was positive (r=0.65 P<0.01). Values declined from 14.4 ± 2.79 (0h) to 6.8 ± 1.98μg g^{-1} rumen fluid (16h). The abundance of Eucarya increased when the cows were returned to the normal diet, reaching 40.3 ± 5.5μg RNA per g rumen fluid at 72h. Archaea decreased following the induction of acidosis, from 1.4 ± 0.4 to 0.4 ± 0.07μg g^{-1} rumen fluid at 0 and 72h respectively. The population encompassed by the families *Methanoplanaceae, Methanomicrobiaceae* and *Methanocorpusculaceae* declined rapidly during the first 16h. (0.54 ± 0.07 to 0.04 ± 0.01μg g^{-1}) and did not recover. In contrast the *Methanobacteriaceae* increased after the perturbation (24.3% of the total Archaea at 0h and 79.8% at 72h). These values were positively correlated with Eucaryal biomass (r = 0.7 P<0.01). *Desulfovibrio* sp. SSU rRNA accounted for an average 1.2% ± 0.59 of the total SSU rRNA abundance. There was a strong positive correlation between the total amounts of *Desulfovibrio* and *Methanobacteriaceae* SSU rRNA (r=0.51 P<0.01) throughout the observation period.

Intestinal ciliates and their endosymbionts from the cockroach hindgut: evolutionary aspects. AHAM Van Hoek, TA Van Alen, VSI Sprakel, JHP Hackstein, GD Vogels (*Deptartment of Microbiology and Evolutionary Biology, University of Nijmegen, NL-6525 ED Nijmegen, The Netherlands)*

Anaerobic ciliates occur in the hindgut of many cockroach species, but only in those cockroaches that also are host to intestinal methanogens. Such ciliates contain, without exception, endosymbiotic methanogenic bacteria [1]. Although all of these anaerobic ciliates seem to be related to *Nyctotherus* sp. behavioural studies (e.g. galvanotaxis) and the morphology of the methanogenic endosymbionts suggest the presence of different protists in the various cockroach species. The rDNA genes of individual ciliates and their methanogenic endosymbionts were analysed in order to study the symbioses. DNA sequencing and restriction analysis of PCR-amplified rDNA genes showed that the ciliates isolated from the various cockroach hosts did differ significantly. The endosymbiotic methanogenic bacteria of these ciliates, also, proved to be different. Thus, in contrast to the situation in aphids with their eubacterial endosymbionts [2], the phylogeny of the anaerobic ciliates and their cockroach hosts [3] does not match. Since the methanogenic endosymbionts also differ substantially, it seems reasonable to conclude that the symbioses between methanogenic bacteria, ciliates, and cockroaches evolved more than once.

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MICROBIAL GENETICS

Transformation of *Butyrivibrio fibrisolvens* strains with pBH$_f$ plasmid. J Kopecný.[1], K Fliegerová[1], K Gregg[2] ([1]*Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Prague 10, Uhoínìves, 104 00, Czech Republic;* [2]*Institute of Biotechnology, University of New England, Armidale, NSW 2351, Australia)*

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
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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