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Metagenomic analysis of methanogen populations in three full-scale mesophilic anaerobic manure digesters operated on dairy farms in Vermont, USA

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HIGHLIGHTS

- 50 246 methanogen 16S rRNA gene sequences analyzed from three manure digesters.
- The combined digester methanogen diversity was estimated at 307 species-level OTUs.
- A single *Methanosarcina*-related OTU (acetrotrophic) was dominant in two digesters.
- One digester had a diverse methanogen profile with representation from four phyla.

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ABSTRACT

The microbial communities that produce biogas as a result of anaerobic digestion of manure remain poorly understood. Using next-generation sequencing, methanogen populations were investigated in three full scale mesophilic anaerobic digesters operated on dairy farms. A combined 50 246 non-chimeric sequence reads covering the V1–V3 hypervariable regions of the methanogen 16S rRNA gene were assigned to 307 species-level operational taxonomic units (OTUs). The Blue Spruce Farms (BSF) and Green Mountain Dairy (GMD) anaerobic digesters were found to have nearly identical methanogen profiles, with the overwhelming predominance of OTU 1 (98.5% and 99.7%, respectively), which showed 99.2% sequence identity to *Methanosarcina thermophila*. In contrast, methanogens from the Chaput Family Farms (CFF) anaerobic digester were more diverse, with five major OTUs belonging to four distinct phylogenetic groups (Methanomicrobiales, Methanosarcinales, Methanoplasmatales, and Methanobacteriales). Differences in management practices and years of operation were hypothesized as potential factors responsible for differences in the methanogen profiles.

1. Introduction

In light of global challenges such as climate change and the exhaustion of fossil fuels, anaerobic digestion of organic waste into methane has become an increasingly more attractive strategy worldwide to produce renewable energy to help meet growing demand. As costs of other energy sources increase and climate policy incentives such as carbon tax or credits come into effect, the profitability of anaerobic digestion is predicted to rise (Zaks et al., 2011). In many Westernized countries, the beef and dairy cattle industries represent a large proportion of the agricultural sector. Cattle consume large amounts of feed and consequently they generate great quantities of manure. While it serves as a valuable source of fertilizer, manure also represents an environmental hazard, as runoff can result in contamination of environmental water sources, and storage of manure is favorable to the production of methane without restricting the release of this greenhouse gas into the atmosphere. Thus, anaerobic digesters provide a sustainable solution to manure management concerns by harvesting methane for energy production, improving fertilizer potency and reducing pollution (Weiland, 2010). Improvements in design, engineering, and management have greatly contributed to making the use of manure anaerobic digester technology financially sustainable. However, performance is still limited. For instance, methane typically constitutes 60–70% of the biogas produced by mesophilic anaerobic digesters. A very large volume of manure is then needed to produce electricity in a cost-effective way, which currently limits profitable operation of a digester to large livestock operations.

Biomethanation, the production of methane from anaerobic digestion of organic substrate, is accomplished by complex microbial communities. These communities consist of specialized microorganisms that each contribute to different stages of the process (Weiland, 2010). Biomethanation starts with the hydrolysis of

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large polymers, such as cellulose, xylan, proteins or lipids for example, to release monomeric subunits. Monosaccharides, amino acids or fatty acids are then fermented into organic acids (acidogenesis), which in turn are further metabolized as a result of acetogenesis into simpler compounds, such as acetate, formate, H_2 and CO_2. The products of acetogenesis are then used as substrates for the synthesis of methane, the last step in the decomposition of organic matter in oxygen-free environments (Thauer et al., 2008). While a number of different bacterial groups contribute to hydrolysis, acidogenesis, or acetogenesis, methanogenesis is exclusively performed by methanogens. Methanogens are a phylogenetically diverse group of archaea that share synthesis of methane as a major product of their energy metabolism.

Since microorganisms within a community are specialized, they are each dependent on others to provide them with substrates and/or metabolize their products to favor their metabolic activity. In order to thrive, each bacterial or methanogen species also requires a specific combination of physical and chemical conditions, such as pH, temperature, and salinity in addition to substrate availability. Thus, while microbial communities from different environments can perform anaerobic digestion through the same general steps of hydrolysis, acidogenesis, acetogenesis and methanogenesis, the particular microbial species that populate them tend to vary between environments depending on physical and chemical conditions.

Since biomethanation is performed by microbial communities, additional improvements in manure digester performance could be accomplished by selecting or manipulating particular groups of microorganisms that populate them. However, communities from anaerobic manure digester remain largely uncharacterized, so a deeper understanding of population structure, as well as metabolic properties and interactions, are necessary in order to successfully improve performance through microbiological manipulation. In the state of Vermont (USA), the construction and operation of anaerobic manure digesters have increased in recent years as a result of subsidies, logistical support, and financial incentives that promote the sale of electricity produced by dairy farms. Since the microbial communities populating anaerobic digesters in this state have not been investigated, this represented a great opportunity to deepen the knowledge base in this field. This report presents the methanogen profiles from three mesophilic anaerobic manure digesters operated on Vermont dairy farms. Investigations were performed using next-generation sequencing technology.

2. Methods

2.1. Anaerobic manure digester sampling

Effluent from anaerobic manure digesters operated on three dairy farms located in Vermont (VT) were collected during the month of June 2011: Blue Spruce Farm (Bridport, VT), Green Mountain Dairy (Sheldon, VT) and Chaput Family Farms (North Troy, VT) (Fig. 1). These farms sell electrical power to Vermont customers through "Cow Power", a division of the utility provider Green Mountain Power (Colchester, VT). Digester samples were maintained on ice after collection, and frozen at −20 °C within 2 h. Samples remained frozen until DNA extraction.

The BSF anaerobic digester is a plug-flow design (GHD Inc, Chilton, WI, USA) with a capacity of 2.27 million liters. It is operated at a temperature of 37.8 °C, with a retention time of 21 days, and has been running since 2006. While dairy cattle manure is the main substrate, whey from a local cheese processing plant is also used. During the month of June 2011, the BSF digester generated 1.29×10^5 kWh of electricity.

The GMD anaerobic digester also has a plug-flow design (GHD Inc, Chilton, WI, USA), with a capacity of 3.8 million liters. It is operated at 38.3 °C, with a retention time of 25–27 days, and has been running since March 2007. Manure from dairy cattle is the main substrate, but it is also supplemented with waste from an ice cream factory. During the month of June 2011, the GMD digester generated 9.49×10^4 kWh of electricity.

The CFF anaerobic manure digester is of complete mix digester design (RCM International LLC, Berkeley, CA, USA), with a capacity of 3.43 million liters. It is operated at 36.1 °C, with a retention time of 30 days, and has been running since August 2010. Dairy cattle manure is the main substrate, with the addition of oil waste from a fish canning plant as a co-substrate. During the month of June 2011, the CFF digester generated 1.42×10^5 kWh of electricity.

2.2. Microbial DNA isolation and PCR amplification of 16S rRNA gene sequences

Microbial DNA from manure digester samples was isolated as described by Yu and Morrison (2004). Methanogen 16S rRNA genomic sequences containing the hypervariable V1–V3 regions were amplified from purified digester microbial DNA by PCR using one pair of universal primers. The forward primer had the following design, from 5′ to 3′: Roche 454 adapter A, four nucleotide barcode, and the Met86F primer (Wright and Pimm, 2003). The reverse primer had the following design, from 5′ to 3′: Roche 454 adapter B, four nucleotide barcode, and the 519R primer (Turner et al., 1999). PCR reactions were performed using the iProof Taq DNA polymerase (BioRad) on a C1000 Thermal Cycler (BioRad) under the following conditions: hot start (4 min, 98 °C), followed by 5 cycles of denaturation (10 s, 98 °C), annealing (30 s, 58 °C) and extension (30 s, 72 °C), then 30 cycles of denaturation (10 s, 98 °C), annealing (30 s, 65 °C) and extension (30 s, 72 °C), and ending with a final extension period (10 min, 72 °C). PCR products were separated by agarose gel electrophoresis, and amplicons of the expected size (~380 bp) were excised for DNA extraction using the QiaexII Gel extraction kit (Qiagen). Two hundred nanograms of methanogen 16S rRNA gene amplicons from each digester were pooled and submitted to the DNA Sequencing Facility at the University of Pennsylvania (Philadelphia, PA, USA) for pyrosequencing using the Roche 454 platform. "Sequence data are available from the NCBI Sequence Read Archive (experiment SRX246946)."

2.3. Computational analysis of nucleotide sequences

Computational analysis of methanogen digester sequence reads was performed using MOTHUR, an open-source bioinformatics software (Schloss et al., 2009). Reads corresponding to methanogen 16S rRNA sequences spanning the entire targeted 86F to 519R region were first selected using trim.flows (Schloss et al., 2011). The command shhh.flows, MOTHUR's implementation of PyroNoise (Quince et al., 2009), was then used to screen for high quality 86F-519R sequence reads according to default quality threshold values, and to create consensus sequences representing digester methanogen phylotypes. Phylotypes were aligned using the align.seqs function, and alignments were refined manually based on visual assessment. The Chimera-slayer and Uchime functions were then used to detect chimeric sequences, which were removed from further analysis. Genetic distance data from the aligned chimera-free methanogen digester phylotypes were generated using dist.seqs, which were then used to group phylotypes into operational taxonomic units (OTU) with the function cluster, based on a 2% genetic distance cutoff. This genetic distance cutoff was estimated from known methanogen species as a representative limit of ge-