

## Potential for functional replacement of methanogenic bacteria by acetogenic bacteria in the rumen environment

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Ruminal fermentation is influenced by interspecies  $H_2$  transfer in which methanogens enhance energy production by fermentative microorganisms by converting  $H_2$  to  $CH_4$ . However, the energy contained in  $CH_4$  represents a 5 to 15% loss of apparent digestible energy loss to the animal and contributes to global methane emissions. The objective of our research is to determine if alternative  $H_2$ -utilizing bacteria, acetogens are capable to replacing the interspecies  $H_2$  transfer function of methanogens when methanogens are inhibited. We have isolated several acetogenic isolates, A10 and  $^3H$ , with low hydrogen thresholds (Boccazzi et al, 1993, Proc Conf Rumen Funct, 22-28). The purpose of this research was to determine if they could utilize  $H_2$  in a mixed culture system.

Serum bottles (120 ml) containing 0.35 g ground alfalfa hay (1 mm) were inoculated with 10 ml of a solution consisting of 60% anaerobic dilution solution:40% fresh rumen contents from a cow consuming a 60% concentrate:40% corn silage diet. Bromoethane sulfonic acid (BES, 5 mM) was added to all serum bottles except the controls. Treatments were : control, control + BES,  $^3H$  + BES and A10 + BES,

where the acetogenic cultures were added to give a final concentration of  $4 \times 10^8$  acetogen cells/ml under a  $CO_2$  atmosphere. Triplicate serum bottles for each treatment and time period were incubated at  $37^\circ C$  and shaken at 200 rpm. Hydrogen and  $CH_4$  concentration in the headspace was measured by gas chromatography using thermal conductivity.

No  $CH_4$  accumulated in serum bottles where BES had been added which indicates that 5 mM BES completely inhibited methanogenesis for up to 74 h. Hydrogen concentrations at 12 h were lower for cultures containing added acetogens. High levels of  $H_2$  in the control of 12 h may have been due to low numbers of methanogens in the inoculum. Concentrations of  $H_2$  were lower for control bottles and bottles containing acetogens at 24 and 74 h. Cultures containing acetogenic isolate  $^3H$  consistently had lower  $H_2$  concentrations than cultures containing acetogenic isolate A10. These data show that acetogens can functionally replace the role of methanogens in interspecies  $H_2$  transfer and that different levels of  $H_2$  control may be obtained by using different acetogenic cultures.

Treatment	$H_2$ ( $\mu M$ )				$CH_4$ ( $\mu M$ )
	0h	12h	24h	74h	74h <sup>1</sup>
Control	10	166	16	0.5	258
Control + BES	8	171	112	23	0.8
$^3H$ + BES	8	48	12	2	1
A10 + BES	9	108	44	21	3

<sup>1</sup> Initial  $CH_4$  concentrations were less than 2  $\mu M$ .