

## DATA NOTE

# The metagenome and metatranscriptome of a biogas-producing microbial community from an agricultural production-scale biogas plant

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## Abstract

**Background:** The production of biogas takes place under anaerobic conditions and involves microbial decomposition of organic matter, with most participating microbes still considered unknown and non-cultivable. Accordingly, metagenome sequencing is currently the only possibility to obtain insights into community composition and the genetic repertoire.

**Findings:** Here, we report the first deeply sequenced metagenome and metatranscriptome of a complex biogas-producing microbial community from an agricultural production-scale biogas plant. We assembled the metagenome and reconstructed most genes involved in the methane metabolism, a key pathway involving methanogenesis populated by low-abundance archaea. This exemplary result indicates sufficient sequencing coverage for most downstream analyses.

**Conclusions:** Sequenced at least one order of magnitude deeper than previous studies, our metagenome data will enable novel insights into community composition and the genetic potential of important community members. Moreover, mapping of transcripts to reconstructed genome sequences will enable the identification of active metabolic pathways in target organisms.

**Keywords:** Biogas; Metagenome; Metatranscriptome; Sequencing; Assembly

## Data description

### Background

Production of biogas by means of anaerobic digestion of biomass is becoming increasingly important as biogas is regarded a clean, renewable and environmentally compatible energy source [1]. Moreover, generation of energy from biogas relies on a balanced carbon dioxide cycle.

The process of biogas production takes place under anaerobic conditions and involves microbial decomposition of organic matter, yielding methane as the main final product of the fermentation process. Complex consortia of microorganisms are responsible for biomass decomposition and biogas production. The majority of the participating microbes are still unknown, as is their influence on reactor performance. Since most of the organisms within biogas communities are non-cultivable by today's conventional microbiological techniques, sequencing of metagenomic total community DNA is currently the only way to obtain unbiased insights into community composition and the genetic potential of key community members.

Here, we report the first deeply sequenced metagenome of an agricultural production-scale biogas plant on the Illumina platform [2]. We sequenced  $27.3\times$  and  $19.3\times$  deeper, respectively, than previous studies relying on 454 [3] or SOLiD [4] sequencing. Metatranscriptomic sequencing of total community RNA complements the metagenome. Combined, these data will enable a deeper exploration of the biogas-producing microbial community, with the objective to develop rational strategies for process optimization.

#### Digester management and process characterization

The biogas plant, located in North Rhine Westphalia, Germany, features a mesophilic continuous wet fermentation technology and was designed for a capacity of  $537\text{ kW}_{el}$  combined heat and power (CHP) generation. The process comprises three digesters: a primary and secondary digester, where the main proportion of biogas is produced, and a storage tank, where the digestate is fermented thereafter.

The primary digester is fed hourly with a mixture of 72 % maize silage and 28 % liquid pig manure. The biogas and methane yields at the time of sampling were at 810.5 and 417.8 liters per kg organic dry matter ( $l/kg\text{ oDM}$ ), respectively. After a theoretical retention time of 55 days, the digestate is stored in the closed, non-heated final storage tank. Further metadata are summarized in Table 1.

#### Sampling and nucleic acid isolation

Samples from the primary digester of the aforementioned biogas plant were taken in November 2010. Prior to the sampling process, approximately 15 L of the fermenter substrate were discarded before aliquots of 1 L were transferred into clean gastight sampling vessels and transported directly to the laboratory.

Aliquots of 20 g of the fermentation sample were used for total community DNA preparation as described previously [5]. A random-primed cDNA library was prepared by an external vendor (vertis Biotechnologie AG). Briefly, total RNA was first treated with 5'-P dependent Terminator exonuclease (Epicentre) to enrich for full-length mRNA carrying 5' CAP or triphosphate structures. Then, first-strand cDNA was synthesized using a N6 random primer and M-MLV-RNase H reverse transcriptase, and second-strand cDNA synthesis was performed according to the Gubler-Hoffman protocol.

#### Sequencing and quality control

We sequenced one metatranscriptome and two metagenome shotgun libraries on Illumina's Genome Analyzer IIx system, applying the Paired-End DNA Sample Preparation Kit (Illumina Inc.) as described by the manufacturer and generating  $2\times 161\text{ bp}$  paired-end reads. On Illumina's MiSeq system, we sequenced three further metagenome shotgun libraries, applying the Nextera DNA Sample Preparation Kit (Illumina Inc.) as described by the manufacturer and generating  $2\times 155\text{ bp}$  paired-end reads. Our sequencing efforts, yielding 35 gigabases in total, are specified in Table 2.

We then used Trimmomatic [6], version 0.32, for adapter removal and moderate quality trimming. After adapter clipping, using Trimmomatic's *Truseq2-PE* and *Nextera-PE* templates, we removed leading and trailing ambiguous or low quality bases (below Phred quality scores of 3). Table 3 summarizes the effect on sequencing depth, more than 25 gigabases of filtered sequence data passed quality control.

### Metagenome assembly and quality assessment

We assembled the metagenome with Ray Meta [7], version 2.3.1, using a  $k$ -mer size of 31 and a minimum contig length of 1,000 bp. This resulted in a total assembly size of approximately 228 megabases in 54,489 contigs, with an N50 value of 9,796 bp. Table 4 summarizes our results.

Mapping,  
Picard-  
tools

We aligned the post-QC sequencing reads to the assembled contigs with bowtie2 [8], version 2.2.4, and used samtools [9], version 1.1, to convert SAM to BAM and thereafter sort the alignment file.

### Gene prediction and annotation

We then used MetaProdigal [10], version 2.6.1, to predict 250,596 protein-coding genes on the assembled contigs. Table 4 also includes these results.

TODO

We blasted all predicted genes against the KEGG database [11], release 72.0, using Protein-Protein BLAST [12], version 2.2.29+. Of the 250,596 predicted genes, 191,766 had a match in the KEGG database, using an Evalue cutoff of  $10^{-6}$ . We determined the KEGG Orthology (KO) for each gene by mapping the top-scoring BLAST hit to its orthologous gene in KEGG, resulting in  $xxx$  genes with an assigned KEGG Orthology.

### Relating the metagenome and the metatranscriptome

We counted aligned reads in predicted genes with BEDTools, version 2.22.0, [13]. Figure 2 shows metagenomic vs. metatranscriptomic coverage in RPKM units.

TODO

## Availability

### Data accession

The datasets supporting the results of this article are available in the European Nucleotide Archive (ENA) under study accession PRJEB8813. Intermediate results for the review process are deposited in the project's GitHub repository [14].

Data needs to be submitted to GigaDB (everything).

### Reproducibility

The complete workflow is organized in a single GNU Makefile and available on GitHub [14]. Starting from the raw read files, available from SRA and/or GigaDB, all data and results can be reproduced by a simple invocation of *make*. Excluding the KEGG analysis, which relies on a commercial license of the KEGG database, all steps are performed using free and open-source software. To further support reproducibility, all tools and dependencies are available in a single Docker container implementing the bioboxes assembly interface, version 0.8, from DockerHub.

TODO

## Discussion

### Potential use cases.

TODO

Metagenomic and metatranscriptomic profiling of the biogas-producing microbial community. Highlight, that methane metabolism pathway is widely covered, but still room for improvement, i.e. sequence deeper. Possibly mention new data generated within the CSP? Tricky to phrase it without trashing this data set.

Identification of metaproteomic data out there (cite Vera, in preparation, and Magdeburg - Fabian).

Ultimate goal: process optimization by biological insights.

#### Competing interests

The authors declare that they have no competing interests.

#### Author's contributions

AB conceived and performed all bioinformatic analyses and wrote the paper. IM investigated all metadata and drafted part of the data description. PB implemented the accompanying Docker container. FE sampled stuff. AW and AA sequenced stuff. AP provided funding. ASch revised the paper. ASch conceived of many of the analyses and revised the paper. ASch and ASch jointly directed the project. All authors read and approved the final manuscript.

**FIXME: Phrasing of middle authors' contributions**

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**ASch: Biogas Marker, Biogas Core**

**Acknowledge Stadtwerke?**

#### Author details

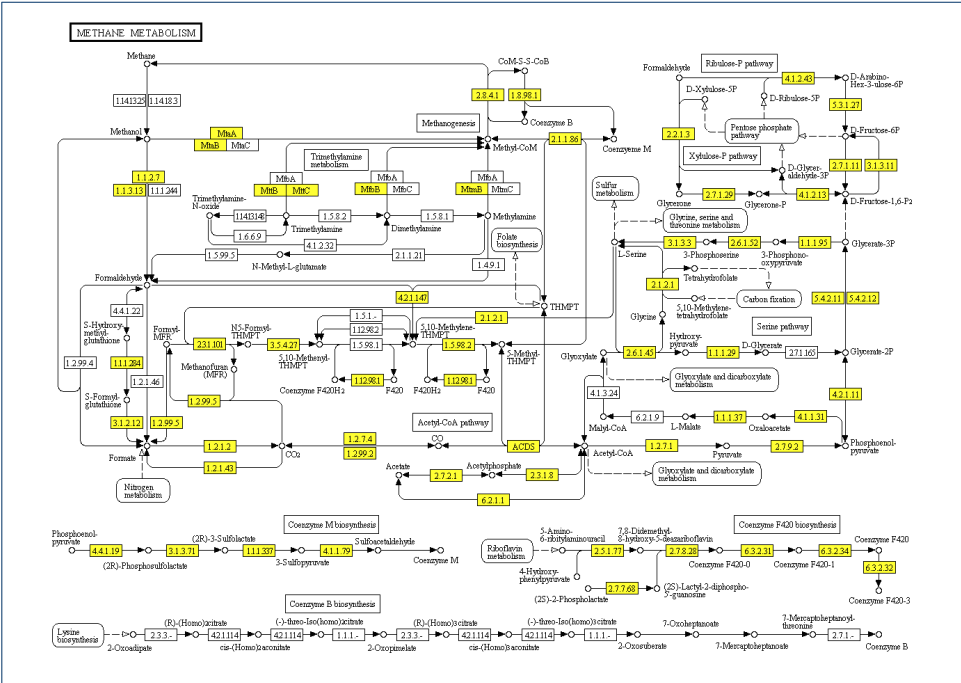
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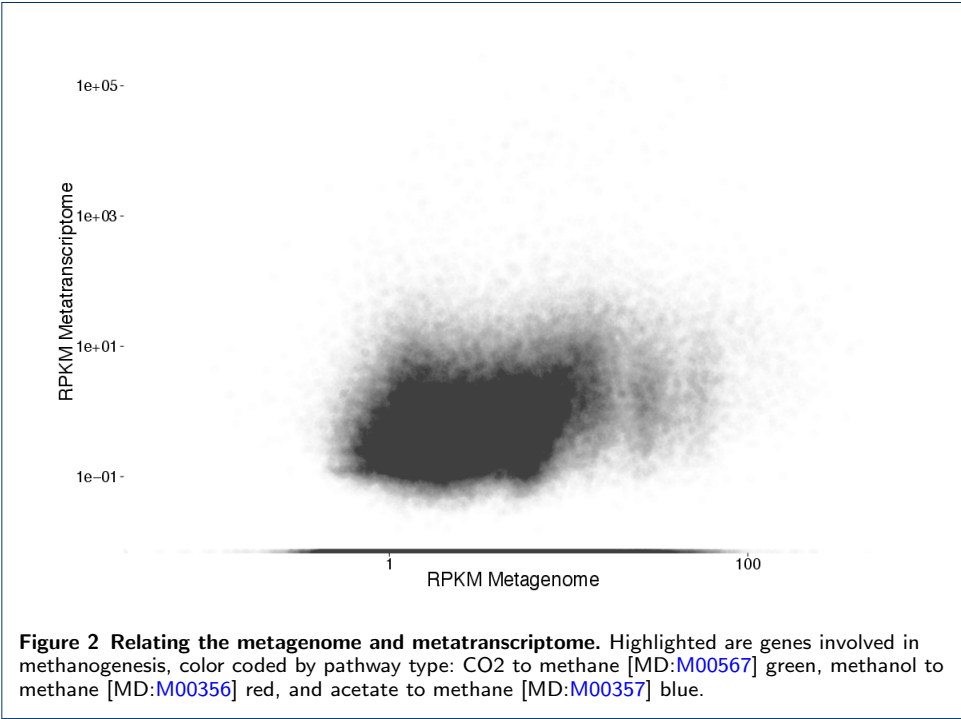
#### Figures

#### Tables



**Figure 1 Methane metabolism pathway analysis.** Genes reconstructed in our assembly, that are involved in the methane metabolism [PATH:map00680], are highlighted in yellow. Base pathway image copyrighted by Kanehisa Laboratories.

Nicer (?) alternative: 2 colors, metagenome and metatranscriptome!



**Figure 2 Relating the metagenome and metatranscriptome.** Highlighted are genes involved in methanogenesis, color coded by pathway type: CO<sub>2</sub> to methane [MD:M00567] green, methanol to methane [MD:M00356] red, and acetate to methane [MD:M00357] blue.

**Table 1** Characteristics of the studied biogas plant. Primary digester, sampling date: Nov 15, 2010.

Process parameter	Sample
Net volume	2041 m <sup>3</sup>
Dimensions	6.4 m high, diameter of 21 m
Electrical capacity	537 kW <sub>el</sub>
pH	7.83
Temperature	40 °C
Conductivity	22.10 mS/cm
Volatile organic acids (VOA)	5327 mg/l
Total inorganic carbon (TIC)	14397 mg/l
VOA/TIC	0.37
Ammoniacal nitrogen	2.93 g/l
Acetic acid	863 mg/l
Propionic acid	76 mg/l
Fed substrates	72 % maize silage, 28 % pig manure
Organic load	4.0 kg oDM m <sup>-3</sup> d <sup>-1</sup>
Retention time	55 d
Biogas yield	810.5 l/kg oDM
Methane yield	417.8 l/kg oDM

**Table 2** Overview of the different sequencing libraries.

Accession	Library name	Library type	Insert size <sup>1</sup>	Cycles	Reads	Bases
ERS697694	GAllx, Lane 6	RNA, TruSeq	202 ± 49	2 × 161	78,752,308	12,679,121,588
ERS697688	GAllx, Lane 7	DNA, TruSeq	157 ± 19	2 × 161	54,630,090	8,795,444,490
ERS697689	GAllx, Lane 8	DNA, TruSeq	298 ± 32	2 × 161	74,547,252	12,002,107,572
ERS697690	MiSeq, Run 1.1	DNA, Nextera	173 ± 53	2 × 155	4,915,698	761,933,190
ERS697691	MiSeq, Run 1.2	DNA, Nextera <sup>2</sup>	522 ± 88	2 × 155	1,927,244	298,722,820
ERS697692	MiSeq, Run 2.1	DNA, Nextera	249 ± 30	2 × 155	3,840,850	573,901,713
ERS697693	MiSeq, Run 2.2	DNA, Nextera <sup>2</sup>	525 ± 90	2 × 155	4,114,304	614,787,564

<sup>1</sup>Fragment sizes determined by Picardtools. <sup>2</sup>This Nextera library was sequenced twice.

**Table 3** Metagenomic and metatranscriptomic sequencing.

Library type	Reads, raw	post-QC	Bases, raw	post-QC
Metagenome (total)	143,975,438	137,365,053	23,046,897,349	17,267,320,221
Metatranscriptome	78,752,308	73,165,986	12,679,121,588	8,455,809,264

**Table 4** Metagenome assembly statistics, minimum contig size of 1,000 bp.

Assembly metric	Our assembly
Total size	228,382,457 bp
Number of contigs	54,489
N50 value	9,796 bp
Largest contig	333,979 bp
Predicted genes	250,596
of these, full-length	172,372 (69 %)
Match in KEGG Genes (10)	241,153
Match in KEGG Genes (1e-3)	200,214
Match in KEGG Genes (1e-6)	191,766
Match in KEGG Genes (1e-9)	184,251
of these, assigned KO	xxx,xxx

KOs to be added asap, see above.