linkage between a phenolic acid and the carbohydrate of hydrolysable tannins. TAH has not previously been reported in bacterial systems, having only ever been described in fungi. The enzyme is considerably smaller than the fungal enzyme - 59 kDa compared with the 186 kDa fungal glycoprotein. *Sel. ruminantium* TAH is induced by the presence of tannins in the growth medium. We have established basic kinetic parameters for TAH using methyl gallate as substrate. TAH has been shown to be released from cells in the presence of Triton X-100, raising the possibility that it may be sequestered in extracellular vesicles. Purified enzyme has been obtained and 13 amino acids were sequenced at the N terminus and 10 amino acids at an internal peptide derived from Endo-Lys C hydrolysis and HPLC purification. From the amino acid sequence information, degenerate primers have been synthesised and used in a PCR reaction to generate a TAH-specific DNA probe. Currently a *Sel. ruminantium* K2 gene library is being screened for the TAH gene which will be sequenced and examined for regulatory motifs that control tannin responsiveness.

In both cases these organisms provide two advantages to the animal. Firstly, by virtue of their growth, they provide essential microbial protein for the animal. Secondly, these organisms help reduce the effective level of dietary tannin and thereby alleviate some of the anti-nutritional effects. There may also be other rumen microbial species that thrive in the presence of phenolic compounds, each forming part of a complex consortium to detoxify tannins. Mixed enrichment cultures have been used to isolate some of these additional species.

Tannin-resistant ruminal bacteria from East African ruminants. AA Odenyo, PO Osuji (*International Livestock Research Institute, P.O. Box 5689, Addis Ababa, Ethiopia)*

Five strains of rumen bacteria resistant to tannin (crude extracts, tannic acid) were isolated from enrichment cultures of the rumen microflora of sheep, goat and antelope established in medium containing high concentration of tannin extracts or tannic acid. One of the strains (ES11) from sheep was a facultatively anaerobic, Gram-positive, *Streptococcus* which grew in medium containing up to 40g l^{-1} tannin extracts or 30g l^{-1} tannic acid. Another strain (ES14.2) was an obligately anaerobic, Gram-variable rod containing rounded bodies, occurring in singles, pairs or short chains which grew in medium containing up to 50g l^{-1} tannin extracts or tannic acid. Three strains EAT 2.1 (antelope), ES 3.1 (sheep), and EG 19 (goat) were curved rods, obligately anaerobic, Gram-negative and highly motile and were characterized as *Selenomonas* species. *Selenomonas* strains grew in medium containing 50g l^{-1} tannin extracts and 50, 70 and 60g l^{-1} tannic acid respectively. The *Streptococcus* strain and one *Selenomonas* strain (EATT2.1) were able to hydrolyze gallic acid, while two *Selenomonas* strains (ES3.1 and EG19) hydrolysed tannic acid but not gallic acid. All isolates were able to grow in medium containing up to 8g l^{-1} condensed tannins. Growth was very slow when soluble carbohydrate was excluded from the medium.

Tannic acid-resistant *Streptococcus* sp. from the rumen. S Muhammed[1,2], T Acamovic[2], CS Stewart[1] ([1]*Rowett Research*

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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)