Peptide Utilisation of the Novel Fluoroacetate-Degrading Ruminal Bacterium

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Fluoroacetate, commercially known as Compound 1080, is widely used to control feral pests in Australia and New Zealand. It is also been isolated in at least 40 species of plants around the world. This results in high incidences of livestock poisoning as a consequence of the animals feeding off these plants. Recently, a fluoroacetate-degrading bacterium was isolated from the rumen of Australian cattle. It is proposed that this bacterium, *Synergistes sp.* strain MFA1, can potentially protect cattle against fluoroacetate poisoning.

Initial findings have shown that this MFA1 strain does not use glucose or other sugars as an energy source, but has demonstrated significant growth and fluoroacetate metabolism in the presence of amino acids (Davis *et al* 2008). Other strains from the *Synergistetes* phylum have also been demonstrated to utilise amino acids in peptide form (McSweeney *et al* 1993). The aim of this study is to investigate the peptide metabolism pathways of the MFA1 strain via growth studies with genomic and analytical chemistry approaches.

The accompanying preliminary annotations from Integrated Microbial Genome (IMG) system of the sequenced draft genome have provided evidence of some prominent peptidase pathways in the strain. This bacterium shares some similar gene annotations for peptide metabolism with other bacteria from the IMG database, in particular, putative proline dipeptidase (Xaa-Pro), which has six predicted genes in the genome.

A growth assay was performed using media containing rumen fluid (10% vol/vol) and other minerals, supplemented with four different sources of amino acids derived from proteolytic digests (0.4%, wt/vol): casamino acids, phytone peptone, tryptone and yeast extract. Casamino acids provide the bacterium with free amino acids, while there are higher abundance of peptides from phytone peptone, tryptone and yeast extract. 20mM of fluoroacetate were also supplied with each treatment. Culture growth was monitored using spectrophotometer at 600 nm and production of fluoride ions using fluoride ion selective electrode was used to measure fluoroacetate metabolism.

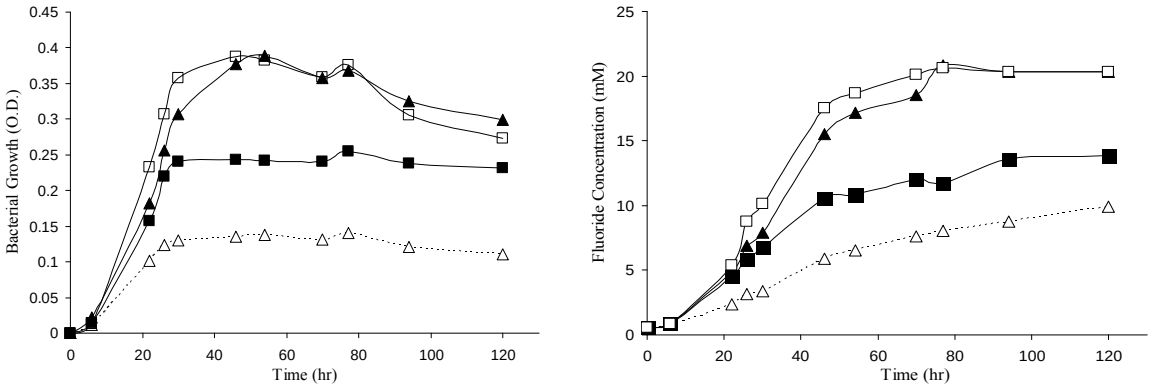

Figure 1: The effect of different sources of amino acids (yeast extract - □; tryptone - ▲; phytone peptone - ■; casamino acids - Δ) on growth and fluoroacetate metabolism of *Synergistes sp.* strain MFA1.

Maximal growth and complete fluoroacetate degradation occurred when amino acids were supplied in peptide form rather than as free amino acids (Figure 1). Peptides in yeast extract and tryptone were more stimulatory than phytone peptone. These results confirm that *Synergistes sp.* strain MFA1 primarily ferments amino acids for growth, but, efficiency of growth and metabolism of fluoroacetate is enhanced when amino acids in peptide forms are supplied.

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