

Interactions Among Ruminal Cellulolytic Bacteria in Defined Cocultures Under Cellobiose Limitation

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Introduction

Cellulose is the major component of plant material and thus a major energy source for forage-fed ruminants. In the rumen, cellulose hydrolysis produces cellodextrins and cellobiose, which can be utilized by both cellulolytic and noncellulolytic ruminal bacteria. Individual species of cellulolytic bacteria differ in their fermentation endproducts and in their interaction with noncellulolytic species, suggesting that the cellulolytics have an effect on both the overall microbial ecology of the rumen, and on the endproducts of the ruminal fermentation. Previously, we have shown that the three predominant species of ruminal cellulolytic bacteria can establish stable tricultures when grown under limiting amounts of cellulose. The success of each species in the competition depends on several factors, including its ability to attach to cellulose; to effectively utilize low concentrations of hydrolytic products; and to produce products that inhibit the growth of competitors. During growth on soluble products of cellulose hydrolysis, the outcome of the competition may be expected to differ, because attachment to cellulose is no longer a factor in the competition. The purpose of this study was to examine the outcome of competition among the three predominant ruminal cellulolytic species in defined coculture with the soluble substrate cellobiose as growth substrate, and to determine how the competition is affected by the presence of noncellulolytic cellobiose-utilizing competitors.

Materials and Methods

Fibrobacter succinogenes S85, *Ruminococcus flavefaciens* FD-1 and *Ruminococcus albus* 7 were simultaneously inoculated in similar (~ 3 mL) quantities into a 139 mL reactor fed a modified Dehority medium that contained ~ 4 g of cellobiose/L. The reactor was continuously sparged with CO₂, and was fed at dilution rates in the range of 0.016 to 0.046 h⁻¹. In some of the experiments, the noncellulolytic bacteria *Selenomonas ruminantium* (either with strain D, or a

mixture of strains D, GA192, HD4, and H18) or *Streptococcus bovis* JB-1 were included in the inoculum. After achievement of steady state (3-5 dilutions), samples (5 mL) of culture were aseptically removed and analyzed for pH, residual soluble sugars (by a phenol-sulfuric acid method), and fermentation endproducts (by HPLC). After concentrating the cells by centrifugation, RNA was extracted from the cells by a chloroform-phenol method, and the recovered RNA hybridized on nylon membranes to species-specific oligonucleotide probes to 16S ribosomal RNA. Hybridized RNA was quantitated by chemluminescence and densitometry. Because the cells in the chemostats at steady state had equivalent growth rates, it was assumed that cell mass corresponding to each species was proportional to the relative abundance of species-specific RNA in the sample. Owing to the unavailability of effective probes for *S. ruminantium* and *S. bovis*, estimates of relative population densities were determined only for the three cellulolytic species, and were expressed as a percentage of the total cellulolytic population. However, the production of lactate by *S. bovis*, or of propionate by *S. ruminantium*, were taken as indicators of their presence in the coculture, as none of the three cellulolytic strains used here produce these compounds in significant amounts.

Results and Discussion

The data on microbial populations are summarized in Table 1. For all of the defined mixed cultures, steady states were achieved in which the species distributions reached constant values. In the three tricultures of the cellulolytic species, *R. albus* was the dominant member of the culture, while *R. flavefaciens* FD-1 and *F. succinogenes* S85 were below the detection limit of the oligonucleotide probe method. Based on previous triculture studies in batch mode on cellobiose (Odenyo et al. 1994) and in cellulose-fed chemostats (Weimer and Chen 1998), it appears that *R. albus* 7 produced a compound that inhibits the growth of the normally more competitive *R. flavefaciens*. This was

confirmed by the demonstration that culture supernatants of *R. albus* 7 reduced the growth rate of *R. flavefaciens* FD-1 in liquid culture.

Inclusion of the succinate-decarboxylating, sugar-fermenting, noncellulolytic *Selenomonas ruminantium* resulted in a dramatic shift of the cellulolytic population away from *R. albus* and toward *R. flavefaciens*. These cultures demonstrated a nearly complete conversion of succinate to propionate, and microscopy revealed the abundance of *S. ruminantium*. Because succinate conversion by *S. ruminantium* does not yield energy necessary to support growth, this species grew in the coculture via effective competition for cellobiose. This reduced the density of *R. albus*, and the resulting decrease in the level of inhibitor enhanced the prominence of *R. flavefaciens*.

Inclusion of sugar-fermenting *Streptococcus bovis* in the cellulolytic triculture resulted in only a slight change in the cellulolytic population at $D = 0.021 \text{ h}^{-1}$ (Table 1). However, at $D = 0.045 \text{ h}^{-1}$, the cellulolytic population was shifted in a manner similar to that observed in the *Selenomonas*-amended cultures.

Conclusions

R. albus can effectively outcompete *R. flavefaciens* and *F. succinogenes* under conditions of cellobiose limitation, but this effect is attenuated by the presence of noncellulolytic cellobiose-utilizing bacteria. In these defined cocultures, *Selenomonas ruminantium* (a major ruminal propionate producer) enhanced *R. flavefaciens* (a major ruminal producer of the propionate precursor, succinate) at the expense of *R. albus*. This effect may counterbalance the suppression of *F. succinogenes* (which also produces succinate) observed previously in cellulose-limited cocultures (Weimer and Chen 1998).

References

- 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. *Appl. Environ. Microbiol.* 60: 3688-3696.
- Weimer, P.J., and J. Chen. 1998. Interactions among ruminal cellulolytic bacteria in defined cocultures under cellulose limitation. U.S. Dairy Forage Res. Ctr. 1997. Res. Sum., USDA-Agricultural Research Service, Washington, DC., p.53-54.

Table 1. Quantitative distribution of cellulolytic species in cellobiose-limited tricultures of *Fibrobacter succinogenes* S85, *Ruminococcus albus* 7, and *R. flavefaciens* FD-1 under steady state conditions in the presence or absence of the noncellulolytic bacteria *Selenomonas ruminantium* or *Streptococcus bovis*.

Culture	D (h^{-1}) ^a	pH	Percentage of the cellulolytic population ^b		
			<i>F.succinogenes</i>	<i>R.albus</i>	<i>R.flavefaciens</i>
<i>F.s.</i> + <i>R.a.</i> + <i>R.f.</i>	0.016	6.36	< 2.1 AB	> 97.1 G	< 0.8 A
	0.026	6.67	< 2.1 AB	> 97.1 G	< 0.8 A
	0.046	6.63	< 2.1 AB	> 97.1 G	< 0.8 A
<i>F.s.</i> + <i>R.a.</i> + <i>R.f.</i> + <i>S. ruminantium</i> D	0.021	6.63	< 2.1 AB	3.3 BC	94.6 F
<i>F.s.</i> + <i>R.a.</i> + <i>R.f.</i> + four strains of <i>S. ruminantium</i> ^c	0.034	6.68	< 2.1 AB	5.8 D	92.2 E
<i>F.s.</i> + <i>R.a.</i> + <i>R.f.</i> + <i>S. bovis</i> JB1	0.021	6.49	< 1.9 AB	3.4 BC	94.7 F
	0.045	6.71	< 1.9 AB	93.6 EF	4.5 CD

^aDilution rate in reciprocal hours

^bValues with different capital letters in column or row differ ($p < 0.05$)

^c*Selenomonas ruminantium* strains D, GA192, HD4, and H18