Microbial Interactions in the Rumen and Their Potential Impact on the Survival of *Escherichia coli* O157

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Introduction

Cellulose and other plant fibre polymers are fermented in the rumen by a cellulolytic biofilm containing a complex mixture of microbial species. Polymer hydrolysis and fermentation are accompanied by the release of plant secondary "defence" chemicals such as tannins, saponins and coumarins. The release and microbial transformation of these compounds [4] modifies the gut environment and the processes therein. Although ruminants have traditionally been seen as "mobile fermentors" [11], there is increasing awareness of their contribution to global warming through methane production and their possible role as reservoirs for pathogens such as *Escherichia coli* O157.

Interactions in the cellulolytic biofilm

Nutritional interactions in the cellulolytic biofilm are based primarily upon interspecies competition for and transfer of nutrients and/or protons. Many saccharolytic bacteria and methanogens stimulate microbial growth and cellulolysis by removing products or supplying nutrients such as branched-chain VFA [reviewed in 27]. For example the propionate-producing bacterium *Selenomonas ruminantium* uses sugars and H_2 produced by the anaerobic cellulolytic fungus *Neocallimastix frontalis* [16] and the presence of *S. ruminantium* enhances cellulolysis (Fig 1a). In contrast, co-culture of cellulolytic species reveals the existence of competitive and inhibitory interactions, co-cultures often demonstrating reduced fibre degradation compared to that achieved by the more active organisms grown axenically. This may be based on competition for cellulose, perhaps through competition for attachment sites as the bacteria spread from their initial point of attachment, or competition for soluble nutrients. Thus, cultures of *Ruminococcus flavefaciens* strains 007 and 17 were found to be more active in degrading fibrous substrates such as barley straw and clover than were cultures of *Fibrobacter succinogenes* strains S85 and BL2 or mixed cultures containing both species [24], possibly reflecting interactions of this type (Fig 1b).

Some cellulolytic bacteria produce anticompetitor compounds [1,12,18,23]. The chitinolytic rumen bacterium *Clostridium tertium* inhibits fungal cellulolysis by an as yet unknown mechanism [14], presumably involving chitinase. The production of bacteriocins by ruminal and other gut bacteria is reviewed elsewhere [Teather *et al*, this symposium]. Work with non-cellulolytic bacteria including *Escherichia coli* suggests that the production of anticompetitors enables bacteria to colonize new habitats [26]. Although inhibitor-susceptible strains may be eliminated in laboratory co-culture [3], they may survive in high numbers in the gut [2]. This may reflect the wide variety of micro-

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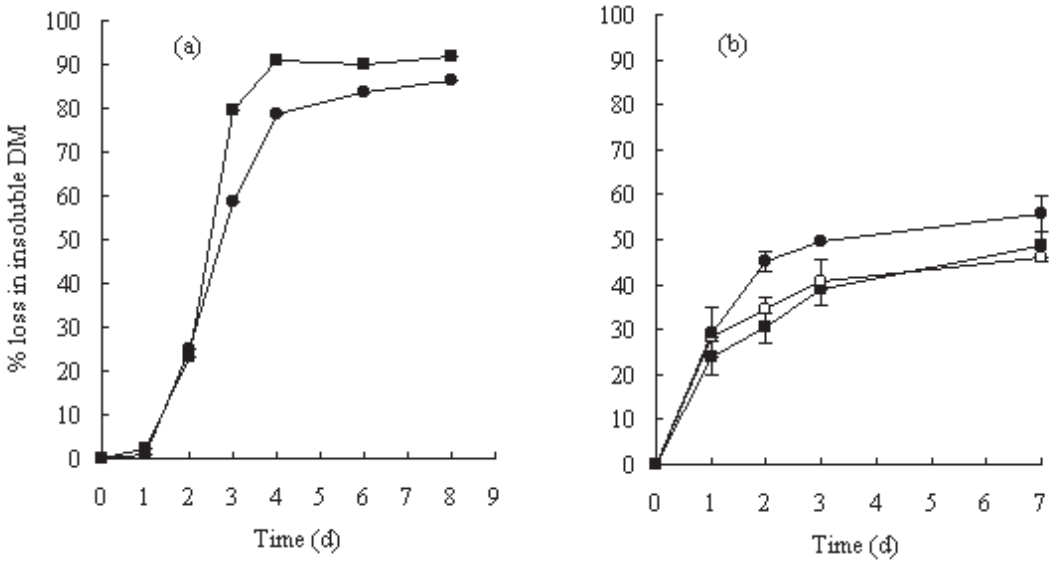


Fig 1. Contrasting effects of co-culture on fibre degradation. a) Cellulolysis by *Neocallimastix frontalis* (●) and *N. frontalis plus Selenomonas ruminantium* JW13 (■)(AJ Richardson, unpublished data cited in [16]). b) degradation of insoluble DM of clover by *Ruminococcus flavefaciens* (strains 17 and 007 mixed) *Fibrobacter succinogenes* (strains S85 and BL2)(■), and mixed cultures containing both species, (O), (Recalculated from [24]). Bars = SD.

habitats present *in vivo*. Also, inhibitor production *in vivo* is presumably regulated to ensure the survival of producers, rather than the complete elimination of competitors. In the rumen, *Ruminococcus* species and anaerobic fungi co-exist, despite the almost total inhibition of fungal growth on cellulose by ruminococci *in vitro* [1,23].

The genetic manipulation of fibre-degrading bacteria is being investigated with a view to enhancing rumen cellulolysis [Krause *et al.*, this symposium]. Establishing these bacteria in the rumen presents a particular challenge. A non-manipulated strain of *R. flavefaciens* pre-incubated with plant fibre to a high population density was rapidly replaced by autocthonous microorganisms on incubation in the rumen [25]. The competitive ability of manipulated bacteria might be enhanced by the ability to produce anticompetitor compounds.

Methane production

Methanogenesis in the ruminant gut is calculated to account for 15-25% of the global methane emission of around 7.5×10^{12} g yr^{-1} [13]. Methane is the major electron sink product of the fibre-degrading cellulolytic-methanogen consortium; by comparison the amylolytic-methanogen consortium produces relatively more propionate and less methane [13]. Classical two-component co-cultures are not an appropriate model for rumen methanogenesis. Thus, the addition of *S. ruminantium* to co-cultures of *N. frontalis* and *Methanobrevibacter smithii* reduced methanogenensis compared to that obtained in the 2-component cultures, presumably because the bacterium, which competes successfully for sugars released upon cellulose hydrolysis (above), produces less H_2 than the fungus (Table 1 and [16]). Methanogenesis is high because bicarbonate is by far the most important extracellular electron acceptor in the ruminant gut; alternative electron

Table 1. Fate of hexose C in mono, di- and tri-cultures grown on cellulose

Inoculum[a]	CO_2[c]	CH_4	Hfor[b]	Hac	Hpr	Hlac	Eth	Hsucc
N. frontalis strain RE 1	10.0	-	23.0	32.0	-	20.0	11.0	4.0
RE1 + *M. smithii* strain PS	10.1	16.6	-	60.8	-	3.1	5.8	3.5
RE1 + PS + *S.ruminatium* strain JW13	7.5	6.0	-	40	36.2	2.1	6.0	-

[a]Species N = Neocallimastix, M = Methanobrevibacter, S = Selenomonas.
[b]Hfor, Hac, Hpr, Eth, Hsucc = formate, acetate, propionate, lactate, ethanol and succinate respectively; - = not detected.
[c]Data shows the C in each product expressed as a percentage of the total C recovered . Methods were as described in [16], incubations were for 7d (AJ Richardson, V Roque, unpublished data).

acceptors including sulphate and nitrate are present in only small amounts in the feed. Unsaturated fatty acids arising from lipid hydrolysis are hydrogenated [11]. Rapid hydrogenation of the phenolic compounds ferulic, coumaric and caffeic acids also occurs, but unlike bicarbonate, these compounds are not renewed as a result of fermentation so their capacity to act as alternative sinks is limited [20].

Plant secondary compounds

The rumen microbes are seen as the "first line of defence" against toxic plant secondary compounds ingested by ruminants [4, Brooker *et al*., this symposium]. In addition to effects on animals, plant defence chemicals may have significant effects on the composition and activity of the rumen microbiota. Tropical legumes such as *Sesbania*, *Acacia* and *Calliandra* have shown marked effects on some microbial species [19,22]. Ciliate protozoa which ingest plant material seem to be particularly susceptible to secondary compounds [19]. Since rumen protozoa utilise cellulose and reduce the contribution of other organisms by predation [11], factors affecting the rumen protozoa potentially have a very significant effect on the rumen fermentation. Some plant metabolites might have species-selective effects similar to those of feedlot antibiotics, and could provide safer alternatives providing they do not result in the selection of strains with enhanced resistance to antibiotics, as has been suggested for some pine-oil based disinfectants [17].

Survival of *Escherichia coli* O157

Recently, concern has arisen about the emergence of human pathogens, such as the verocytotoxic *Escherichia coli* serotype O157, for which farm animals may act as a reservoir, shedding of the pathogen in faeces resulting in contamination of the human food chain. This serotype does not produce disease symptoms in adult animals, although neonatal cattle may be susceptible [5].

The survival of *E. coli* O157 was reduced in cattle dosed with commensal *E. coli* and *Proteus mirabilis* strains recovered from ruminant faeces [28]. *Pseudomonas aeruginosa*

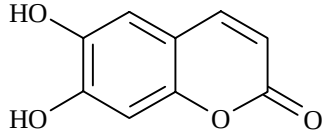
from the rumen of sheep inhibited growth of *E. coli* O157 *in vitro* [8]. The *P. aeruginosa* strains from different animals produced different bacteriocins (pyocins), which may have an ecological role in determining the dominant biotype present. Two pigments from *P. aeruginosa* (pyocyanin and fluorescein) were shown to inhibit both commensal and O157-serotype strains of *E. coli*. Pyocyanin had the greater effect but, as reported previously [10], was more inhibitory in the presence of oxygen than its absence. It seems that some of the bacteria interacting most directly with *E. coli* in the ruminant gut are other relatively minor members of the gut microbiota. Variations in the presence and numbers of these bacteria may contribute to differences between animals in the survival and shedding of *E. coli*. The co-excretion of such bacteria may also influence the survival of *E. coli* in shed faeces and in the soil. Pyocyanin would presumably be active if present in faeces exposed to air.

In relation to shedding, the dynamics of passage of *E. coli* through the gut will be influenced by whether or not this bacterium associates with the biofilms within the system. The liquid phase of the gut contents flows faster than the digesta [9], so planktonic bacteria would be expected to pass through the gut more rapidly than bound ones. There is also a possibility that attachment to the gut epithelium, whether in the rumen or in other gut compartments, could provide continuous re-inoculation of the gut contents. Investigations of the ecology of *E. coli* in the ruminant gut will be aided by the use of reporter genes, particularly constructs expressing the green fluorescent protein of the jellyfish *Aequorea* [9] combined with detection by fluorescence-activated cell sorting.

Escherichia coli O157 can be recovered from the rumen and the colon of infected animals, and it is clear that the carriage of this bacterium may be influenced by the composition of the diet [15]. The mechanisms involved in these dietary effects are not well understood. The growth of *Escherichia coli* is known to be reduced in the presence of high concentrations of VFA [21], and this is the most obvious way in which the rumen fermentation of the feed would affect numbers of *E. coli*. However, it has recently been shown that exposure to high concentrations of VFA, similar to those found in the rumen of cattle fed grain, may increase the ability of *E. coli* to survive exposure to the acidic secretions of the abomasum, resulting in increased numbers of this bacterium in the ruminant colon and faeces [6].

Separate work has also illustrated how the transformation of dietary plant secondary compounds by gut anaerobes may affect the growth and survival of *E. coli*. The hydroxycoumarin glycoside esculin is hydrolysed by bacteria, including some ß-glucosidase-producing *Prevotella*, *Butyrivibrio* and *Selenomonas* species. Compared to gut anaerobes, the rate of growth of commensal and serotype O157 strains of *E. coli* proved to be relatively susceptible to coumarin aglycones, especially esculetin, the product of esculin hydrolysis (Fig 2). The effect of the simultaneous presence of VFA arising from fermentation appears to be additive [7]. When *E. coli* strain 12900 (an O157 strain which

Fig 2. Esculetin



lacks the genes encoding verocytotoxins) was incubated with mixed viable rumen microorganisms in the presence of esculin, a selective 2,000 fold decrease occurred in the viable numbers of *E. coli* 12900 compared to the numbers of viable anaerobes [7]. Key questions about any such process for practical application will include whether the effect persists on prolonged exposure, and whether resistant strains also prove to be resistant to therapeutic antibiotics. At present, most purified plant secondary compounds are too expensive for use as bacterial inhibitors but their cost would decrease dramatically if important applications were found.

Conclusions

In future, investigations using PCR-amplified 16S rDNA genes, reverse-transcriptase PCR and other molecular techniques will help us gain a deeper understanding of the complex microbial interactions of the ruminant gut. Controlling these interactions offers opportunities not only for feeding the world's poor, but also for limiting the adverse ecological consequences of agricultural practice.

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