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Effects of phenolic acids on the proteolytic activity of the rumen bacteria *Butyrivibrio fibrisolvens*

D Debroas, G Blanchart

INRA-ENSAIA, Laboratoire de Zootechnie, 2, av de la Forêt-de-Haye, 54505 Vandœuvre-lès-Nancy, France

Proteins bound to the cell walls are hardly hydrolyzed by the ruminal microorganisms. Martin and Akin (1988) noted the inhibitory effect of phenolic acids on the polysaccharidases. However, it has never been demonstrated that they are involved in the degradation of plant-cell-wall proteins. This paper aims to determine whether the phenolic acids released during the degradation of plant cell walls do affect the proteolytic activity of *Butyrivibrio fibrisolvens*.

The proteolytic activities of the culture medium of *B fibrisolvens* were measured using azocasein as a standard substrate. The culture medium was recovered in the middle of the exponential phase and centrifuged at 6 000 *g* for 20 min at 4°C. The azocasein solution (final concentration 0.2% w/v) was dissolved in 100 mM phosphate buffer (pH 7) previously boiled under a CO_2 current. The phenolic acids (pCA: *p*-coumaric acid; CA: cinnamic acid; FA: ferulic acid; VA: vanillic acid; PA: protocatechuic acid) were added at final concentrations of 0.05, 0.1 and 0.2 (w/v) (Martin and Akin, 1988). The final ratios (mg of phenolic acid/mg of protein) were 0.25, 0.5, 1. The cell-free culture medium and the substrate were mixed (1:1) and incubated for 3 h at 39°C in anaerobic conditions (80% N_2, 10% H_2, 10% CO_2). The reaction was stopped and the proteins were precipitated with 10% trichloroacetic acid. The test tubes were then centrifuged at 30 000 g for 30 min at 4°C. One ml of 1 M NaOH was then added to 1 ml supernatant. The optical density is proportional to the weight of hydrolyzed azocasein and was measured at 450 nm.

The protease activity of *B fibrisolvens* was inhibited by pCA, CA and FA (table I). For pCA and CA, the inhibitory action increased with the phenolic acid concentration. The inhibitory effect of FA decreased when the ratio between phenolic acid and substrate reached 1. On the contrary, VA could only be considered as a proteolysis inhibitor when this ratio exceeded 0.5. PA stimulated the proteolysis at all the tested concentrations.

Apart from the activating action of PA on the proteolytic activity of *B fibrisolvens*, lignin monomers have an inhibitory effect on the proteolytic activity of this bacterium. According to Fahey and Jung (1983) phenolic monomers could also be able to bind to substances such as nitrogen compounds. The present work did not show whether these bonds affect protease activity or/and the resistance of protein to protease activity.

Fahey GC, Jung HG (1983) *J Anim Sci* 57, 220-225

Martin SA, Akin DE (1988) *Appl Environ Microbiol* 54, 3019-3022

Table I. Effects of phenolic acids on the proteolytic activity of *B fibrisolvens* R = phenolic acid (mg)/protein (mg). The proteolytic activities are expressed in % of control without phenolic acid ($n = 3$).

Phenolic acids	*R*		
	0.25	*0.5*	*1*
pCA	78.1	68.5	62.0
CA	82.7	52.3	48.5
FA	51.5	49.0	91.8
VA	107.7	95.8	87.5
PA	180.6	171.8	158.2