Plasmid pBH_f was constructed for detoxification of plant-derived fluoroacetate in the rumen [1]. Unfortunately, the dehalogenase activity expressed in the *Butyrivibrio fibrisolvens* strain OB156 was not high enough to protect living ruminants against fluoroacetate intoxication. The aim of this work was to find out which new strains could be transformed with plasmid pBH_f and which could be used to achieve higher levels of dehalogenase expression. Strains of *Butyrivibrio fibrisolvens* were isolated from sheep, cow and red deer digesta in NSW and Queensland, Australia and Canada. From 95 electroporated strains, 14 strains were transformed and showed resistance to erythromycin. Only 6 transformed strains were expressing dehalogenase. The level of the enzyme expression varied from 23% to 149% of the dehalogenase specific activity produced by *Butyrivibrio fibrisolvens* OB156. The stability of the plasmid in some new transformants was tested and in strain JK 10/1 the plasmid loss was about 15% per 100 generations against no loss in strain OB156.

From a taxonomic point of view, the species *Butyrivibrio fibrisolvens* covers a wide range of different subspecies [2]. Transformed isolates of *Butyrivibrio fibrisolvens* showed differences in their morphology (size, curved and straight rods) as well as in different sugar utilization (fructose, maltose, mannitol, ribose, sorbitol and trehalose). Fermentation products were same in most cases (formate, butyrate, lactate and ethanol). Transformed strains fit to description of *Pseudobutyrivibrio ruminis* [3] but show differences in DNA sequences (RJ Forster, personal communication).

The transformed strains will be tested under in vivo conditions.

1. Beard CE, Hefford MA, Forster RJ, Sontake S, Teather RM, Gregg, K (1995) *Curr Microbiol* 30, 105-109
2 Hudman JF, Gregg K (1989) *Curr Microbiol*, 19, 313 - 318
3. Van Gylswyk NO, Hippe H, Rainey FA (1996) *Int J Syst Bacteriol* 46, 559-563

A novel mobile chromosomal element conferring Tc^R in rumen *Butyrivibrio* species. TM Barbosa[1], KP Scott[1], K Forbes[2] HJ Flint[1] ([1]*Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, Scotland. AB21 9SB;* [2]*Department of Medical Microbiology, University of Aberdeen, Foresterhill. Aberdeen, AB25 2ZD, UK)*

The rumen contains an unusually dense and diverse microflora and is a potentially significant site for horizontal gene transfer. Extensive gene transfer between different strains, species and genera could be an important factor in the ecology and evolution of rumen microorganisms, increasing their capacity for rapid adaptive change, and may also be significant in the dissemination of antibiotic resistance genes and of transgenes in the environment. At present little is known about the capacity of rumen microorganisms for natural genetic exchange. Naturally occurring antibiotic resistant strains have, however, been isolated for many of the predominant obligately anaerobic bacterial species and can provide important information on gene transfer mechanisms.

Two out of three tetracycline resistant strains of *Butyrivibrio fibrisolvens* isolated from the rumen were found to be able to donate tetracycline resistance to a

rifampicin resistant mutant of the *B. fibrisolvens* type strain 2221 at remarkably high frequencies (up to 10^{-1} per recipient) in anaerobic filter matings. The recipient strain shows an auto-aggregation phenotype but it is not yet established whether this contributes to the high transfer rates observed.

Tc^R *B. fibrisolvens* were found to carry chromosomal sequences giving detectable hybridisation to *tet*(M) or *tet*(O) probes. Chromosomal DNA from Tc^R transconjugants did not however hybridise strongly to *tet*(M) or *tet*(O) probes although a new, weakly hybridising band was detected, suggesting that a second tetracycline resistance determinant was being transferred. Comparison of total genomic DNA from transconjugants and the recipient by pulsed-field gel electrophoresis was consistent with the acquisition of a mobile chromosomal element [1]. The mobile element is about 50kb in size and has a preferred insertion site in the *Butyrivibrio* genome. The element did not hybridise with probes carrying the *int/xis* gene of Tn*916*, or with the integrase gene of Tn*5252*, and may represent a novel type of transferable element in Gram-positive bacteria. This is the first example of a chromosomal element from an obligately anaerobic rumen species.

1. Scott KP, Barbosa TM, Forbes KJ, Flint HJ (1997) *Appl Environ Microbiol* 63, 3405-3411

Restriction-modification systems in ruminal bacteria: occurrence and some evolutionary implications. P Pristas, P Javorsky (*Institute of Animal Physiology, Slovak Academy of Sciences, Soltesovej 4-6, 040 01 Kosice, Slovakia)*

Type II restriction modification systems involve a DNA methyltransferase and an endonuclease of the same recognition sequence specificity. It is generally accepted that these systems act primarily to protect bacteria from foreign DNA, particularly from infection by bacteriophages. The study of the biology of restriction-modification systems has revealed some general features and it has been shown that the composition of the bacterial chromosome and the restriction-modification systems present within cells are evolutionarily linked [1]. Restriction endonucleases have been found in bacteria from all taxonomic and ecological groups. Ruminal bacteria have been shown to be a promising source of these enzymes. Up to now ten restriction endonucleases have been isolated from bacteria of this ecological group. Our studies on variability of endonucleolytic activity in *S. ruminantium* have demonstrated a high frequency and diversity of restriction endonucleases in this species, and at least ten different specificities have been characterized [2]. In addition the observed frequency of restriction endonucleases, which were present in more than one-third of strains tested, is higher than observed in bacteria from other ecological groups. A high frequency of restriction endonucleases in *S. ruminantium* can also be inferred from the analysis of DNA. Using the method of Karlin *et al.* [1], it was shown that average counts of perfect 4- and 6-base pairs palindromes observed within *S. ruminantium* DNA are lower than in other bacteria, and even lower than those observed among phage DNAs. The observed low frequency of short palindromes is therefore in good agreement with the high frequency of restriction endonucleases observed in this genus. Similarly other ruminal bacteria