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

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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 directly determine the ability of three predominant ruminal cellulolytic bacteria to compete with one another for growth-limiting concentrations of cellobiose, an important intermediate in the enzymatic depolymerization of cellulose and cellodextrins.

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 139 mL) continuously fed a modified Dehority medium supplemented with cellobiose (4 g/L) as sole energy source and with yeast extract (1 g/L). 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. For all experiments, samples were withdrawn from the reactor at various times for measurement of residual soluble sugars (by the anthrone colorimetric reaction), soluble fermentation products (by HPLC), and cell mass (estimated from nitrogen content of cell pellets). Populations of individual species were determined in three ways: i) by characteristic fermentation products (succinate for S85 or FD-1, ethanol for 7); ii) by signature membrane fatty acids (pentadecanoic acid for S85, 13-methyltetradecanoic acid for FD-1, and hexadecanoic acid for 7); and iii) using oligonucleotide probes homologous to characteristic sequences of 16S rRNA. RNA isolated from each sample was slot blotted on nylon membranes, hybridized to digoxigenin-labeled probe, and reacted with chemiluminescent substrate (Boehringer-Mannheim Genius system). X-ray film exposed by the chemiluminescent reaction was scanned by a densitometer.

Results

The outcomes of the different coculture experiments are summarized in Table 1. As expected from its higher affinity (lower $S_{0.5\mu max}$ value) for cellobiose, *R. flavefaciens* FD-1 readily outcompeted *F. succinogenes* S85, regardless of whether both species were co-inoculated into the chemostat or FD-1 was added to an established steady-state culture of S85.

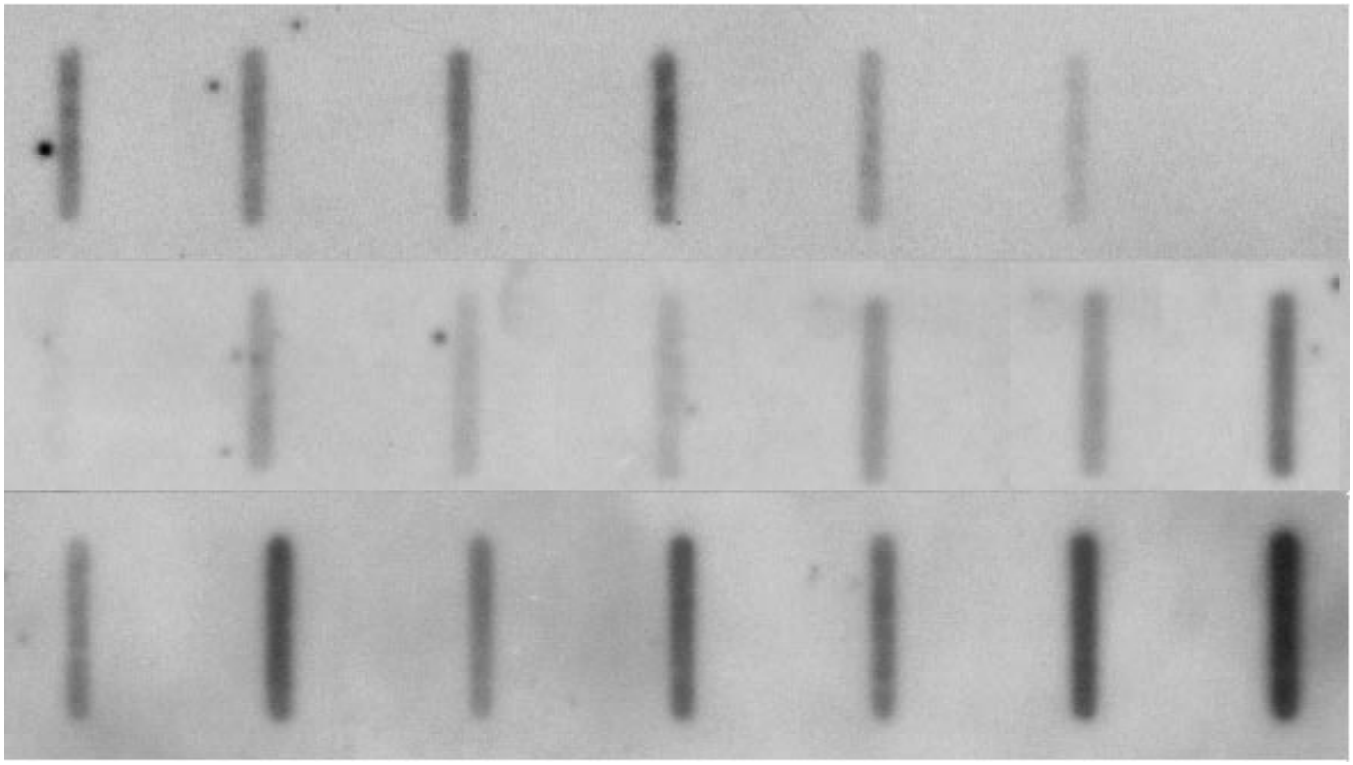
R. albus 7 outcompeted *F. succinogenes* S85 (Fig.1) or *R. flavefaciens* FD-1, despite its poorer affinity for cellobiose. The success of 7 against S85 is not due to production of an inhibitor, as both 7 and S85 grew to approximately equal populations when co-inoculated into batch cultures. Instead, it appears that 7 was able to adapt to more rapid growth at lower cellobiose concentrations. The takeover of a steady-state culture of S85 by 7 was accompanied by a reduction in the steady-state concentrations of soluble sugars from 0.63 mM glucose equivalents in the S85 monoculture to 0.49 mM glucose equivalents at steady state in the 7-dominated culture. Moreover, the selected population recovered from the chemostat at the end of the run had a μ_{max} similar to the original 7 culture (0.48 h^{-1}), but its $S_{0.5\mu max}$ value (0.23 mM) was five-fold lower than that of the original 7 culture (1.21 mM).

The mechanism underlying the success of 7 against FD-1 remains to be elucidated. Another *R. albus* strain

(8) is known to produce an inhibitor of FD-1, and the success of 7 against FD-1 may be due to production of an inhibitor as well. However, co-inoculation of 7 and FD-1 into vials containing an excess of cellobiose has produced inconsistent results (monocultures of 7, or binary cultures in which both species are present in substantial quantities).

Conclusions

R. flavefaciens FD-1 outcompetes *F. succinogenes* S85 for cellobiose by a classical pure and simple competition mechanism based on its higher affinity for this substrate. *R. albus* 7 outcompetes S85, apparently due to selection in the chemostat of a population of 7 with a higher affinity for cellobiose. *R. albus* 7 also outcompetes *R. flavefaciens* FD-1, but it is not yet clear if this interaction involves the production of an inhibitor.



culture (D = 0.07 h^{-1}) of F. succinogenes S85 challenged with R. albus 7 at 47 hours. Top panel: Slot blot of 5 ng of RNA from culture samples at indicated times, detected by chemiluminescence after hybridization with oligonucleotide probes. S85 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 cellobiose-limited chemostats.[a]

Inoculation order	D (h^{-1})[b]	pH	Cellobiose (mM glucose equiv.) Initial	1st st.st.[c]	2nd st.st.[d]	Dominant strain	Assay method and lower detection limit[e]
Co-inoculation:							
S85 + FD-1	0.067	6.24	17.85	2.28	—	FD-1	MFA (4.5)
7 + FD-1	0.024	6.36	24.51	0.24	—	7	FP (0.4), MFA (4.5), RNA (1.0)
7 + S85	0.070	6.40	23.12	0.76	—	7	FP (0.2), MFA (10.9), RNA (1.0)
Sequential:							
S85, then FD-1	0.088	6.73	6.09	0.39	0.13	FD-1	MFA (3.1), RNA (1.0)
FD-1, then S85	0.169	6.62	16.98	1.09	1.09	FD-1	MFA (3.1), RNA (1.0)
FD-1, then 7	0.067	6.49	14.26	0.96	1.36	7	FP (0.8), MFA (3.2), RNA (1.0)
7, then FD-1	0.167	6.71	12.89	0.09	0.09	7	FP (0.8), MFA (3.2), RNA (1.0)
S85, then 7	0.070	6.36	23.12	0.63	0.49	7	FP (0.2), MFA (10.9), RNA (1.0)
7, then S85	0.166	6.54	12.89	0.08	0.08	7	FP (0.4), MFA (10.9), RNA (1.0)

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

[b]Dilution rate in reciprocal hours.

[c]Steady-state before challenge with second strain

[d]Steady-state after challenge with second strain

[e]Assay method: MFA = signature membrane fatty acids; FP = characteristic fermentation products; 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.