Competition Among Different Species of Ruminal Cellulolytic Bacteria in Cellulose-Limited Chemostats

Y. Shi and P.J. Weimer

Introduction

Cellulose is the major component of forage cell walls, and its digestion by ruminal microorganisms provides the bulk of the volatile fatty acids (VFA) used by the forage-fed ruminant animal for energy and milk production. Ruminal cellulose digestion is thought to be mediated primarily by three bacterial species: *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *R. albus*. The most commonly studied laboratory strains of these three species display quantitatively similar cellulolytic capabilities. However, because these species produce different ratios of fermentation endproducts, the relative populations and/or activities of these species may have profound effects on VFA ratios in the rumen. Little is known about the interactions among these species, particularly under conditions of substrate limitation that predominate in the rumen. The purpose of this study was to examine directly the competition among these three cellulolytic species for growth rate-limiting concentrations of cellulose.

Methods

F. succinogenes S85, *R. flavefaciens* FD-1, and *R. albus* 7 were used for growth experiments. Continuous cultures were performed at 39°C under CO_2 in a stirred reactor (working volume 875 mL) continuously fed a modified Dehority medium supplemented with cellulose (4.4 - 7.1 g/L) as sole energy source. The cellulose-containing medium was delivered as a CO_2-segmented slurry to prevent settling of cellulose in the pump lines. Two types of experiments were conducted: co-inoculation experiments in which two species were mixed aseptically and then inoculated into the reactor and "challenge" experiments in which one species was added to a steady-state chemostat culture of another species. Samples were withdrawn at various times and were analyzed for residual cellulose by a modified NDF method, and for soluble sugars, fermentation endproducts, and total cell mass as described in the previous report. The relative amount of each bacterial species was determined by three different methods (characteristic fermentation products, characteristic membrane fatty acids, and oligonucleotide probes homologous to 16S rRNA sequences specific for each species) as described in the previous report.

Results

The outcomes of the different coculture experiments are summarized in Table 1. *R. flavefaciens* FD-1 supplanted *F. succinogenes* S85 regardless of whether the strains were co-inoculated or an established steady-state culture of S85 was challenged with FD-1. The results are consistent with our previous observations that FD-1 adheres more rapidly and completely to cellulose and has a higher affinity for all cellodextrins (G2-G6) tested. Although S85 is capable of growth on glucose, the free glucose concentration in the culture was insufficient to sustain growth at the dilution rate of the chemostat.

Under the conditions tested, *R. albus* 7 was able to co-exist in binary culture with either *F. succinogenes* S85 or *R. flavefaciens* FD-1. In the case of FD-1, this co-existence may reflect a balance between factors favoring FD-1 (more rapid adherence to cellulose and more favorable growth kinetics on low concentrations of cellodextrins) and those favoring 7 (possible production of an inhibitor). In the case of S85, the coexistence with 7 is more complex. These two strains, in many cases, have similar growth rates on equivalent concentrations of cellodextrins and similar in vitro kinetics of adherence to cellulose, although growing cultures of S85 always show more complete attachment of cells to cellulose than do growing cultures of 7. *R. albus* 7 may benefit from the active efflux of longer chain cellodextrins that is routinely performed by S85.

Conclusions

Competition studies in cellulose-limited chemostats reveal that *R. flavefaciens* FD-1 readily outcompetes *F. succinogenes* for limiting amounts of cellulose, probably due to its more avid adherence to cellulose and its more rapid growth on the products of cellulose hydrolysis. By contrast, *R. albus* 7 was able to co-exist with either of the other two species, suggesting

that the interactions among these species are not based on pure and simple competition for cellulose, but instead reflect a combination of physiological characteristics of these species.

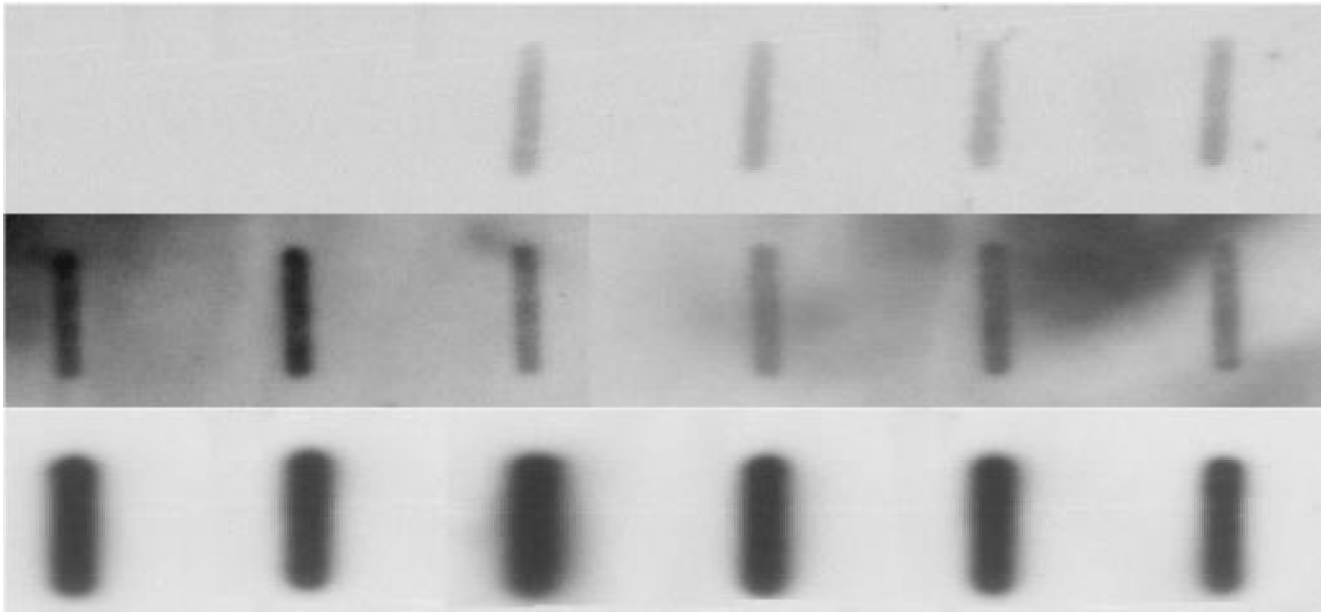

Figure 1. Cell populations in a cellulose-limited chemostat culture (D = 0.03 h^{-1}) of F. succinogenes S85 challenged with R. albus 7 at 90 hours. Top panel: Slot blot of 5 ng of RNA from culture samples at indicated times, detected by chemiluminescence after hybridization with oligonucleotide probes. A3c = F. succinogenes probe; RAL196 = R. albus probe; EUB338 = universal eubacterial probe that detects both species. Bottom panel: Estimate of cell populations in the chemostat, determined from RNA-specific oligonucleotide probes (RNA) or from membrane fatty acid composition of cell pellets (MFA).

Table 1. 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) Initial	Steady-state	Culture composition[c] S85	FD-1	7	Assay method and lower detection limit[d]
Co-inoculation:								
S85 + FD-1	0.049	6.44	4.50	1.23	< 4.5	> 95.5	—	MFA (4.5)
7 + FD-1	0.016	6.79	7.08	6.55	—	85.1	14.9	RNA (1.0)
7 + S85	0.024	6.27	4.44	2.39	89.9	—	10.1	RNA (1.0)
7 + S85	0.020	6.10	5.59	3.53	78.1	—	21.9	RNA (1.0)
Sequential:								
S85, then FD-1	0.030	6.48	4.35	1.42	< 4.5	> 95.5	—	MFA (4.5)
FD-1, then 7	0.029	6.52	5.22	1.78	—	89.2	10.7	RNA (1.0)
					—	94.3	5.7	MFA (3.2)
S85, then 7	0.031	6.50	5.66	1.40	90.7	—	9.3	RNA (1.0)

[a]Steady-state data only. Complete time-course data not shown.

[b]Dilution rate in reciprocal hours.

[c]Estimated composition at end of incubation, calculated from characteristic biomarkers indicated in (d).

[d]Assay method: MFA = signature membrane fatty acids; RNA = oligonucleotide probes to species-specific 16S rRNA. Values in parentheses indicate minimum percentage of the population of the subordinate strain detectable by the method.