TOWARDS GENETIC MODIFICATION OF RUMEN BACTERIA FOR IMPROVED PASTURE FIBRE DIGESTION

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Improved microbial fibre digestion holds the potential to increase production in the Australian beef and sheep industries. The introduction of genes for improved fibre-degradation to rumen bacteria has been impeded by the lack of suitable genetic transformation systems. Genetic transformation of *Butyrivibrio fibrisolvens* is prerequisite for the expression of recombinant fibre-degrading genes within this group of rumen microorganisms. *Escherichia coli*/ *B. fibrisolvens* shuttle vectors have been described for *B. fibrisolvens* (1, 2, 3), however these vectors are limited in host range or utility. We have investigated the potential of the broad host range plasmid pUB110 to enable the expression of recombinant fibre-degrading genes in *B. fibrisolvens* strains.

MATERIALS AND METHODS

B. fibrisolvens strains were grown anaerobically in M2 medium (4) containing 0.5% w/v cellobiose. Plasmid DNA was isolated by alkaline lysis (5) from *Bacillus subtilis* or transformed *B. fibrisolvens* strains and then used to electroporate *B. fibrisolvens* (2).

RESULTS AND DISCUSSION

B. fibrisolvens strains isolated from the rumen of sheep (AR9, AR11, AR51), arctic reindeer (E14), cattle (H17c), white-tailed deer (OB156), water buffalo (LP1028), and goat (LP1309) were successfully transformed with the plasmid vector pUB110 and/or recombinant derivatives carrying hemicellulase (pUB*xynA*) or cellulase (pUB*celD*) cDNAs isolated from the anaerobic rumen fungus *Neocallimastix patriciarum* (Table 1).

Table 1. *B. fibrisolvens* strains transformed with pUB110 and recombinant derivatives.

	Plasmid Constructs		
Strain(s)	pUB110	pUB*xynA*	pUB*celD*
AR9, E14	+		
AR11	+	+	
AR51, H17c, LP1028, LP1309, OB156	+	+	+

The successful transformation of *B. fibrisolvens* strains with recombinant fibre-degrading genes will allow testing of the hypothesis that modified rumen bacteria may contribute to the improvement of fibre-digestion in livestock.

REFERENCES

1. Ware, C.E. *et al*. 1992. Current Microbiol. 24, 193-197.

2. Whitehead, T.R. 1992. Lett. Appl. Microbiol. 15, 186-189.

3. Beard, C.E. *et al.* 1995. Current Microbiol. 30, 105 - 109.

4. Hobson, P.N. 1969. In: Methods in Microbiology. (Eds J.R. Norris and D. W. Ribbons) (Academic Press: London). pp. 133-149.

5. Bron, S. 1990. In: Molecular Biological Methods for *Bacillus*. (Eds C.R. Harwood and S.M. Cutting) (Wiley and Sons: Chichester). pp. 140-141.