

A novel host-vector system for *Butyrivibrio fibrisolvens* and its possible use for developing more fibrolytic rumen bacteria

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It has been considered as a main target in gene modification of rumen bacteria to enhance their cellulolytic and hemicellulolytic activities. However, no significant progress has been made because of the lack of effective gene transfer systems for rumen bacteria. We have embarked on a project to develop a host-vector system for *Butyrivibrio fibrisolvens*, a predominant xylanolytic bacterium in the rumen, and to modify the species into a more fibrolytic bacterium by heterologous gene expression.

A cryptic plasmid pRJF2 was isolated from a deer rumen bacterium *B. fibrisolvens* OB157 and sequenced. Its size was 2971 bp with a region appearing to control its replication (1.8 kb). Shuttle vectors, pYK2 (4.4 kb) and pYK3 (6.1 kb), for transferring DNA between *E. coli* and *B. fibrisolvens* were constructed by ligating the replication region of pRJF2 into pUC18 and pBR322, respectively. A fragment containing *erm* from pAM β 1 was inserted into pYK2 as a selectable marker to construct another shuttle vector pYK4 (7.9 kb). These vectors harvested from *E. coli* were used to transform *B. fibrisolvens* OB157 and another strain OB156 by electroporation. *erm* was expressed well in *B. fibrisolvens* OB156 but not in OB157, while *amp* and *tet* from pUC18 and/or pBR322 were not expressed in both the hosts. This shows

that pYK4 and *B. fibrisolvens* OB156 are available for transforming *B. fibrisolvens*.

A *Eubacterium ruminantium* xylanase gene previously cloned and sequenced was used as target gene for amplification and expression in *B. fibrisolvens*. The gene was truncated in its C-terminus, fused with a stop codon linker and ligated into pYK4. The resultant chimeric plasmid pYK4X was electroporated into *B. fibrisolvens* OB156. Transformants were screened for *erm* expression, elevated xylanolysis, zymogram and southern analysis. No significant change in growth rate on xylan was observed after the recombination. However, an increased accumulation of reducing sugars was noted in the medium of the recombinant *B. fibrisolvens*. Agarose gel electrophoresis showed the recombinant to have a high copy number of the target gene, while the xylanolytic activity was elevated 2-3 times. Xylanolysis by both the recombinant and parent strains was repressed by glucose, cellobiose or xylose, though xylanolysis elevation (2-3 fold) did not change. An alternative promoter for expression of this foreign xylanase gene, proteolysis of the translated product and for regulating xylanase production in originally xylanolytic *B. fibrisolvens* should be explored to promote higher and more stable gene expression.