

pH-dependent cellulose-attachment by *Fibrobacter succinogenes* monitored by competitive PCR

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ABSTRACT

In vitro fermentation studies were conducted to evaluate effects of initial medium pH on the extent of attachment on pure cellulose by one of major ruminal cellulolytic bacteria: *Fibrobacter succinogenes* via competitive PCR. After incubation for 10 min, the numbers of *F. succinogenes* attached to cellulose were 10⁹/g dry matter (DM) of cellulose at all pH levels. Thereafter, the numbers of *F. succinogenes* attached to cellulose did not change to an appreciable extent until 48 h at higher initial pH. However, at low initial medium pH (5.8), the numbers of *F. succinogenes* attached to cellulose gradually decreased and drastically dropped at 48 h incubation (10⁵/g DM). This may be the result of higher detachment rate or lower growth when *F. succinogenes* was exposed to initial medium pH less than 6.0. Higher initial pH resulted in higher DM disappearance. However, changes in the rate of *in vitro* cellulose disappearance were not synchronized with changes in the number of attached *F. succinogenes*.

KEY WORDS: *Fibrobacter succinogenes*, cell numbers, cellulose attachment, competitive PCR, initial pH

INTRODUCTION

Fibrobacter succinogenes is considered as a representative cellulolytic bacteria in the rumen (Forsberg et al., 1997). Quantitative confirmation of the rate and extent of fibre attachment *in vivo* is necessary in order to estimate the contribution of the representative cellulolytic species to ruminal fibre digestion. We recently developed competitive PCR assays that facilitate the rapid and accurate enumeration of *F. succinogenes*, without the need for culturing (not published).

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Application of these assays to the analysis of fibre-associated populations could provide quantitative information on these species *in vivo*. Moreover, such analysis may confirm which species play the largest role in plant fibre digestion in the rumen.

In the present study, we monitored the influence of initial *in vitro* medium pH on the attachment of *F. succinogenes* to cellulose using competitive PCR assays.

MATERIAL AND METHODS

Ruminal contents were obtained from a rumen cannulated Holstein steer which was fed twice daily with a mixture of 40% concentrate and 60% timothy hay. Ruminal content was homogenized under anaerobic condition, strained through four layers of cheese-cloth, and then mixed with a buffer solution (McDougall, 1948). Half a gram of Sigmacell[®] cellulose (Sigma-Aldrich Corporation, USA) was added to the medium and finally initial pH was adjusted to 5.8, 6.2 and 6.8 with a sodium carbonate solution. All the tubes in triplicates were incubated at 39 °C for 0, 10 min, 2, 4, 8, 12, 24 and 48 h.

To maximize DNA extraction efficiencies, physical disruption (Reilly et al., 1998) was used to extract DNA from freeze-dried cellulose samples, which were obtained by centrifugation after respective incubation time. The numbers of *F. succinogenes* attached to Sigmacell[®] cellulose (Sigma-Aldrich Corporation, USA) were enumerated by competitive PCR assay. Species specific primer sets for *F. succinogenes* were used to obtain PCR products. Proper internal control was constructed by using restriction enzyme (Ssp I and Sma I).

A detachable pressure transducer and digital readout voltmeter (Laurel Electronics, Inc., CA, USA) were used to measure the headspace gas pressure of fermenting cultures. *In vitro* DM digestibility was measured by centrifugation method. Data were analysed using the general linear model (GLM) procedure of the Statistical Analysis System Institute, Inc. (SAS) (1985).

RESULTS AND DISCUSSION

Almost maximum microbial attachment was achieved within 10 min of incubation regardless of initial medium pH as shown in Figure 1. There was a slow increase in the number of attached microbes at medium and high initial pH until 4 h incubation, and thereafter the numbers declined to a small extent. However, low initial medium pH (5.8) caused a sharp decline in the numbers of attached *F. succinogenes*.

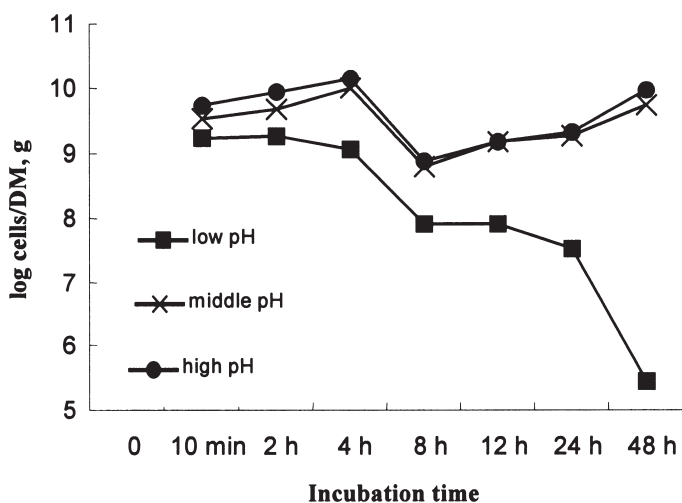


Figure 1. Influence of initial medium pH on the number of *F. succinogenes* attached to cellulose

Data on DM digestibility (Table 1) study indicated that little disappearance of cellulose occurred at pH values of less than 6.0. On the other hand DM disappearance increased with incubation time at medium or high medium pH. Disappearance of the incubated cellulose (DM digestibility) increased linearly up to 48 h, but the trend was not synchronized with changes in bacterial mass attached to the cellulose. Probably, this is due to the delayed increase in fibrolytic enzyme activities. Similar results have been presented by others (Koike et al., 2003).

The results of this study clearly indicate that attachment of *F. succinogenes* on cell surface is pH-dependent, and failure of attachment or enhanced detachment of major cellulolytic microbes may explain at least part of reduced fibre digestion under lower rumen pH, which is a commonly observed phenomenon with high concentrate and low forage diets.

Table 1. *In vitro* DM digestibility (%) as influenced by different initial pH

Incubation time, h	Low pH		Middle pH		High pH		SEM ¹
	ave	stdev	ave	stdev	ave	stdev	
0	4.47 ^a	0.42	5.00 ^a	0.75	4.93 ^a	0.56	0.340
2	4.78 ^a	0.72	4.44 ^a	1.03	3.63 ^a	1.10	0.557
4	2.96 ^a	0.59	4.01 ^a	0.62	3.33 ^a	0.50	0.331
8	3.68 ^a	0.37	6.14 ^b	1.27	7.30 ^b	0.50	0.470
12	4.53 ^a	0.71	7.68 ^b	0.49	8.38 ^b	0.11	0.289
24	7.63 ^a	0.25	16.80 ^b	0.72	18.64 ^c	0.76	0.359
48	9.70 ^a	0.24	28.11 ^b	0.07	39.22 ^c	0.48	0.180

¹SEM, standard error of means

^{a,b,c} means in the same row with different superscripts differ ($P < 0.01$)

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