ruminal bacteria.

The effect of the pyrethroid insecticide supermethrin on the growth and cyanogenic activity of eight ruminal bacterial species *Streptococcus bovis* AO 24/85, *S. xylosus* 310, *Enterococcus faecium* 2, *Lactobacillus plantarum*, *Megasphaera elsdenii* 4MJ, *Selenomonas ruminantium* A17, *Fibrobacter succinogenes* 16J and *Bacteroides ruminicola* 3/3 in pure culture was examined in this study. Bacteria grown in two concentrations of supermethrin (0.66 and 6.6mg ml^{-1}) showed a similar growth rate and resistance to supermethrin. Production of $cyan^-$ from supermethrin occurred in all strains of ruminal bacteria examined, but the enzymatic activity varied considerably both with species and with the concentration of supermethrin.

In our experiments with the lactogenic strains *L. plantarum* S20 and S15, we found no effect of Delor 105 in concentrations 0.2, 2.0 and 20μg ml^{-1} on growth and lactic acid production. The mixed ruminal microorganisms cultured in RGC medium were resistant to 0.2 and 2μg ml^{1} Delor 105. The concentration of total volatile fatty acids were not significantly changed. Although the PCB concentrations detected in silage did not affect the silage preservative lactogenic bacteria and mixed ruminal bacteria, their cumulative toxic effects on the animal and, through the food chain, on humans, are still of considerable importance.

Survival in the face of tannins - a microbial perspective. JD Brooker, IK Skene, L O'Donovan, J McCarthy (*Animal Science Department, University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Australia)*

Tannins represent a complex group of anti-nutritional plant phenolic substances that inhibit effective utilisation of many potentially valuable forages by browsing and grazing animals, especially in the arid regions of Australia. These compounds form indigestible tannin-protein complexes, cause the loss of minerals by chelation, inhibit rumen microbial growth and may have direct toxic effects on the rumen epithelium. However, some wild ruminants have developed a unique resistance to tannins that enables them to utilise woody shrubs and other forages. Domestic ruminants do not enjoy this advantage. Experimentally, crude inoculations of total rumen bacteria from feral goats to domestic ruminants have shown that this capacity is transferable, but more detailed information is needed on individual bacterial species and the molecular mechanisms of tannin resistance.

From feral goats browsing Acacia, we have isolated and identified two rumen microbial species than can tolerate high levels of tannins and may derive energy for growth from metabolism of these compounds. One species, *Streptococcus caprinus*, hydrolyses protein tannin complexes and produces pyrogallol from the decarboxylation of gallate, the major component of hydrolysable tannins. This species also produces large quantities of extracellular polysaccharide (EPS) in the presence of tannins. Tannin induction of EPS biosynthesis in *S. caprinus*, and identification of key genes involved is currently under investigation.

The other ruminal species, *Selenomonas ruminantium* K2 expresses an esterase activity that has been identified as tannin acyl hydrolase (TAH) (EC.3.1.1.20). This hydrolyses the ester

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*