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Author:	E. Hoedt, P. Ó Cuív, L. Wheeler, P. Lacaze, and M. Morrison
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A novel isolate of *Methanosphaera* sp. isolated from cattle possesses a large genome compared to methanogens isolated from other gut environments

E. Hoedt[1, 2], P. Ó Cuív[2], L. Wheeler[2], P. Lacaze[3], and M. Morrison[2]
[1]School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Australia, 4072
[2]Diamantina Institute, University of Queensland, Brisbane, Australia, 4102
[3]Millenium Science, Mulgrave, Victoria, 3170
Corresponding author: m.morrison1@uq.edu.au

As part of the continued efforts to reduce livestock methane emissions, much effort has been focused on autotrophic methanogens that use CO_2 and H_2 for growth (e.g. *Methanobrevibacter* spp., *Methanobacterium* spp., *Methanomicrobium* sp., and *Methanoculleus* sp.) (Attwood *et al.* 2011, Janssen *et al.* 2008). However, relatively little attention has been paid to the heterotrophic members of the community that use a greater variety of carbon compounds to support methane formation. A core focus of my PhD project is to isolate and characterise new isolates of these heterotrophic methanogens, using a combination of culture-based and genomic methods with a view to understand their role and control of methane formation. I have isolated a bovine *Methanosphaera* sp. from the rumen digesta of a Brahman steer grazing native forage in northern Australia (hereafter referred to as BMS), while the macropodid *Methanosphaera* sp. isolate I isolated (hereafter referred to as WGK6) was obtained from a foregut digesta sample collected from a Western grey kangaroo culled in south Western Australia. The human strain (*Methanosphaera stadtmanae*, hereafter referred to as DSMZ) was isolated in North America and it is the type strain for *Methanosphaera* spp.. Substrate utilization studies have revealed that BMS and the human type strain are only able to produce methane when cultured with methanol plus hydrogen. However strain WGK6 is metabolically more versatile, because it is capable of using either hydrogen or ethanol to reduce methanol to methane. These findings suggest that the use of ethanol by WGK6 is not a universal characteristic of *Methanosphaera* spp. and is a specific adaptation to life in the macropodid foregut. I have since produced a draft genome sequence for WGK6 and by comparison with the genome for the human isolate confirmed there is a genetic basis for this difference because WGK6 encodes genes for using ethanol but the DSMZ strain does not. To further determine the similarities and differences at a genome level between these three species, the BMS genome has recently been sequenced at the Diamantina Institute using the PacBio RS2 “continuous long read” technology. This type of sequencing technology is new to Australia, and has the advantage of producing complete or near complete microbial genomes. The BMS genome is estimated to be 2.9 Mbp in size and is currently assembled into 2 contiguous sequences. The size of the BMS genome is almost twice that of the human and macropodid isolates (~1.8 Mbp) and my preliminary comparisons show the human and kangaroo isolates are much more similar in size and gene organization (synteny) to each other, rather than to BMS. Because the BMS genome is much larger in comparison with the human and kangaroo isolates, there would appear to be many more features (genes) needed for colonization and persistence of these archaea in ruminant livestock. For these reasons, my PhD research should not only provide a greater understanding of methanogen-host interactions, but should also support the identification of novel, host-specific targets to inhibit these methanogenic archaea in ruminants.

Attwood GT, *et al.* (2011) *Animal Feed Science and Technology* 166–167(0):65-75.
Janssen PH & Kirs M (2008) *Applied and Environmental Microbiology* 74(12):3619-3625.