AEGC Australasian Exploration Geoscience Conference Brisbane 2021

Microbial methane production in the Surat Basin, Queensland

Bronwyn C. Campbell
Macquarie University & CSIRO
North Ryde, NSW
Bronwyn.Campbell@mq.edu.au

Se Gong
CSIRO
Lindfield, NSW

Paul Greenfield
CSIRO
North Ryde, NSW

David J. Midgley
CSIRO
Lindfield, NSW

Ian T. Paulsen
Macquarie University
North Ryde, NSW

Simon C. George
Macquarie University
North Ryde, NSW

SUMMARY

A large proportion of the methane found in coal seams is produced by microbial communities. Despite coal seam methane being a valuable resource for human energy security, the processes employed by these microbes in the production of methane are still not well understood.

The best understood part of the coal degradation process is the final step of methane production, which is performed by microbes known as 'methanogens'. As methanogens are present in many other anoxic environments, such as the digestive systems of animals, and the substrates they can work with are relatively simple, our understanding of the role of the methanogens within coal seam communities is much more complete than for the other microbes present.

In order to investigate which types of methanogens are present within the Surat Basin, Queensland, and thus which substrates are likely being made available to them by other microbes during coal degradation, two datasets of coal seam microbial community DNA underwent a process of filtering and assembly to extract DNA sequences for key methane-producing genes (mcrA).

This study used small variations within these methane-producing genes to identify different groups of methanogens within the coal seam community, and found that there are two distinct methane-producing communities within the same basin at different locations.

Key words: methane, coal, microbe, methanogenesis, biodegradation

INTRODUCTION

A global transition to less carbon intensive sources of energy is underway, with substantial economic and technological hurdles still to be faced. Coalbed methane is a useful bridging fuel for this energy transition due to its lower greenhouse gas emissions relative to thermal coal (Hardisty, Clark and Hynes 2012; Schandl *et al*. 2019), and lack of other pollutants associated with coal such as nitrogen oxides, sulfur dioxide, and particulates (Markandya and Wilkinson 2007).

Over the last few decades it has become apparent that significant amounts of coal bed methane are produced by microbial activity (Strąpoć *et al*. 2008). This discovery of microbial involvement in methane production has led to interest in enhancing their production of methane *in situ*. In general, these modifications have taken the form of microbial augmentation with microbial communities from non-coal environments (Wang *et al*. 2016), addition of catalytic compounds (Beckmann *et al*. 2016), or nutrient amendments (Jones *et al*. 2010). Despite this work, our understanding of the degradation pathways involved in producing methane from coal still remains poorly understood, aside from the final steps of methane production.

Methanogens, the microbes capable of producing methane, share a common gene known as 'mcrA'. The mcrA gene codes for a particular enzyme (methyl coenzyme M reductase) which is responsible for the final step in methane production. Extracting this gene from coal seam water samples and looking for small variations in their DNA sequences allows for the identification of the different species of methanogens found in these communities. With this idea in mind, DNA samples were extracted from coal seam water produced by two wells in the Surat Basin (Figure 1), the metagenomes of these samples were sequenced, and the mcrA genes were extracted, thus allowing us to compare the methanogenic species present in each.

Figure 1. Location of the Surat Basin in Australia, and approximate location of the sampled wells referenced in Greenfield *et al*. (2019).

METHODS

This study uses 'Kelpie', a tool that extracts and assembles genes from metagenomic reads using a process analogous to an *in silico* polymerase chain reaction (Greenfield *et al*. 2019).

Metagenomic datasets of coal seam microbial communities from two wells intersecting the Walloon Subgroup in the Surat Basin (referred to as Well A and Well B) were processed with Kelpie to extract the diverse mcrA genes found in these metagenomes. Four different sets of mcrA primers were used to give Kelpie information about conserved regions of the mcrA gene. The primer sets used are known as ME (Hales *et al.* 1996), MCR (Springer *et al*. 1995), mlas-mod – F and mcrA-rev – R

(Angel *et al*. 2012) and ML (Luton *et al*. 2002). The MCR primer was chosen for final use due to its ability to detect the most complete set of mcrA data, after the alteration of one base (MCR-f adjusted to 5'-TWYGAYCARATHTGGYT-3').

NCBI BLAST (a program for comparing biological sequence data to a database; https://blast.ncbi.nlm.nih.gov/Blast.cgi) was then used to identify close relatives for these mcrA sequences from within the GenBank nucleotide collection.

RESULTS AND DISCUSSION

Although this study looked at diversity of mcrA genes, for ease of discussion these genes are referred to, and treated as, proxies for the methanogens in the communities. Seven distinct taxonomic groupings of methanogens were found to be present in the dataset from Well A, and eight were observed in the dataset from Well B (Figure 2; Table 1). Of these, only two were present in both datasets: one belonged to a putative Methanomicrobia sp. (MCR9), and the other was likely from a Methanosarcinaceae sp. (MCR10). The small number (two) of methanogens observed in both wells relative to those exclusive to just one well (eleven) suggests that connectivity between these wells is limited.

The methanogens exclusive to one well only (either Well A or Well B; Figure 2) mainly had close relatives restricted to the hydrogenotrophic process of methanogenesis, meaning they produce methane by reducing carbon dioxide with hydrogen (Kallistova *et* al. 2017; Evans *et al*. 2019). Supporting the hypothesis of these two wells having limited connectivity is how distantly related the majority of these methanogens are. Well A is dominated by methanogens from the Methanomicrobiales order, whereas Well B is dominated by methanogens from the Methanobacteriaceae family (Figure 2). Nevertheless, as these are all likely to be hydrogenotrophic methanogens, the methanogenic substrates being utilised and produced by the microbial communities from both wells are likely similar. Conversely, those methanogens shared between wells may be using non-hydrogenotrophic methanogenic processes and substrates.

The Methanosarcinaceae sp. (MCR10) that is present in both wells (Figure 2) has close relatives capable of producing methane, either via hydrogenotrophic methanogenesis, acetoclastic methanogenesis (whereby methane is produced by disproportionation of acetate; Kallistova *et al*. 2017; Evans *et al*. 2019), or methylotrophic methanogenesis (whereby methane is produced by disproportionation of methylated molecules; Kallistova *et al*. 2017; Evans *et al*. 2019). The other methanogen present in both wells (MCR9) is both the most abundant in mcrA reads and also the least well classified, being only classified down to the class level (Table 1). If these methanogens are indeed involved in non-hydrogenotrophic methanogenesis, this may advantage them through avoiding competition with the dominant hydrogenotrophs.

CONCLUSIONS

This study has provided initial insights into the diversity and distribution of mcrA genes from two wells in the Surat Basin. The dominant methanogens differing between the wells suggests either a lack of connectivity, differences in water chemistry, or some other unknown abiotic driver. Both groups included numerous hydrogenotrophs, suggesting that the non-methanogen communities from each well may be producing similar methanogenic precursors at both sites.

FUTURE RESEARCH

This study would benefit from greater investigation of the poorly classified methanogen (MCR9) to determine its possible roles in methane production, and comparison with 16S rRNA genes to determine whether all the methanogens identified by this method are also found in general prokaryote surveys. Additionally, a more detailed understanding of the local geology, particularly the region between the two wells, would be useful in determining whether the communities are indeed disconnected. Finally, the use of the Kelpie software tool provides the opportunity to analyse existing datasets of microbial communities from other coal seams internationally, as well as other functional genes of interest. Analysis of metagenomic data from other coal seams would provide a greater context for understanding these Surat Basin communities, and assist in placing them in their international context. Using Kelpie to analyse other key genes present in existing coal seam metagenomic datasets would assist in assigning functions to different taxa within those communities, providing a more complete understanding of the degradation pathways from coal to methane.

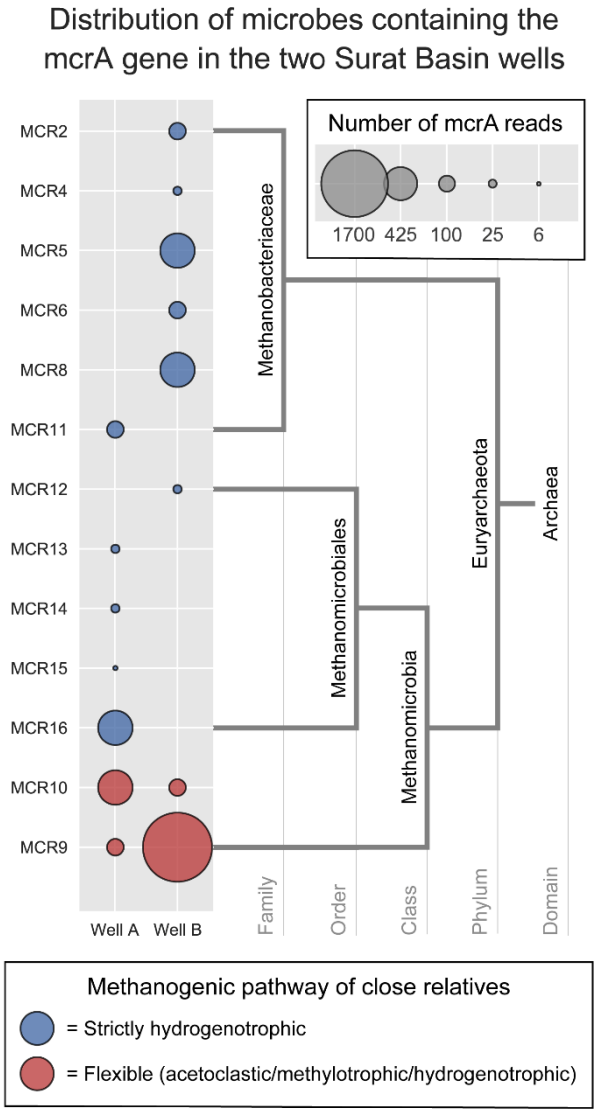

Figure 2. Distribution of the different groupings of distinct methanogens across both Surat Basin wells.

Table 1. Taxonomic details for each distinct grouping of methanogens found in the Surat Basin wells. The family of the nearest BLAST relatives for each methanogen is displayed in bold. Sequence match details and GenBank accession of closest relative are provided in brackets.

Taxon ref.	**Family of relatives (if known)** (% cover, % ident, accession)	**Environment of closest relative**
MCR2	**Methanobacteriaceae** (95, 99.33, KX234781)	Coal seam gas well, China
MCR4	**Methanobacteriaceae** (99, 96.79, HE647307)	Earthworm digestive tract
MCR5	**Methanobacteriaceae** (96, 97.57, KX234759)	Coal seam gas well, China
MCR6	**Methanobacteriaceae** (98, 98.03, AB842184)	Hot spring, Japan
MCR8	**Methanobacteriaceae** (100, 98.71, KF761097)	Rice paddy soil, China
MCR9	**Methanomicrobia** (95, 99.55, KT314685)	Petroleum reservoir, China
MCR10	**Methanosarcinaceae** (99, 97.34, KX233742)	Rice paddy soil
MCR11	**Methanobacteriaceae** (99, 74.95, FJ226712)	Biogas plant
MCR12	**Methanomicrobiales** (96, 98.32, KF595606)	Intertidal mudflat, China
MCR13	**Methanomicrobiales** (96, 97.26, JN185013)	Soda lake sediments, India
MCR14	**Methanomicrobiales** (100, 96.35, JX648567)	Mud volcano, Taiwan
MCR15	**Methanomicrobiales** (99, 96.75, JX097231)	Tidal flat sediments, Germany
MCR16	**Methanomicrobiales** (100, 96.55, JX097240)	Tidal flat sediments, Germany

ACKNOWLEDGMENTS

Ms Bronwyn Campbell was supported by a Macquarie University Research Training Program scholarship, a CSIRO Energy strategic research initiative and a PESA Horstman Federal Postgraduate Scholarship.

REFERENCES

Angel, R., Claus, P. and Conrad, R., 2012, Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions: The ISME Journal, 6, 847-862.

Beckmann, S., Welte, C., Li, X., Oo, Y.M., Kroeninger, L., Heo, Y., Zhang, M., Ribeiro, D., Lee, M., Bhadbhade, M., Marjo, C.E., Seidel, J., Deppenmeier, U. and Manefield, M., 2016, Novel phenazine crystals enable direct electron transfer to methanogens in anaerobic digestion by redox potential modulation: Energy & Environmental Science, 9, 644–655.

Evans, P.N., Boyd, J.A., Leu, A.O., Woodcroft, B.J., Parks, D.H., Hugenholtz, P. and Tyson, G.W., 2019, An evolving view of methane metabolism in the Archaea: Nature Reviews Microbiology, 17, 219-232.

Greenfield, P., Tran-Dinh, N. and Midgely, D., 2019, Kelpie: generating full-length `amplicons' from whole-metagenome datasets: PeerJ, 6, e6174.

Hales, B.A., Edwards, C., Ritchie, D.A., Hall, G., Pickup, R.W. and Saunders, J.R., 1996, Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis: Applied and Environmental Microbiology, 62:2, 668-675.

Hardisty, P.E., Clark, T.S. and Hynes, R.G., 2012, Life cycle greenhouse gas emissions from electricity generation: a comparative analysis of Australian energy sources: Energies, 5, 872–897.

Jones, E.J.P., Voytek, M.A., Corum, M.D. and Orem, W., 2010, Stimulation of methane generation from nonproductive coal by addition of nutrients or a microbial consortium: Applied & Environmental Microbiology, 76, 7013–7022.

Kallistova, A.Y., Merkel, A.Y., Tarnovetskii, I.Y. and Pimenov, N.V., 2017, Methane formation and oxidation by prokaryotes: Microbiology, 86:6, 661-683.

Luton, P.E., Wayne, J.M., Sharp, R.J. and Riley, P.W., 2002, The mcrA gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill: Microbiology, 148, 3521-3530.

Markandya, A. and Wilkinson, P., 2007, Electricity generation and health: The Lancet, 370, 979–990.

Schandl, H., Baynes, T., Haque, N., Barrett, D. and Geschke, A., 2019, Final Report for GISERA Project G2 - Whole of Life Greenhouse Gas Emissions Assessment of a Coal Seam Gas to Liquefied Natural Gas Project in the Surat Basin, Queensland, Australia: CSIRO, Australia.

Springer, E., Sachs, M.S., Woese, C.R., Boone and D.R., 1995, Partial gene sequences for the A subunit of methyl-coenzyme M Reductase (*mcr*I) as a phylogenetic tool for the family Methanosarcinaceae: International Journal of Systematic And Evolutionary Microbiology, 45:3, 554-559.

Strąpoć, D., Picardal, F.W., Turich, C., Schaperdoth, I., Macalady, J.L., Lipp, J.S., Lin, Y.S., Ertefai, T.F., Schubotz, F., Hinrichs, K.U., Mastalerz, M. and Schimmelmann, A., 2008, Methane-producing microbial community in a coal bed of the Illinois Basin: Applied and Environmental Microbiology, 74, 2424–2432.

Wang, H., Lin, H., Rosewarne, C.P., Li, D., Gong, S., Hendry, P., Greenfield, P., Sherwood, N. and Midgley, D.J., 2016, Enhancing biogenic methane generation from a brown coal by combining different microbial communities: International Journal of Coal Geology, 154–155, 107–110.