Fluoroacetate Degradation by Naturally Occurring Rumen Bacteria

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Fluoroacetate is a plant toxin which poisons thousands of livestock every year in Australia and overseas. Rumen bacteria genetically engineered to express an enzyme which degrades fluoroacetate have been shown to provide protection from the toxic effects of fluoroacetate (Gregg *et al.* 1994*).* Prior to this investigation, only one naturally occurring microorganism has been found to degrade fluoroacetate in an anaerobic environment (Visscher *et al* 1994). Here we present evidence for naturally occurring bacteria in the rumen of cattle which are able to degrade fluoroacetate.

Anoxic rumen digesta from cattle in Queensland were enriched in the presence of 20 mM sodium fluoroacetate. Production of fluoride ions was used to monitor the degradation of fluoroacetate. Sterile, autoclaved inoculum and no fluoroacetate controls were included. Isolation of fluoroacetate degrading bacteria was facilitated using a colorimetric plate assay, which localised regions of fluoride release from colonies on solid media. Culture growth was monitored by optical density at 600 nm. Short chain fatty acid analysis was performed using gas chromatography with flame ionisation detection.

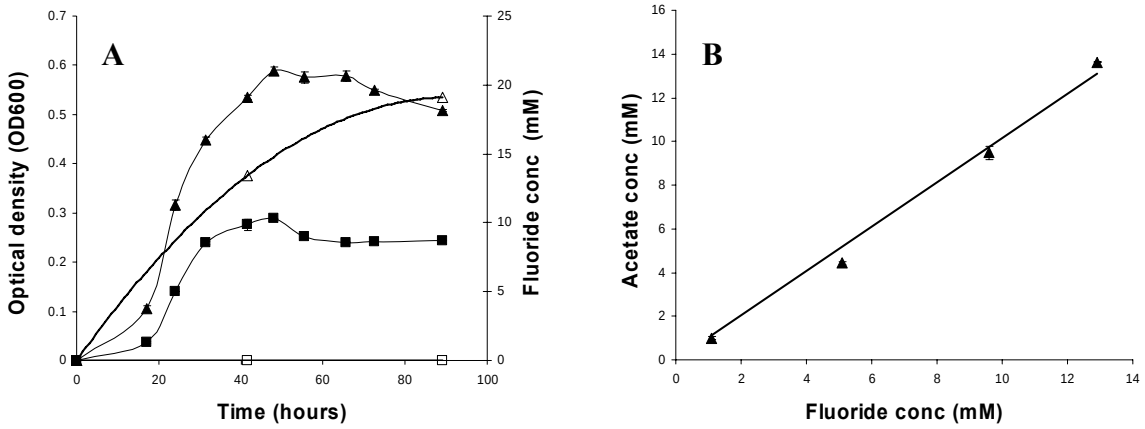

Figure 1A. *In vitro* degradation of fluoroacetate by a rumen bacterial isolate. Culture growth with 20 mM fluoroacetate (solid triangle) and fluoride concentration (open triangle); culture growth without fluoroacetate (solid square) and fluoride concentration (open square).
Figure 1B. Net production of acetate and release of fluoride by a rumen bacterial isolate

The presence of 20 mM fluoroacetate increased the growth of the bacterial isolate and was accompanied by defluorination of fluoroacetate (Figure 1A). The bacteria only grew and degraded fluoroacetate when supplied with amino acids in the form of protein digests. Analysis of short chain fatty acids showed that the major fermentation products were acetate, propanoate and isovalerate. The addition of fluoroacetate lead to an increase in the net production of acetate which correlated strongly with the stoichiometric release of fluoride i.e. degradation of fluoroacetate (Figure 1B, r^2=0.99). This data suggests that fluoroacetate is degraded to acetate and fluoride. Taken together with other data from our lab, this is the first report where microbial reductive defluorination is able to be repeatedly demonstrated. Further studies on the isolate have revealed that growth and defluorination can be enhanced by several compounds (data not show), pointing to the potential to increase fluoroacetate degradation *in vivo* through dietary intervention. Our interests in the future will focus on strategies to promote naturally occurring bacteria populations in the rumen in an attempt to reduce the fatal incidence of fluoroacetate poisonings.

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Gregg K. et al. (1994). *Nature Biotechnology* 12: 1361-5
Visscher, P.T., Culbertson, C.W. and Oremland, R.S. (1994). *Nature* 369: 729-31

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