

## Methane matters in animals and man: from beginning to end



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**Methanogenic archaea resident in the mammalian gastrointestinal tract have long been recognised for their capacity to participate in interspecies hydrogen transfer, with commensurate positive effects on plant biomass conversion. However, there is also still much to learn about these methanogenic archaea in regards to their metabolic versatility, host adaptation, and immunogenic properties that is of relevance to host health and nutrition.**

### Methane, man and best laid plans

The methane club has an exclusive membership, principally restricted to the Domain Archaea and more specifically, the Euryarchaeota. Five orders of methanogens have long been recognised: the Methanopyrales, Methanococcales, Methanobacteriales, Methanomicrobiales, and Methanosarcinales<sup>1</sup>. However, the membership has recently been expanded to include the Methanocellales<sup>2</sup>

as well as the provisionally named ‘*Methanoplasmatales*’<sup>3</sup>. Members of the methane club are very popular, invited to join virtually all anaerobic microbial communities and especially those where sulphate is limiting. Popular hangouts include moist soil biomes, fresh water sediments and rice paddies, landfills, the gastrointestinal tracts of invertebrate and vertebrate animals, anaerobic lagoons and waste management facilities<sup>4–6</sup>. Indeed, the number and distribution of these hangouts have dramatically increased in recent decades in response to human population growth and urbanisation, as well as the intensification of agriculture to feed a hungry world; but the hangover has arrived. We are now being challenged to reduce methane gas emissions, and in particular, methane emissions from livestock production systems, which are attributed with producing ~20% of global methane emissions<sup>7</sup>, in response to global concerns about our impacts on the environment and climate change. Additionally, the resurgent interest in the microbiota we share our body with, and their

impacts on our health and well-being, extends to the methane club<sup>8</sup>. For these reasons, there is a renewed interest in gut methanogens, but is it more of the same or something new? We contend that there is still much to be learned about members of the methane club and their behaviour in the digestive tracts of animals and man, from beginning to end.

## Separating the sheep from the goats: methane and livestock

As herbivores, ruminants rely upon their microbial communities within the rumen-reticulum to not only deconstruct plant biomass, but provide the schemes of anaerobic fermentation necessary to support the formation of protein-yielding and energy-yielding nutrients such as microbial biomass and short chain fatty acids<sup>9,10</sup>. Methanogens have long been recognised to support these processes via minimising  $pH_2$ , with the concept of 'interspecies hydrogen transfer' (IHT) first demonstrated by Bryant and Wolin<sup>11</sup> using culture based experiments with rumen bacteria and methanogens. Much of the subsequent research focused on the taxonomic and ecological variations among methanogen communities as affected by diet, animal breed, and production system. In general terms, these studies have shown that while autotrophic *Methanobrevibacter* spp. are often numerically predominant there is also a relatively diverse population of heterotrophic archaea present in these animals<sup>6,12</sup>. In recent years, the application of 'omics' approaches has provided new insights into the roles the archaea might play in rumen function. Poulsen *et al.* (2013) showed that the reduced methane emissions from dairy cows fed rapeseed oil could be attributed to a selective suppression of the '*Methanoplasmatales*', with coincident decreases in transcripts encoding for methylotrophic methanogenesis from the rumen contents of these animals<sup>13</sup>. New Zealand and US-DOE researchers have also studied the rumen microbiota of sheep stratified with respect to methane production, and demonstrated that the trait is heritable<sup>14</sup>. Using a combination of metagenomic and metatranscriptomic methods they found no significant differences in total methanogen numbers between the 'low' and 'high' methane producers, although there were differences in the relative abundances of the methylotrophic *Methanosphaera* spp. (increased in 'low methane' sheep) and the hydrogenotrophic *Methanobrevibacter gottschalkii* clade (increased in 'high methane' sheep). The metatranscriptomic data revealed that 7/10 genes coordinating the hydrogenotrophic pathway were significantly increased in high methane producing sheep. Collectively, these findings suggest that while the inhibition of select populations of methanogens can mitigate livestock methane emissions, it is also a heritable trait, suggesting host-mediated effects on the rumen microbiota. In that context, 'high methane' emitting animals have been postulated to possess a longer retention of feed within the rumen as well as alterations in the bacterial 'ruminotype' increasing the levels of ruminal hydrogen, with coordinate elevated expression of genes encoding the hydrogenotrophic pathway and greater methane yield<sup>15,16</sup>. It seems intuitive then to further suggest that the increased relative abundance of hydrogen-dependent

methylotrophic methanogens in 'low methane' animals relates to their capacity for growth when the bacterial ruminotype favours less hydrogen production during fermentation<sup>12,17</sup>.

## Differences downunder: the low methane emitting macropodids

The macropodids (kangaroos, wallabies, pademelons and relatives) bear some similarity to ruminants in so far as their reliance on forestomach colonisation by microbes for plant biomass conversion and nutrient provision. In contrast, the foregut microbiota resident in these animals releases relatively low amounts of methane compared to sheep<sup>18,19</sup>. Although these observations were initially proposed to reflect the absence of methanogenic archaea within the macropodid forestomach, several studies have now demonstrated the presence of *Methanobrevibacter*, *Methanosphaera*, and '*Methanoplasmatales*' archaea, albeit at numbers substantially less than found for ruminant livestock ( $\sim 10^6$  g.sample<sup>-1</sup> c.f.  $\sim 10^8$  g.sample<sup>-1</sup>)<sup>6</sup>. Our group has now produced an axenic culture of *Methanosphaera* sp. (strain WGK6) from foregut digesta collected from a Western grey kangaroo (*Macropus fuliginosus*). Like the human strain *Methanosphaera stadmanae* DSM-3091, WGK6 uses methanol for methane formation, energy production and growth. However, the annotated draft sequence of the WGK6 genome suggests the macropodid isolate possesses some unique features that may support a greater metabolic versatility than previously characterised from studies of the human strain. So it seems that the adaptations to herbivory in the 'low methane' emitting macropodids includes the maintenance of *Methanosphaera* spp., which also seem to be present in greater abundance in ruminant animals individually confirmed to be 'low methane' emitters.

## Humans and methanogens: a docile partnership or secret frenemies?

Methanogens are consistently identified from human subjects deemed healthy or suffering from disease; however the relationships between the diversity of methanogen community members and the health status of the host are still unclear. Early studies determined that like other mammals the human large bowel was colonised by hydrogenotrophic *Methanobrevibacter* spp. (principally *Mbb. smithii*) and the methylotrophic *Methanosphaera* spp. (principally *Msp. stadmanae*<sup>20</sup>). More recently, the analysis of human microbiota samples from subgingival, intestinal or vaginal mucosae has further expanded the diversity of methanogenic archaea to include a new species of *Methanobrevibacter* (*Mbb. oralis*), as well as two isolates of methylotrophic archaea (*Candidatus* 'Methanomethylophilus alvus' and *Methanomassiliicoccus luminyensis*) affiliated with the newly defined order *Methanoplasmatales*<sup>21,22</sup>. Interestingly, our own unpublished studies, as well as the findings of Poulsen *et al.*<sup>13</sup>, Dridi *et al.*<sup>21</sup> and Borrel *et al.*<sup>22</sup> show these archaea are capable of using methylated amines arising from phosphatidylcholine metabolism to support growth. In that context, establishment of the *Methanoplasmatales* in the human large bowel might be of clinical relevance for persons known to

possess relatively high levels of trimethylamine-oxide in blood, because of its association with cardiovascular disease pathogenesis (reviewed by Morrison<sup>23</sup> and Brugère<sup>24</sup>). However, there is also mounting evidence from cross-sectional studies that variations in archaeal communities at different body sites might impact human health<sup>25–27</sup>. For instance, patients with periodontitis have been found to harbour large numbers of methanogenic archaea, in addition to acetogenic and sulphate-reducing bacteria within subgingival periodontal pockets<sup>28</sup>. Blais Lecours *et al.*<sup>29</sup> also confirmed that both *Mbb. smithii* and *Msp. stadtmanae* can be immunostimulatory in animal models of respiratory disease, with the latter provoking a stronger immune response. Furthermore, Blais Lecours *et al.*<sup>30</sup> reported that while the total numbers of methanogenic archaea are less in patients suffering from inflammatory bowel disease (IBD), the prevalence of *Msp. stadtmanae* was greater in these patients, and healthy human subjects produced an antigen-specific IgG response to this archaeon. These results suggest that *Msp. stadtmanae* prevalence and/or abundance may be a biomarker of gut dysbiosis, being more prevalent in persons with an altered ‘low hydrogen’ fermentation scheme. This hypothesis warrants more detailed examination as part of well-designed clinical studies of IBD and perhaps, other chronic inflammatory diseases.

## Summary

Despite the widespread recognition of the roles methanogenic archaea play in gut environments, there is still much to learn about their metabolic versatility, host adaptation, and immunomodulation. Recent research of the methylotrophic archaea from three divergent mammalian hosts suggests that methane matters in animals and man, from beginning to end!

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## Biographies

**Emily Hoedt** is a PhD student at The University of Queensland School of Chemistry and Molecular Biosciences, and is supervised by



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**Paul Evans** is a Postdoctoral Fellow, based at the Australia Centre for Ecogenomics at University of Queensland, Brisbane and his research interests are related to microbial ecology in anaerobic environments. His research involves examinations of novel microbes from a range of environments including ruminants, permafrost soils and coal bed methane aquifers. Paul is specifically interested in archaea that populate these anaerobic environments and how they interact with other members of the microbial community to produce energy and make their living.

**Stuart Denman** is a research scientist with the CSIRO in the Agriculture Flagship. He has been actively involved in projects that use molecular methods to detect and monitor key microbial populations within the rumen. His current research focus is on microbial metagenomics and uses advanced molecular and bioinformatic techniques to ascertain the interactions and functional changes that take place in the rumen microbiome as they pertain to methane abatement strategies.

**Chris McSweeney** is a Senior Principal Research Scientist at CSIRO and leads research into the gut microbiology of livestock, humans and native animals. His current research is focussed on the

molecular basis of hydrogenotrophy in gut microbial ecosystems with emphasis on the function of methanogenic archaea. The aim is to eventually modify the ecosystem to reduce methane emissions from ruminant livestock.

**Dr Páraic ÓCuív** is a gut microbiologist at The University of Queensland Diamantina Institute where he has a long standing interest in host-microbe interactions as they relate to the aetiology of chronic gut diseases. He is an expert in gut microbiology, microbial genetics and functional metagenomics and he currently leads the isolation and functional characterisation of microorganisms from the human gut as part of the Australian Healthy Microbiome Project.

**Professor Mark Morrison** is trained as a microbiologist with a specific interest in the role that microbes play in affecting the health and well-being of humans and animals. After nearly 20 years within US academia, he returned to Australia in 2006 initially as a Science Leader within CSIRO, and now as Chair and Group Leader in Microbial Biology and Metagenomics at The University of Queensland Diamantina Institute. His work since returning to Australia has emphasised the use of 'omics' technologies to produce new insights into the microbial world, and from which, improved methods for monitoring and adjustment of the gut microbiota might be achieved; with the goal of improving host animal health and well-being.

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