Genome Watch

Methanogens Implicated by DNA Evidence

Matthew Kellom

This Genome Watch article highlights recent findings that expand the known diversity of methanogenic archaea and the metagenomic evidence that led to their identification and cultivation.

Methane is an important component of the global carbon cycle. Approximately two-thirds of global methane emissions are produced by archaea known as methanogens[1]. Methanogenesis is an anaerobic process found in anoxic environments including aquatic sediments, landfills and animal digestive systems. Until recently, methanogens were only known to be members of the archaeal superphylum Euryarchaeota, but metagenome-based studies offered clues for where and how to cultivate novel lineages. Discovery of methanogenesis marker genes in metagenome-assembled genomes (MAGs) has revealed a more diverse population than previously thought[2]. Genes encoding the methanogenesis protein methyl-coenzyme M reductase (MCR) were found in MAGs annotated from the archaeal phyla Asgardarchaeota, Hadarchaeota, and Thermoproteota. These assembled genomes also contain genes for alternative electron-accepting pathways from what was canonically known for methanogens.

Three recent studies detail the cultivation and experimental evidence that led to the descriptions of novel Thermoproteota lineages that are capable of methyl-reducing methanogenesis[3–5]. The samples were collected from hot spring and oilfield environments, which harbour conditions observed in metagenomic studies to host highly diverse MCR-containing genomes. Once in culture, researchers assessed their physiological properties, including the confirmation of methane production with an array of experimental methods.

One of the recently described methanogens, *Candidatus* Methanodesulfokora washburnensis strain LCB3, was cultured with a medium designed specifically to enrich for its metabolism[3]. The predicted metabolisms of Ca. M. washburnensis MAGs were used to determine the metabolic requirements conducive to LCB3 cultivation. The genome variation between LCB3 and related MAGs from Washburn Hot Springs in Yellowstone National Park (YNP) indicates a species population composed of several strains, all capable of methyl-reducing methanogenesis. In the case of LCB3, methanogenesis was confirmed experimentally with a combination of bioorthogonal non-canonical amino acid tagging (BONCAT) and fluorescence *in situ* hybridization (FISH) to show anabolic activity under methanogenic conditions. However, unlike most cultured methanogens, transcriptional data suggests that LCB3 may utilize electron donors other than hydrogen. With differences from previously cultured methanogens, the 16S rRNA gene of LCB3 branches from deep within the known Thermoproteota phylogeny, establishing its importance as a model for studying methanogen evolution.

Another methanogen from a YNP hot spring, *Candidatus* Methanosuratincola verstraetei strain LCB70[4], has closely related 16S rRNA genes found in a wide-range of global anoxic environment metagenomes. In laboratory cultures, LCB70 produced methane when supplied with methanol and hydrogen, and in fact lacks genes for the more studied carbon dioxide-reducing or acetoclastic pathways of methanogenesis. When hydrogen is not supplied to enriched cultures containing methanogens and methanol degraders, LCB70 seems capable of acquiring hydrogen from coexisting organisms. A stable isotope tracer experiment with ^{13}C-methanol showed probable methanol metabolism producing carbon dioxide, hydrogen and acetate, which supplies LCB70 and other methanogens with metabolic reactants. Gene expression data also suggests that LCB70 may use lactate as an electron donor, although the exact mechanism of lactate utilization requires further investigation.

In contrast to its hot spring relatives, *Methanosuratincola petrocarbonis* LWZ-6 was isolated from oil-produced water in the Shengli oilfield (China)[5]. Although cultured from a geographically and biogeochemically different environment, analysis of LWZ-6 methanogenic properties, metagenomically-described relatives and genome content reveals remarkable metabolic similarity with that of LCB3 and LCB70. Contrary to previous metagenome-based predictions, LWZ-6 was not capable of fermentation alone, but was found to utilize hydrogen produced by *Acetomicrobium* sp. CY-2 when grown in co-culture.

Together, these reports of novel methanogenic lineages and the metagenomic evidence that led to their discovery are exemplary cases of metagenome-guided cultivation. These cultures unlock future biochemical discovery through experimentation and expand our

understanding of methanogenesis and global methane emissions.

*Matthew Kellom**
DOE Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

**e-mail: mkellom@lbl.gov*

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