

## ***In vitro* degradation of [<sup>14</sup>C]lignocellulose by polycentric and monocentric ruminal anaerobic fungi is inhibited differently by phenolic monomers**

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Lignocellulose-derived phenolic monomers inhibit fibrolytic ruminal bacteria (Varel and Jung, 1986, Appl Environ Microbiol, 52, 275-280). Also, they inhibit *in vitro* fibre degradation by mixed anaerobic fungi (AF) selected from ruminal fluid with antibiotics (Akin and Rigsby, 1985, Agron J, 77, 180-182). Since AF contribute significantly to feed intake and fibre digestion in ruminants (Gordon and Phillips, 1993, Lett Appl Microbiol, 17, 220-223), it is important to determine the effect of phenolics on fibre degradation by different species of AF.

Polycentric (PC) fungi were isolated from cattle ruminal contents (Phillips, 1989, Roles of Protozoa and Fungi in Ruminant Digestion, RA Leng *et al*, eds, 247-249), whereas monocentric (MC) fungi were isolated from sheep (Phillips and Gordon, 1989, Appl Environ Microbiol, 55, 1695-1702). [<sup>14</sup>C]Lignocellulose was prepared from oat seedlings (*Avena sativa*) incubated with [<sup>14</sup>C]glucose (Crawford and Crawford, 1988, Meth Enzymol, 161, 18-31). After washing the substrate to remove protein and cell solubles, the <sup>14</sup>C-label was located in structural  $\beta$ -glucan. Degradation rates of [<sup>14</sup>C] lignocellulose (Gordon, 1990, Microbial and Plant Opportunities to Improve Lignocellulose Utilisation by Ruminants, DE Akin *et al* eds, 301-309) were determined with phenolic monomers at a concentration of 1 g/l. The pH

of all media was adjusted to 6.6-6.8 before inoculation with fungi.

Degradation rates of [<sup>14</sup>C]lignocellulose (dpm x 10<sup>3</sup> solubilised per 24 h ; n = 3, mean  $\pm$  standard error) in the absence of phenolics were : 40  $\pm$  4.1 (LM1), 34  $\pm$  2.4 (SM1), 23  $\pm$  1.3 (NM1), 34  $\pm$  2.3 (CR2), 24  $\pm$  2.0 (CM3), 28  $\pm$  2.1 (CF2). Either very little inhibition or no effect was found for syringic acid, syringaldehyde, vanillic acid or vanillin, whereas the greatest inhibition was found with ferulic acid (FA), *p*-coumaric acid (pCA) and hydroxybenzaldehyde (HBA). The MC strains LM1 and SM1 were most sensitive to phenolics, whereas the PC strains CR2 and CM3 were least sensitive to these compounds. The MC strain NM1 and the PC strain CF2 both showed an intermediate level of inhibition. The three MC strains and the PC strain CF2 were equally sensitive to the three phenolics, whereas the two polycentric *Orpinomyces* spp. were least sensitive to FA, compared with pCA and HBA. Unpublished results with *Neocallimastix* sp. LM1 show that the phenolics are toxic to the fungi rather than acting against the fungal fibrolytic enzymes. Since tropical pasture grasses are higher in lignocellulose content, and hence phenolic monomers, than temperate grasses (Wilson, 1994, J Agric Sci, 122, 173-182), we suggest that the growth of *Orpinomyces* spp. should be encouraged in the rumen for improved production from poor tropical pastures.

Fungus	% inhibition by phenolic monomers (mean $\pm$ SE)		
	FA	pCA	HBA
MC <i>Neocallimastix</i> sp. LM1	70 $\pm$ 2.1	72 $\pm$ 2.7	70 $\pm$ 4.9
<i>Piromyces</i> sp. SM1	67 $\pm$ 2.6	71 $\pm$ 3.8	69 $\pm$ 4.7
<i>Caecomyces</i> sp. NM1	53 $\pm$ 1.8	51 $\pm$ 10.7	47 $\pm$ 6.2
PC <i>Orpinomyces joyonii</i> CR2	25 $\pm$ 7.0	34 $\pm$ 11.4	36 $\pm$ 9.1
<i>O. intercalaris</i> CM3	8 $\pm$ 7.5	13 $\pm$ 7.8	14 $\pm$ 10.3
<i>Anaeromyces</i> sp. CF2	58 $\pm$ 11.8	39 $\pm$ 6.2	45 $\pm$ 8.0