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-

mals in the Rocky Mountain regions of North America are particularly important. Such losses are, however, reduced when dietary adaptation leads to accelerated rates of metabolism of the compounds by ruminal microbes. A previously undetected group of nitro-toxin degrading bacteria from cattle rumen contents have now been isolated. Increased concentrations of these organisms were selected when diets containing the toxins were supplied, supporting the concept of their role in this adaptation [1]. These bacteria are obligately anaerobic, non-fermentative, non-motile, Gram-positive, non-sporing rods that obtain energy for growth via anaerobic respiration mechanisms. They require an electron acceptor, such as the nitro-toxins or other oxides of nitrogen, for growth. One strain, from the group of four isolates studied, reduced nitrate and nitrite, with ammonia as a product. When both nitrate and nitropropanol were available, biphasic growth occurred and nitrate was used for nitropropanol. A few other acceptors such as dimethyl sulfoxide or trimethyl amine-oxide also were used and the expected reduced products were recovered; however, various other potential acceptors that were tested, such as sulfate or fumarate, were not utilized. Electron donors that supported growth include hydrogen, formate and lactate, while none of a wide variety of carbohydrates or other potential electron donors were functional. Oxidized vs. reduced absorption spectra of cell-free extracts indicated the presence of a c-type cytochrome with absorption maxima at 411nm in the oxidized state and at 421, 523 and 554nm in the dithionite-reduced form. Analysis of the 16S rRNA gene sequence provided evidence that these are indeed novel organisms and a new genus and species is being proposed to accommodate them.

1. Anderson RC, Rasmussen MA, Allison, MJ (1996) *Appl Environ Microbiol* 62, 3885-3886

RUMEN MANIPULATION

Effect of the microbial additive Levucell® SC on microbial activity in the rumen during the stepwise adaptation of sheep to high concentrate diet.

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Changes occuring in the rumen after excessive intake of starch have been extensively studied, but little is known about modifications during stepwise adaptation of animals from low to high concentrate diets. Since yeast cultures have been shown to stabilize the rumen pH, they could be used to improve the adaptation of the microbial ecosystem during the period of change to diets rich in cereals. The objective of this study was to monitor the enzyme activities of microorganisms as sheep adapted gradually from all-forage to high-barley diets and to evaluate the effect of including *Saccharomyces cerevisiae* CNCM I-1077, Paris (SC), in the form of a commercial product Levucell® during stepwise adaptation.

The effect of the addition of SC (10^7 cfu ml^{-1} of rumen content) was studied with four ruminally fistulated wethers in a 2 x 2 Latin square design. The animals received twice daily an initial diet of 90% hay and 10% soyabean meal. At weekly intervals and for five weeks, increasing