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Jørgensen, Vibeke Rudkjøbing; Dueholm, Morten Simonsen; Knutsson, Simon; Nierychlo, Marta Anna; Kristensen, Jannie Munk; Petriglieri, Francesca; Dottorini, Giulia; Yashiro, Erika; Karst, Søren Michael; Albertsen, Mads; Nielsen, Per Halkjær

Publication date:
2019

[Link to publication from Aalborg University](#)

Citation for published version (APA):

Jørgensen, V. R., Dueholm, M. S., Knutsson, S., Nierychlo, M. A., Kristensen, J. M., Petriglieri, F., Dottorini, G., Yashiro, E., Karst, S. M., Albertsen, M., & Nielsen, P. H. (2019). *Global reference database of microbes in anaerobic digesters*. Abstract from IWC - 16th World Conference on Anaerobic Digestion, Delft, Netherlands.

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Global reference database of microbes in anaerobic digesters

V. Rudkjøbing*, M. Dueholm*, S. Knutson*, M. Nierychlo*, J. Kristensen*, F. Petriglieri*, G. Dottorini*, E. Yashiro*, S. Karst*, M. Albertsen*, P. H. Nielsen*

*Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg University, Fredrik Bajers Vej 7H, 9220 Aalborg, Denmark

Abstract

In this study, we aim to establish an anaerobic digester-specific reference database, comprised of full-length microbial 16S rRNA gene sequences from anaerobic digesters across the world (the Global Database of Microbes in Anaerobic Digesters, www.midasfieldguide.org/en/global-database-of-microbes/). The project utilizes a novel high-throughput, primer-free sequencing method to reveal the true microbial composition without being skewed by primer choice. The database is based on samples from more than 25 countries, making it the largest of its kind to date. The included digester types cover various process designs and a range of substrate feed compositions (with a primary focus is wastewater sludge). Here we present an overview of procedures for sampling, sample handling, sequencing, and the improved 16S rRNA gene reference database and taxonomy.

Keywords: full-length 16S rRNA; reference database; global survey

Session: Microbiology of anaerobic digestion/ (meta)genomic research

Introduction

Wastewater is increasingly viewed as a resource, with anaerobic digester technology being routinely implemented for biogas production. Anaerobic digestion has for many years been considered a great social and environmental opportunity, promising to make waste handling sustainable. Despite this great promise, it has only very recently been possible to maintain energy neutral and economically successful plants.

One problem that should be addressed before the full potential of anaerobic digesters can be reached is that the anaerobic digestion process is largely operated as a black box. Although microorganisms are key to the process, our knowledge about their identity and function is limited. Recent advances in sequencing technologies makes it feasible to analyse the composition of the microbial communities, but currently most of the microbes in digesters remains unidentified. This is largely because the current databases are underpopulated, ecosystem skewed, biased by primers and the taxonomy of some species is not fully resolved.

The MiDAS field guide is a freely available online tool, created to overcome these challenges (<http://www.midasfieldguide.org/>). Comprised by a collection of profiles for key organisms present in activated sludge plants, anaerobic digesters, and related wastewater treatment systems, the MiDAS field guide makes it possible to e.g. search the most dominant genera in thermophilic digesters, and evaluate their distribution and likely function in the ecosystem (e.g. Figure 1).

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Canonical Name	Phylum	Digester-Mesophilic	Digester-Thermophilic	Fermentation	Acetogen	Methanogen	Fatty acids	Sugars	Proteins/Amino acids
Methanothermobacter	Euryarchaeota		70.3						
Methanosarcina	Euryarchaeota	0.1	23.5	●					
G35_D8	Bacteroidetes	0.1	6.5						
Coprothermobacter	Firmicutes		5.4	●	●				
A55_D21	Firmicutes		5.3						
Candidatus Microthrix	Actinobacteria	1.8	3.1	●		●	●	●	●
Gelria	Firmicutes	0.6	2.3						
Anaerobaculum	Synergistetes		2	●	●				
Tetrasphaera	Actinobacteria	1.2	1.9	●					
Fervidobacterium	Thermotogae		1.6	●					
RB349	Chloroflexi		1.5						
B55_F	Firmicutes		1.1						
Clostridium sensu stricto 1	Firmicutes	1.4	1.1	●					
Romboutsia	Firmicutes	1.5	1.1	●	●				
Dechloromonas	Proteobacteria	0.5	1	●					
Methanobrevibacter	Euryarchaeota	1.9	0.9			●	●	●	●
Candidatus Amarillinum	Chloroflexi	0.1	0.8						
Candidatus Promineofilum	Chloroflexi	0.5	0.8	●	●	●	●	●	●
Rhodobacter	Proteobacteria	0.7	0.8	●			●	●	●
Fodinibacter	Actinobacteria	0.1	0.7				●	●	●

● POS ● NEG ● Variable ● Not assessed

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Figure 1. An example of some of the information that is available in the MiDAS field guide, indicating that *Methanothermobacter* is predominant in thermophilic digester reactors, and gives an indication of the metabolism.

Previous MiDAS versions were based on long-term surveys of Danish wastewater treatment plants and anaerobic digesters, and contains a manually curated taxonomy with annotations for the abundant and important genera in the ecosystems (McIlroy et al., 2017, 2015). In 2018, campaigns were initiated to further expand the MiDAS field guide and taxonomy, as the Global Databases of Microbes in Anaerobic Digesters and Wastewater Treatment Systems were launched. Here we present an overview of procedures for sampling, sample handling, sequencing, and improved reference database and taxonomy for the Global Database of Microbes in Anaerobic Digesters.

Results

Samples for the Global Database of Microbes in Anaerobic Digesters were obtained from more than 25 countries across the world. This makes this project the largest of its kind to date. For all participating countries, at least one national representative was selected to coordinate sampling multiple anaerobic digesters (15-20 plants/country, sampled in duplicates), and take charge of conservation and shipment of the samples. The coordinators received standardized protocols for sampling and handling of samples, and pre-labelled tubes containing preserving liquid for samples during shipment. Basic plant information was collected along with samples and filled into a

standardized metadata sheet (samples without affiliated information were not accepted into the database).

Upon arrival, DNA and RNA was extracted from the samples and used for full length 16S rRNA sequencing using a novel high-throughput, primer-free method (Karst et al., 2018). In addition to this, DNA was extracted from two subsamples from all plants, and used for 16S rRNA amplicon sequencing, the results of which were provided to the plant operators (data not included here).

The full-length 16S rRNA gene sequences that resulted from this novel sequencing procedure of RNA and DNA, were not clustered into OTUs - instead the database was constructed using the unique sequences, also called exact sequence variants (ESVs) of the microbial community. This will provide a database that represents the true microbiota. The taxonomy of the database is based on the SILVA database and its new releases, in combination with the existing MiDAS taxonomy, and will continuously be updated. The MiDAS taxonomy is carried over to the global database, since this manually curated taxonomy includes annotations for many taxa that would otherwise not be identified with publically available taxonomies, but are abundant and important genera in the ecosystems. The new taxonomy will create unique IDs for all ESVs and act as placeholders until valid names appear with time.

The end result should be a highly curated ecosystem-specific reference database that will be useful for everyone working in the field. It promises to provide unique identification tags, and thus a universal language, for all future analyses of anaerobic digesters, and with time to link function to specific species, e.g. via updated versions of the MiDAS field guide.

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