Institute, Bucksburn, Aberdeen AB21 9SB, UK; [2]Scottish Agricultural College, King St. Aberdeen, AB24 5UD, UK)

The nutritive value of many tropical and sub-tropical plants consumed by ruminants is influenced by the presence of hydrolysable or condensed tannins. These compounds affect the growth of many rumen microorganisms, but tannin-resistant *Streptococcus* spp. have been isolated from the gut or faeces of ruminants and other herbivores (see also Brooker *et al.* this symposium). For example, the bacterium *Streptococcus caprinus*, isolated from feral goats fed *Acacia*, grows in the presence of high concentrations of tannic acid, a hydrolysable tannin [1]. The aim of this study was to measure the effect of tannic acid on cellulose degradation by mixed rumen microorganisms and to demonstrate whether the addition of *S. caprinus* strain TPC 2.3 to these incubations reduced the effects of tannic acid on cellulose degradation and fermentation. In addition, a representative tannic-acid resistant bacterium was isolated from incubations that did not receive *S. caprinus*, and the characteristics of this organism were compared with those of *S. caprinus* and *S. bovis*.

When microcrystalline cellulose (Avicel) was incubated with mixed rumen microorganisms in batch culture in the basal medium of Hungate and Stack [2], around 40% of the cellulose was solubilised in 96h. The presence of 1.5mM tannic acid almost completely inhibited cellulolysis. When *S. caprinus* strain TPC 2.3 was added to these incubations together with the rumen microorganisms, this bacterium established in high numbers but did not reduce the inhibitory effect of tannic acid on cellulolysis. In those incubations which contained tannic acid, the accumulation of fermentation products was greater in the presence of *S. caprinus* than in its absence.

Mixed rumen microorganisms from a cow fed a mixed hay-concentrate diet were incubated with cellulose (Avicel) plus tannic acid, and the predominant tannic-acid resistant bacteria were then isolated using an anaerobic medium contained in petri-plates and overlaid with a solution of tannic acid (0.2%, w/v). Strain SM1 was chosen as being representative. Protein fingerprinting (SDS-PAGE) of this isolate and of *S. bovis* strain 26 and *S. caprinus* strain TPC 2.3 showed that the three bacteria were similar in this respect. Tests in API 32 identification kits showed that strain SM1 fermented a range of sugars but that in common with *S. caprinus* strain TPC 2.3 and in contrast to *S. bovis* strain 26, strain SM1 fermented mannitol. It appears that the fermentation characteristics and taxonomic relationships of the tannic-acid resistant streptococci from the rumen are worthy of more detailed investigation.

1. Brooker JD, O'Donovan LA, Skene I, Clark K, Blackall L, Muslera P (1994) *Lett Appl Microbiol* 18, 313-318
2. Hungate RE, Stack RJ (1982) *Appl Environ Microbiol* 44, 79-83

Characterization of 2,3 DHP-degrading activity in cell free extracts of the rumen bacterium *Synergistes jonesii*. MT Rincon[1], MJ Allison[2], MG Domínguez-Bello[1] (*[1]IVIC, Laboratorio de Fisiología Gastrointestinal, CBB, A postal 21827, Caracas 1020A, Venezuela; [2]National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010, USA)*

The rumen bacterium *Synergistes jonesii* degrades toxic dihydroxypyridine (DHP)

compounds derived from mimosine. This activity prevents intoxication of ruminants consuming *Leucaena leucocephala*. In laboratory cultures, degradation typically occurs between day 2 and 5 of incubation, after the late exponential phase of growth has been reached. In this work, two systems in which cell free extracts of *S. jonesii* degrade 2,3 DHP, under N_2 or H_2 gas phases, are characterised.

Cell free extracts were obtained from 12l cultures grown in medium 64-A. Culture pellets were washed in phosphate buffer (0.1M) containing dithiothreitol (2mM), resuspended in the same buffer containing PMSF (1mM), passed twice through a french press and incubated for 20min on the bench with DNAse (2 crystals) in a solution of $MgCl_2$ (4.8mM). After ultracentrifugation, supernatants were stored at -70 °C, until use. Extracts were diluted 100-fold in phosphate buffer (50mM), dithiothreitol (1mM), and 2,3 DHP (5mM) for enzyme assays. Degradation was assessed by a colorimetric method in which the aromatic ring of 2,3 DHP is complexed with ferric ions. The complex is not formed when aromaticity is lost. Hydrogenase activity in the extracts was determined using methyl viologen (0.5mM) as a substrate. VFA were determined by gc (carbowax HP20 column).

Extracts were unable to degrade 2,3 DHP in the suspension buffer under N_2 or H_2, unless electron donor compounds were added to the reaction mixture. Activity was observed if pyruvate (19mM) was added under the N_2 atmosphere, or if methyl viologen (0.5mM) was added under the H_2 atmosphere. Different extract preparations varied in their 2,3 DHP-degrading activity in these two systems. Addition of NADH or cytochrome c did not alter the degradation activity in either of the two systems, and substitution of pyruvate for NADH in the N_2 system did not allow degradation of 2,3 DHP. These results suggested that NADH is not a reducing intermediate, or cytochrome c an electron transporter. Cell free extracts were able to reduce methyl viologen. This hydrogenase activity may play an important role in the reduction of the 2,3 DHP ring, in the system H_2/methyl viologen. Previous evidence that propionate, acetate and ornithine may be formed from 2,3 DHP, led to testing the formation of VFA after complete degradation of 2,3 DHP in both the N_2/pyruvate and H_2/methyl viologen systems. Formation of VFA was not detected when degradation of 2,3 DHP ocurred. In the presence of N_2/pyruvate there was only one peak corresponding to acetate (which may be formed from pyruvate), and with H_2/methyl viologen no VFA were formed after the degradation of 2,3 DHP was completed. It is still unclear whether the ring of the 2,3 DHP is really opened in the system N_2/pyruvate and only modified by reduction in the system H_2/methyl viologen.

Properties of a new species of rumen bacteria that appears to be important in degradation of forage nitrotoxins. RC Anderson, MA Rasmussen, MJ Allison (*National Animal Disease Center, Agricultural Research Service, U.S. Dept. of Agriculture, Ames, IA 50010, USA)*

The nitro-toxins, 3-nitro-1-propanol and 3-nitro-1-propionate, are found in a diversity of plants (especially in the genus *Atragalus*). Losses due to these compounds in the productivity of grazing ani-