellobiose), or modified cellulosic materials. These included ball-milled cellulose (a cellulose ground until all of its crystalline structure—that is, the ordered arrangement of individual chains of cellulose into a crystalline lattice—is lost); acid-swollen cellulose (in which crystallinity is disrupted by prolonged treatment in dilute acid); or even carboxymethylcellulose (a cellulose that has been chemically modified to make it completely water-soluble; Fig. 2). We chose to use microcrystalline cellulose, a commer-

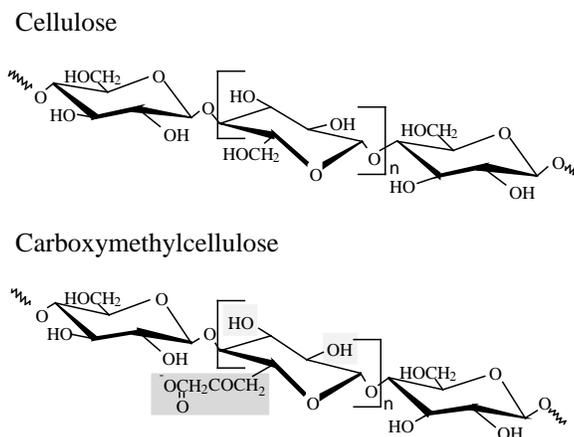


Figure 2. Monomeric structures of cellulose and of sodium carboxymethylcellulose (NaCMC), a soluble cellulose ether often used for assay of cellulolytic activity. Although the carboxymethyl group of NaCMC is shown at carbon 6 of the glucosyl moiety, it may also be located at carbons 2 or 3 (shaded); the amount and position of the carboxymethyl substituents are determined by the synthetic conditions used. The number n of repeating units can be up to 3000 for cellulose or 1500 for NaCMC. The latter compound resembles cellulose well enough to fool cellulolytic enzymes (and microbiologists who believe that with this substrate they are studying cellulose degradation). Ruminal bacteria will hydrolyze NaCMC, but cannot utilize the hydrolytic products. Thus NaCMC can be used for assaying one type of cellulase activity, but cannot be used for growth studies.

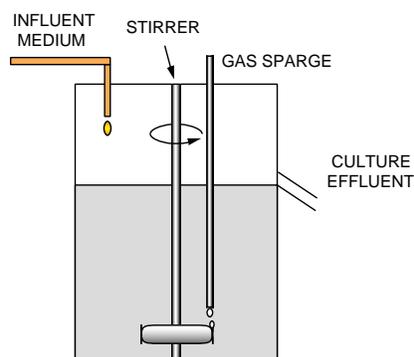


Figure 3. Schematic view of a continuous culture device. Continuous supply fresh nutrient allows us to maintain microbes in an active, exponential growth phase.

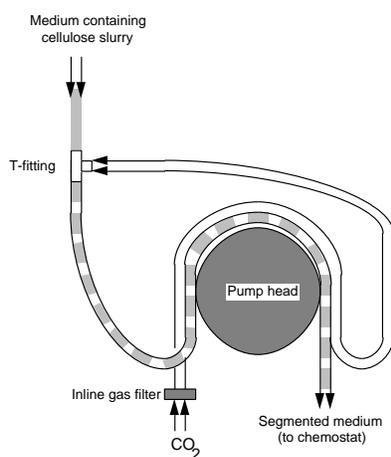


Figure 4. Segmented slurry delivery of cellulose. The device permits delivery of cellulose to a culture as a growth-limiting nutrient, which permits the application of chemostat theory and the determination of fundamental growth and fermentation parameters. To quote Tom Wolfe, "It took us forty years to get this far!"

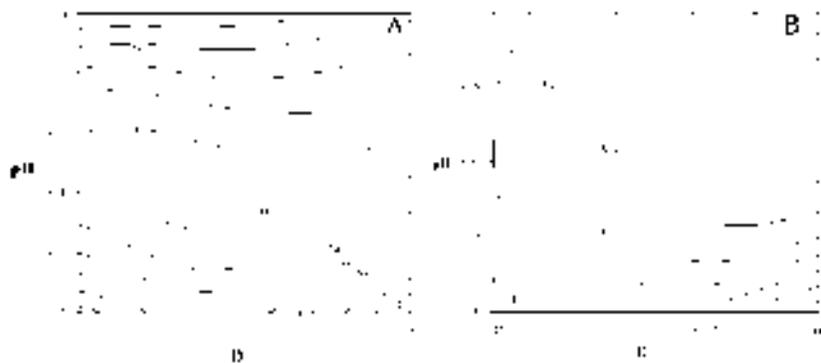


Figure 5. Response surfaces for per cent of added cellulose digested (A) and for microbial growth yield (B; g cells/g cellulose consumed) for *R. flavefaciens* FD-1 as a function of dilution rate (D , equivalent to microbial growth rate) and pH. The contours were obtained by statistical fitting of data from 20 different chemostat runs, each at a different combination of D and pH (Shi and Weimer, 1992). Response surfaces have also been generated for *F. succinogenes* S85 (Weimer 1993).

cial derivative of cotton, that has been treated to reduce its particle size (to simplify handling) without sacrificing its two most important physical properties: insolubility in water and crystalline structure.

The Benefits of Continuous Culture

Initially we wanted to determine the fundamental fermentation and growth parameters of each of the major ruminal cellulolytic species, specifically:

- How fast would each digest cellulose?
- What are the product balances and growth yields of each?
- How much substrate would each require to remain alive in the absence of growth?
- How were all these parameters affected by the growth rate of the organism and by important environmental factors such as pH?

The most accurate way to make these determinations is to grow a bacterial species in continuous culture, using a type of bioreactor known as a chemostat (Fig.3). In this technique, growth medium is supplied at a discrete rate to a vessel of constant working volume whose contents are continuously mixed. By selecting the proper concentration of nutrient, one can obtain a homogeneous culture in which all of the microbes are maintained at the same growth rate, a rate equal to the dilution rate (i.e., flow rate divided by working volume). The ability to pre-select the growth rate of the organism distinguishes the chemostat from batch cultures (the most common

way of growing microbes in the laboratory). In batch culture, the bacteria are sealed in a tube with nutrient media, and growth proceeds in a mostly uncontrolled fashion through a classic sequence of phases (lag, exponential growth, stationary, death). Exponential growth occurs only at the organism's maximal growth rate. This type of growth kinetics is not representative of the real world, because in nature microbes normally grow at submaximal rates, usually as a consequence of nutrient limitation (Slater 1988).

Chemostats have been an important tool for microbiologists for fifty years and have been used to determine the growth parameters for many microbial species. They have been used to characterize the growth of numerous ruminal species—including some cellulolytic ones—but using soluble substrate. We wanted to examine the growth of cellulolytic bacteria while they were growing on cellulose. So our first challenge was to develop a new type of bioreactor that would allow us to undertake continuous culture studies using cellulose as the growth-limiting nutrient.

The Segmented Slurry Delivery System

The continuous culture device we developed relied on a new type of delivery system, which we called "segmented slurry delivery," in which a well-mixed culture medium containing microcrystalline cellulose particles was broken into tiny segments separated by small bubbles of CO_2 (Fig.4). The cellulose is retained within the segments of medium by the high surface tension of the aqueous media and is reproducibly delivered to the reactor via a peristaltic pump at the low rates required for these types of growth studies. This apparatus enabled us to determine the effect of microbial growth rate and pH on the fermentation parameters for two major species of ruminal cellulolytic bacteria, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* (Shi and Weimer 1992; Weimer 1993). These data have allowed us to identify the optimum growth rate and pH for cellulose digestion by each organism (Fig. 5). Moreover, these experiments pro-

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vide some important information that was not suspected from earlier studies. I’ll give three examples that have implications for the rumen fermentation and for prospects of its manipulation.

Cellulases: Soluble or Cell-Bound?

Numerous references in the literature indicate that batch cultures of ruminal cellulolytic bacteria grown with excess cellulose substrate often contain extracellular cellulase enzymes in amounts sufficient for their purification and biochemical characterization. Yet it also has been known for a long time that ruminal fluid itself lacks measurable levels of cellulases. The latter observation was always justified by the claim that cellulose in the digesta completely adsorbed cellulases secreted by the ruminal bacteria.

Cultures in our cellulose-limited chemostats also lack extracellular cellulase activities. Microscopic examination of the cellulose particles in these cultures reveals that they are completely covered by adherent cellulolytic bacteria. In other words, there are no available sites on the cellulose to adsorb secreted cellulases (Fig. 1). This indicates that ruminal cellulolytic bacteria that are actively growing and actively digesting cellulose do not secrete their cellulolytic enzymes but instead retain them at the cell surface. From an ecological standpoint, this makes perfect sense: it gives the cellulolytics the first crack at the products of cellulose digestion, and it helps the cellulolytic en-

zymes survive in an environment so rich in extracellular proteases that most feed protein is degraded before it can pass out of the rumen. In light of the localization of cellulolytic enzymes and its ecological advantages, it is likely that attempts to “improve” cellulolytic species to secrete large amounts of cellulase will not be a productive strategy to enhancing forage digestion.

Relative Capacities for Cellulose Digestion

A second interesting conclusion arising from our chemostat studies involves the relative cellulolytic capabilities of different bacterial species. Our data show that *F. succinogenes*—regarded for decades (with little evidence) as the king of the ruminal cellulolytic bacterial species—in fact is inferior to *R. flavefaciens* in terms of the maximum rate of digestion of crystalline cellulose (Shi and Weimer 1992; Weimer 1993). Studies in other labs have indicated that the third major ruminal cellulolytic species, *R. albus*, has a maximal rate of cellulose digestion somewhat lower than those of the other two species (Pavlostathis et al. 1988b). Yet when one compares these rates with those of other cellulolytic species, one finds that all the three predominant species of ruminal cellulolytic bacteria degrade cellulose much more rapidly than do almost any nonruminal species—including some “benchmark” cellulolytic species (e.g., the fungus *Trichoderma reesei*) used for commercial production of cellulases (Table 1). The data reinforce the notion that the predominant ruminal cellulolytic species are a specialized, highly adapted group of microbes whose cellulolytic capabilities allow them to overpower introduced species in competition for cellulose. This conclusion is in accord with an interesting observation by Varel et al. (1995), who introduced a huge inoculum (a six liter fermentor-grown culture) of the *Clostridium longisporum* into the emptied rumens of three alfalfa-fed steers. This strain of *C. longisporum* had been isolated from the rumen and, while it does not extensively colonize cellulose, it degrades cellulose well in pure culture. Despite this, Varel et al. (1995)

Table 1. Comparison of rate constants for digestion of crystalline cellulose by various ruminal and nonruminal microorganisms.¹

| Organism | Source | Substrate ² | Rate constant (h ⁻¹) |
|----------------------------------|------------|------------------------|----------------------------------|
| <i>Clostridium thermocellum</i> | Nonruminal | AV | 0.16 |
| <i>Ruminococcus flavefaciens</i> | Ruminal | SC | 0.08 |
| <i>Fibrobacter succinogenes</i> | Ruminal | SC | 0.07 |
| <i>Ruminococcus albus</i> | Ruminal | AV | 0.05 |
| <i>Cellulomonas uda</i> | Nonruminal | AV | 0.027 |
| <i>Cellulomonas flavigena</i> | Nonruminal | AV | 0.006 |
| White-rot fungi (5 species) | Nonruminal | Cot | < 0.004 |
| Brown-rot fungi (8 species) | Nonruminal | Cot | < 0.002 |

¹Data from various sources, tabulated in Weimer (1996)

²AV = Avicel microcrystalline cellulose PH 101, SC = Sigmacell 20 microcrystal cellulose, and Cot = cotton cellulose.

“... genetic engineering of acid-tolerant cellulolytic species may represent a useful strategy for improving fiber digestion in modern ruminants ...”

found that within 24 hours of introduction, this strain was not detectable any of the three rumens. The message to an introduced organism is clear: “Enter ye hither, and leave all hope behind!”

Our attempts to better relate laboratory-scale microbiology to in vivo conditions requires that the relationship between pH and cellulolysis be revisited. In vitro studies clearly show the inability of ruminal cellulolytic bacteria to grow at pH values below 6 (Russell and Dombrowski 1980). But today’s dairy and beef animals are geared to high production, which is usually achieved by including high proportions of grain in the diet, with consequent rapid fermentation and low ruminal pH. Under such conditions, fiber digestion is strongly inhibited. Despite a strong selective pressure in the natural environment, there is no indication that these feeding regimes have resulted in the adaptation or development of acid-tolerant cellulolytic species (Slyter 1986). Thus genetic engineering of acid-tolerant cellulolytic species may represent a useful strategy for improving fiber digestion in modern ruminants (Russell 1988). One way to do this—the introduction of cellulolytic capabilities into acid-tolerant noncellulolytic bacteria that normally inhabit the rumen—may be a worthwhile approach, but it is likely that efficient cellulose digestion will occur only if the engineered species display some of the same desirable characteristics of the predominant ruminal cellu-

lytic species: adherence of cells to the cellulose surface; retention of cellulolytic enzymes at the cell surface; and ability of the enzymes to degrade authentic crystalline cellulose. In addition, the introduced strain would have to be able to compete effectively during times when the rumen pH rises above pH 6 and the native species attempt to regain control of the cellulose fermentation.

Profiles of Fermentation Products

A third conclusion from chemostat studies is that each of the predominant ruminal cellulolytic species has a characteristic profile of fermentation products that changes only slightly with growth rate and pH. This is in marked contrast to noncellulolytic species such as *Streptococcus bovis* and *Selenomonas ruminantium*, whose products vary tremendously with growth rate (Table 2). The relative constancy of product ratios for individual cellulolytic species suggests that control of the proportions of VFAs in the rumen may be achieved by controlling the relative populations of cellulolytic species.

This notion is consistent with the known effects of monensin, an antibiotic widely used in beef production (and which is used in replacement heifers but not in lactating dairy cows). Monensin is a proton ionophore that has a number of effects, one of which is a selective inhibition of Gram-positive bacteria (including most hydrogen-producing ruminal bacteria) and resulting enhance-

Table 2.
Reported ranges of molar yields in fermentation products of several ruminal bacteria.

| | n ^a | mol product/mol glucose equivalent | | | | | Reference |
|----------------------------------|----------------|------------------------------------|-----------|------------|-----------|-----------|-----------|
| | | succinate | acetate | propionate | ethanol | lactate | |
| Cellulolytic | | | | | | | |
| <i>Fibrobacter succinogenes</i> | 22 | 0.74-1.28 | 0.16-0.56 | ---- | ---- | ---- | b |
| <i>Ruminococcus flavefaciens</i> | 20 | 0.45-0.80 | 0.64-0.99 | ---- | ---- | ---- | c |
| <i>Ruminococcus albus</i> | 6 | ---- | 0.85-0.91 | ---- | ---- | ---- | d |
| Noncellulolytic: | | | | | | | |
| <i>Streptococcus bovis</i> | 2 | ---- | 0.07-0.79 | ---- | 0.05-0.64 | 0.31-1.67 | e |
| <i>Selenomonas ruminantium</i> | 7 | 0.05-0.62 | 0.44-1.2 | 0.24-1.00 | ---- | 0.10-1.60 | f |

^aNumber of chemostat runs, each with a different dilution rate and/or pH

^bWeimer 1993

^cShi and Weimer 1992

^dPavlostathis et al. 1988a

^eRussell

^fMelville et al. 1988

“... enhancing ruminal acetic acid production to encourage mammary lipogenesis for production of high-fat milk valued in the cheese industry.”

ment of Gram-negative bacteria that produce succinate or propionate (Schelling 1984, Russell and Strobel 1989). In a forage-fed animal, treatment with monensin should lead to a reduction in the population of *Ruminococcus* species (which produce primarily acetate) relative to *F. succinogenes* (which produces primarily succinate, the major precursor of propionate in the rumen); the net effect would be a decrease in the ratio of ruminal acetate relative to ruminal propionate. In fact, a lowering of the acetate/propionate ratio is one of the distinguishing effects of monensin treatment (Schnelling 1984).

While monensin has significantly improved animal production in the beef industry, it makes sense to consider alternative options to control the ruminal microbial population in dairy cattle for two reasons. The first concerns public opinion regarding the real or imagined dangers of antibiotic use in farm animals and milk's historical position in the public mind as the purest of foods. The second involves opportunities for manipulating the ruminal fermentation to provide the cow with a mix of fermentation products most suitable to produce milk with properties demanded by today's consumers. One example of this would be enhancing ruminal acetic acid production to encourage mammary lipogenesis for production of high-fat milk valued in the cheese industry. Increasing the relative proportion of ruminococci in the rumen may be one way to achieve this end.

Interactions Among Ruminal Cellulolytic Bacteria

What are the prospects of controlling the populations of the different cellulolytic species in the rumen? We decided to take a look at this, and as usual, we started with a reductionist approach aimed at determining the outcome of interactions among the three predominant cellulolytic species and identifying the mechanism underlying these interactions. Again, we employed the chemostats for these studies because in the chemostat we can more accurately

mimic the nutritional status of the bacteria. The pioneering work of Waldo et al. (1972) revealed that ruminal cellulose digestion follows first-order kinetics with respect to cellulose concentration. In other words, as long as environmental conditions in the rumen are favorable for growth of the cellulolytic microbes, the rate of cellulose digestion will not be limited by the microbes' cellulolytic capabilities, but by the availability of the cellulose substrate.

We have built upon these studies by demonstrating that available surface area (rather than mass of cellulose per se) is the actual determinant of the digestion rate in vitro (Weimer et al. 1990). It is therefore clear that, if we wish to examine interactions among ruminal cellulolytic bacteria, we should do it under conditions of cellulose limitation (i.e., in a chemostat), not cellulose excess (i.e., in batch culture). Again, this is a departure from the half-dozen or so studies in which defined mixed cultures of ruminal cellulolytic species were grown in media containing excess concentrations of various substrates.

In our studies, we grew the three predominant ruminal cellulolytic species in different binary (two-species) combinations, using either cellulose or cellobiose (a soluble cellulose building block) as the growth-limiting nutrient. We chose these substrates because the cellulolytic species could potentially compete for both the insoluble cellulose and the soluble products of cellulose hydrolysis. For comparative purposes, we also grew the same combinations in batch culture containing excess (nonlimiting) amounts of the same substrates. We assessed the population dynamics of each species using a variety of techniques: unique fermentation products; characteristic membrane fatty acids; and, most useful of all, oligonucleotide probes directed toward strain-specific sequences in 16S ribosomal RNA (Odenyo et al. 1994).

One of the most striking results of these findings is that the three cellulolytic species showed no synergism in the degradation of these simple substrates. The operant interaction was competition, not cooperation. Moreover, the outcome of the competition depended

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upon both the substrate and its availability. When substrate was in excess (i.e., in batch culture), each paired combination of species co-existed in roughly equal population sizes.

Under substrate limitation, we expected competition to be much more intense. Theoretical ecological principles predict that one species should outcompete the other for simple soluble nutrients such as cellobiose. These principles further dictate that the outcome of the competition should be dependent solely on the two so-called Monod growth parameters: the maximal rate of growth (m_{max}) and the affinity constant (K_s , the concentration of substrate that permits growth at half the maximal rate). We determined these parameters from batch culture studies, and the data suggested that the outcome of the competition should be: *R. flavefaciens* FD-1 > *F. succinogenes* S85 > *R. albus* 7 (Shi and Weimer 1996a). In fact, we found that, indeed, one species always took over the culture (Shi and Weimer 1996b). As expected, *R. flavefaciens* always supplanted *F. succinogenes*. However, *R. albus* surprised us by usually (but not always) supplanting either *F. succinogenes* or *R. flavefaciens*. When we characterized the population remaining in the chemostat at the end of a competition won by *R. albus*, we found that that this species had adapted to have a lower K_s value than the original strain,

so that the theoretical principles held up. Interestingly, *F. succinogenes* or *R. flavefaciens* never showed this adaptation. We believe that the inherent adaptability of *R. albus* is one factor that gives it a selective advantage in the rumen.

The competition between these species for cellulose was more complex. Inoculation of two species usually yielded stable cocultures, with one strain predominating and the other present in lesser, but significant, amounts (Table 3). *R. flavefaciens* always was more numerous than were *F. succinogenes* or *R. albus*, apparently because it can colonize the cellulose more rapidly than can the other two species, and because it can grow more rapidly on cellodextrins (the intermediate products of cellulose hydrolysis) (Shi and Weimer 1996c). *R. albus*, which did so well in competition for cellobiose, always was less abundant than was *R. flavefaciens* or *F. succinogenes*, apparently because it was less effective in colonizing and hydrolyzing cellulose, and was probably reduced to growing on soluble products released by the other species during cellulose hydrolysis. The data suggest that as the substrate becomes increasingly complex, the number of factors that can determine the outcome of competition increases. This contributes to the establishment of several species with similar, but not identical, degradative capabilities.

“The data suggest that as the substrate becomes increasingly complex, the number of factors that can determine the outcome of competition increase.”

Table 3.

Outcome of binary cocultures of *F. succinogenes* S85, *R. flavefaciens* FD-1, and *R. albus* 7 in cellulose-limited chemostats.^a

| Inoculation order | D (h ⁻¹) ^b | pH | Residual cellulose (g/L) | | Culture composition ^c | | |
|-------------------|--------------------------------------|------|--------------------------|--------------|----------------------------------|-------|------|
| | | | Initial | Steady-state | S85 | FD-1 | 7 |
| Co-inoculation: | | | | | | | |
| S85 + FD-1 | 0.049 | 6.44 | 4.50 | 1.23 | <4.5 | >95.5 | — |
| 7 + FD-1 | 0.016 | 6.79 | 7.08 | 6.55 | — | 85.1 | 14.9 |
| 7 + S85 | 0.024 | 6.27 | 4.44 | 2.39 | 89.9 | — | 10.1 |
| 7 + S85 | 0.020 | 6.10 | 5.59 | 3.53 | 78.1 | — | 21.9 |
| Sequential: | | | | | | | |
| S85, then FD-1 | 0.030 | 6.48 | 4.35 | 1.42 | <4.5 | >95.5 | — |
| FD-1, then 7 | 0.029 | 6.52 | 5.22 | 1.78 | — | 89.2 | 10.7 |
| S85, then 7 | 0.031 | 6.50 | 5.66 | 1.40 | 90.7 | — | 9.3 |

^aSteady-state data only. Complete time-course data not shown.

^bDilution rate in reciprocal hours.

^cEstimated composition at end of incubation based on RNA probe data., except for the S85/FD-1 cocultures which are based on a less sensitive method employing characteristic membrane fatty acids.

Onward, to the Real World

Our laboratory studies have revealed some of the interactions among the ruminal cellulolytic bacteria. The time is now ripe for expanding these studies to include "real" substrates (forages) and the rumen itself. Our work is being aided enormously by use of rRNA-directed oligonucleotide probes to identify individual cellulolytic species. In this work we seek to answer four main questions:

- Do in vitro and in sacco digestion methods yield similar kinetic results for different cows or different diets?
- What are the quantitative associations among digestion kinetics, VFA ratios, VFA concentrations, specific microbial populations, and milk quality?
- How much variation in these parameters is due to the diet, and how much is due to the cow?
- How well can we control specific populations by feeding and management strategies (e.g., diet or frequency of feeding).

By combining this information with that gained from more fundamental studies on cell-wall structure, we will ultimately be able to predict the outcome of changes in cell wall structure on the aspects of forage digestion that impact milk production.

References

- Bryant, M.P. 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. *Fed. Proc.* 32:1809-1813.
- Hungate, R.E. 1966. *The rumen and its microbes.* Academic Press, New York.
- Odenyo, A.A., R.I. Mackie, D.A. Stahl, and B.A. White. 1994. The use of 16S rRNA-targeted oligonucleotide probes to study competition between ruminal fibrolytic bacteria: development of probes for *Ruminococcus* species and evidence for bacteriocin production. *Applied and Environmental Microbiology* 60:3688-3696.
- Pavlostathis, S.G., T.L. Miller, and M.J. Wolin. 1988a. Fermentation of insoluble cellulose by continuous cultures of *Ruminococcus albus*. *Applied and Environmental Microbiology* 54:2655-2659.
- Pavlostathis, S.G., T.L. Miller, and M.J. Wolin. 1988b. Kinetics of insoluble cellulose fermentation by continuous cultures of *Ruminococcus albus*. *Applied and Environmental Microbiology* 54:2660-2663.
- Russell, J.B., and R.L. Baldwin. 1979. Comparison of maintenance energy expenditures and growth yields among several rumen bacteria grown in continuous culture. *Applied and Environmental Microbiology* 37:537-543.

- Russell, J.B., and D.B. Dombrowski. 1980. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. *Applied and Environmental Microbiology* 39:604-610.
- Russell, J.B., and H.J. Strobel. 1989. The effect of ionophores on ruminal fermentation. *Applied and Environmental Microbiology* 55:1-6.
- Russell, J.B., and D.B. Wilson. 1988. Potential opportunities and problems for genetically engineered rumen microorganisms. *Journal of Nutrition* 118: 271-279.
- Schelling, G.T. 1984. Monensin mode of action in the rumen. *Journal of Animal Science* 58:1518-1527.
- Shi, Y., and P.J. Weimer. 1992. Response surface analysis of the effects of pH and dilution rate on *Ruminococcus flavefaciens* FD-1 in cellulose-fed continuous culture. *Applied and Environmental Microbiology* 58:2583-2591.
- Shi, Y., and P.J. Weimer. 1996a. Utilization of individual cellooligosaccharides by three predominant species of ruminal cellulolytic bacteria. *Applied and Environmental Microbiology* 62:1084-1088.
- Shi, Y., and P.J. Weimer. 1996b. Competition among ruminal cellulolytic bacteria for cellobiose under substrate-unlimited and substrate-limited conditions. *Applied and Environmental Microbiology* (submitted).
- Shi, Y., and P.J. Weimer. 1996c. Competition among ruminal cellulolytic bacteria for cellulose under substrate-unlimited and substrate-limited conditions. *Applied and Environmental Microbiology* (submitted).
- Slater, J.H. 1988. Microbial populations and community dynamics. p.51-74 In: J.M. Lynch and J.E. Hobbie (eds.) *Microorganisms in action: concepts and applications in microbial ecology.* 2nd ed., Blackwell Scientific Publications, Oxford, U.K.
- Slyter, L.L. 1986. Ability of pH-selected mixed ruminal microbial populations to digest fiber at various pHs. *Applied and Environmental Microbiology* 52: 390-391.
- Smith, L.W., H.K. Goering, and C.H. Gordon. 1972. Relationships of forage compositions with rates of cell wall digestion and indigestibility of cell walls. *Journal of Dairy Science* 55:1140-1147.
- Varel, V.H., J.T. Yen, and K.K. Kreikemeier. 1995. Addition of cellulolytic clostridia to the bovine rumen and pig intestinal tract. *Applied and Environmental Microbiology* 61:1116-1119.
- Waldo, D.L., L.W. Smith, and E.L. Cox. 1972. Model of cellulose disappearance from the rumen. *Journal of Dairy Science* 55:125-129.
- Weimer, P.J. 1993. Effects of dilution rate and pH on the ruminal cellulolytic bacterium *Fibrobacter succinogenes* S85 in cellulose-fed continuous culture. *Archives of Microbiology* 160:288-294.
- Weimer, P.J. 1995. Why don't ruminal bacteria digest cellulose faster? *Journal of Dairy Science*, in press.
- Weimer, P.J., J.M. Lopez-Guisa, and A.D. French. 1990. Effect of cellulose fine structure on the kinetics of its digestion by mixed ruminal microflora. *Applied and Environmental Microbiology* 56:2421-2429.

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