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Identification of rumen methanogens, characterization of substrate requirements and measurement of hydrogen thresholds

A thesis presented in partial fulfilment of the requirements for the degree of
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Abstract

In New Zealand, exported farmed commodities derived from ruminants make up about one-third of the nation's economy. However, farming ruminants creates a significant environmental impact by emitting methane which is a by-product of the microbial fermentation occurring in the rumen. Accumulated methane in the atmosphere is considered to be an important contributing factor to global warming and climate change. Methanogenic archaea, collectively called methanogens, inhabiting the rumen are responsible for the production of ruminal methane. These organisms are capable of anaerobically reducing CO₂ to CH₄, using H₂, formate, methanol, a range of methyl-compounds, or acetate as electron-donors. Currently, all known methanogens that have been isolated from a diverse range of habitats are classified into 28 genera and 113 species based on the study of pure cultures and analysis of small subunit rRNA gene sequence data. Less than 10% of these species were isolated from the rumen and these reflect only a small portion of the true rumen methanogen diversity that has been determined by cultivation-independent methods. This project has been derived from the necessity to characterise genome sequences of a greater diversity of rumen methanogens than is currently covered in public culture collections. 14 methanogen strains were isolated as pure cultures and identified based on 16S rRNA and *mcrA* gene sequences in order to create a comprehensive phylogenetic tree comparing the genetic distances between the newly identified strains and the few named species. Strains 229/11, AbM4, M1, SM9, G16, D5, BRM9, YCM1, ISO3-F5, and A4 were then selected to be characterised for their substrate requirements for growth, by systematically omitting single or multiple components from the growth medium. Finally, the threshold levels of hydrogen, below which the methanogens fail to use it as a substrate, were measured for these strains by gas chromatography. Overall, the H₂ thresholds of rumen methanogens fell within the range between 0.5 and 5.8 Pa. *Methanobrevibacter*, the most predominant group of methanogens

occurring in the rumen, had relatively higher H₂ thresholds compared to the genus *Methanosphaera*, a group of methanogens frequently isolated from New Zealand ruminants, and the genus *Methanobacterium*.

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