Deeply sequenced 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. Most of the participating microbes are still unknown and non-cultivable. Accordingly, shotgun metagenome sequencing currently is the method of choice to obtain insights into community composition and the genetic repertoire.

Findings: Here, we report on the deeply sequenced metagenome and metatranscriptome of a complex biogas-producing microbial community from an agricultural production-scale biogas plant. We assembled the metagenome and, as an example application, show that we reconstructed most genes involved in the methane metabolism, a key pathway involving methanogenesis performed by methanogenic Archaea. This result indicates that there is sufficient sequencing coverage for most downstream analyses.

Conclusions: Sequenced at least one order of magnitude deeper than previous studies, our metagenome data will enable new 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, Anaerobic digestion, Wet fermentation, Methanogenesis, Metagenomics, Metatranscriptomics, Sequencing, Assembly

Data description

Background

Production of biogas by 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.

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. Because most of the organisms in biogas communities are non-cultivable by today’s conventional microbiological techniques, sequencing of metagenomic total community DNA currently is the best way to obtain unbiased insights into community composition and the metabolic potential of key community members.

Here, we describe the deeply sequenced metagenome and metatranscriptome of an agricultural production-scale biogas plant on the Illumina platform [2].
sequenced the metagenome 27X and 19X deeper, respectively, than previous studies applying 454 or SOLiD sequencing [3, 4], which focused primarily on community composition.

Metatranscriptomic sequencing of total community RNA, 230X deeper than previously reported [5], complements the metagenome. Combined, these data will enable a deeper exploration of the biogas-producing microbial community, with the objective of developing 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 characterized recently [6]. It was designed for a capacity of 537 kW\textsubscript{e} 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 oDM), respectively. After a retention time of 55 days, the digestate is stored in the closed, non-heated final storage tank. Further information is summarized in Table 1.

**Sampling and library construction**
Samples from the primary digester of the biogas plant were taken in November 2010. Before 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.

For the metagenome, aliquots of 20 g of the fermentation sample were used for total community DNA preparation as described previously [7].

For the metatranscriptome, 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 [8].

### Table 1
Characteristics of the studied biogas plant’s primary digester at the sampling date 15 November 2010

| Process parameter | Sample |
|-------------------|--------|
| Net volume        | 2,041 m\textsuperscript{3} |
| Dimensions        | 6.4 m high, diameter of 21 m |
| Electrical capacity | 537 kW\textsubscript{e} |
| pH                | 7.83 |
| Temperature       | 40 °C |
| Conductivity      | 22.10 mS/cm |
| Volatile organic acids (VOA) | 5,327 mg/l |
| Total inorganic carbon (TIC) | 14,397 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 % liquid pig manure |
| Organic load      | 4.0 kg oDM m\textsuperscript{−3} d\textsuperscript{−1} |
| Retention time    | 55 days |
| 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* | Cycles | Reads | Bases |
|-----------|--------------|--------------|--------------|--------|-------|-------|
| ERS697694 | GAIx, Lane 6 | RNA, TruSeq  | 202 ± 49     | 2 × 161 | 78,752,308 | 12,679,121,588 |
| ERS697688 | GAIx, Lane 7 | DNA, TruSeq  | 157 ± 19     | 2 × 161 | 54,630,090  | 8,795,444,490  |
| ERS697689 | GAIx, Lane 8 | DNA, TruSeq  | 298 ± 32     | 2 × 161 | 74,547,252  | 12,002,107,572 |
| ERS697690 | MiSeq, Run A1† | DNA, Nextera | 173 ± 53     | 2 × 155 | 4,915,698   | 761,933,190   |
| ERS697691 | MiSeq, Run A2‡ | DNA, Nextera‡ | 522 ± 88     | 2 × 155 | 1,927,244   | 298,722,820   |
| ERS697692 | MiSeq, Run B1† | DNA, Nextera | 249 ± 30     | 2 × 155 | 3,840,850   | 573,901,713   |
| ERS697693 | MiSeq, Run B2‡ | DNA, Nextera‡ | 525 ± 90     | 2 × 155 | 4,114,304   | 614,787,564   |

* Insert sizes determined with Picard tools. † Partial runs. ‡ This Nextera library was sequenced twice.
Analyzer IIX system, applying the Paired-End DNA Sample Preparation Kit (Illumina Inc.) as described by the manufacturer to generate $2 \times 161$ 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 to generate $2 \times 155$ bp paired-end reads. Our sequencing efforts, yielding 35 Gbp in total, are summarized in Table 2.

### Metagenome assembly
Prior to assembly, we used Trimmomatic [9] version 0.33 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 Gbp of sequence data passed quality control.

We assembled the metagenome with Ray Meta [10] version 2.3.1, trying a range of $k$-mer sizes from 21 to 61 in steps of 10. To estimate the inclusivity of the set of assemblies, we aligned the post-quality-control sequencing reads to the assembled contigs with bowtie2 [11] version 2.2.4. We then used samtools [12] version 1.1 to convert SAM to BAM, sort the alignment file and calculate the mapping statistics. Given the total assembly size and contiguity and the percentage of mapped back metagenomic reads, we selected the assembly produced with a $k$-mer size of 31. Here, we assembled approximately 228 Mbp in 54,489 contigs greater than 1,000 bp, with an N50 value of 9,796 bp. 77 % (79 %) of metagenomic (metatranscriptomic) reads mapped back to this assembly.

### Gene prediction and annotation
We used MetaProdigal [13] version 2.6.1 to predict 250,596 protein-coding genes on the assembled contigs. We compared the protein sequences of all predicted genes with the KEGG database [14] release 72.0 using Protein-Protein BLAST [15] version 2.2.29+. Of the 250,596 predicted genes, 191,766 (76.5 %) had a match in the KEGG database using an E-value 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 109,501 genes with an assigned KO. Table 4 summarizes our results.

### Relating the metagenome and the metatranscriptome
To illustrate potential use cases, we first counted the number of reads within genes using BEDTools [16] version 2.22.0. The metagenomic and metatranscriptomic coverage of the methane metabolism pathway is shown in Fig. 1. This shows that we have assembled the majority of genes involved in the methane metabolism from our metagenomic data, with accompanying metatranscriptomic data suggesting active gene expression for many.

For a second example, we calculated the reads per kilobase per million mapped reads (RPKM) for each gene as a crude measure for abundance (metagenome) or expression (metatranscriptome). Figure 2 relates the two and highlights all genes assigned to any of the three known types of methanogenic pathways. Hydrogenotrophic methanogenesis, that is, the reduction of $\text{CO}_2$ with hydrogen, appears to be highly expressed in the reactor analyzed, which is in agreement with results obtained by 454 amplicon and metatranscriptome sequencing [5].

### Table 3 Metagenomic and metatranscriptomic sequencing and quality control (QC)

| Library type          | Reads, raw | Reads, post-QC | Bases, raw | Bases, 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   |
| Mapped DNA reads       | 105,461,596 (77 %)|
| Mapped RNA reads       | 57,436,058 (79 %)|
| Predicted genes        | 250,596      |
| Of these, full-length  | 172,372 (69 %)|
| Match in KEGG Genes    | 191,766      |
| Of these, assigned KO  | 109,501      |
| Of these, in KEGG pathways | 61,100    |
Fig. 1 Methane metabolism pathway analysis. Genes reconstructed in our assembly that are involved in the methane metabolism [PATH:ko00680, (http://www.genome.jp/kegg-bin/show_pathway?ko00680)], are highlighted: genes with only metagenomic support are in yellow and genes with metatranscriptomic support as well, suggesting active gene expression, are in orange. Methane is synthesized from CO$_2$, methanol or acetate. KEGG pathway map courtesy of Kanehisa Laboratories

Discussion

We report extensive metagenomic and metatranscriptomic profiling of the microbial community from a production-scale biogas plant. Given the unprecedented sequencing depth and established bioinformatics, our data will be of great interest to the biogas research community in general and microbiologists working on biogas-producing microbial communities in particular.

In a first applied study, our metagenome assembly was used to improve the characterization of a metaproteome generated from biogas plant fermentation samples and to investigate the metabolic activity of the microbial community [17].

By sharing our data, we want to actively encourage its reuse. This will hopefully result in novel biological and biotechnological insights, eventually enabling a more efficient biogas production.

Availability of supporting data

Data accession

Raw sequencing data are available in the European Nucleotide Archive (ENA) under study accession PRJEB8813 (http://www.ebi.ac.uk/ena/data/view/PRJEB8813). The datasets supporting the results of this article are available in GigaScience’s GigaDB [2].

Reproducibility

The complete workflow is organized in a single GNU Makefile and available on GitHub [18]. All data and results can be reproduced by a simple invocation of make. To further support reproducibility, we bundled all tools and dependencies into one Docker container available on DockerHub [19]. docker run executes the aforementioned Makefile inside the container. Reproduction
requires roughly 89 GiB memory and 83 GiB storage, and takes less than 24 hours on 32 CPU cores.

Excluding the KEGG analysis, which relies on a commercial license of the KEGG database, all steps are performed using free and open-source software.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
AB conceived and performed all bioinformatic analyses and wrote the manuscript. IM investigated all metadata and drafted part of the data description. PB implemented the accompanying Docker container. FE collected the study material. ASch and AScz jointly directed the project and extensively revised the manuscript. All authors read and approved the final manuscript.

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