Optimisation of 16S rDNA amplicon sequencing protocols for microbial community profiling of anaerobic digesters

Kirkegaard, Rasmus Hansen; McIlroy, Simon Jon; Larsen, Poul; Karst, Søren Michael; Albertsen, Mads; Nielsen, Per Halkjær

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Optimisation of 16S rDNA amplicon sequencing protocols for microbial community profiling of anaerobic digesters

Rasmus Kirkegaard, Simon McIlRoy, Poul Larsen, Søren M. Karst, Mads Albertsen, and Per H. Nielsen
Center for Microbial Communities, Aalborg University, Denmark

Introduction

To understand the ecology network in anaerobic digesters it is necessary to produce a representative overview of the microbial community. In this study we develop a method for reliable and reproducible identification and quantification of microorganisms involved in biogas production. We test the effect of changing the parameters in a DNA extraction dependent approach to community profiling.

Methods

Extraction

Reactor temperature

Bead beating

FastDNA® SPIN kit for Soil

Input (µL)

Optimal

Standard

Intensity (mJ)

16S rRNA gene

Primers

Sampling

Extraction

Sample prep

Sequencing

Bioinformatic

Validation

PCR independent assessment using Illumina TruSeq shotgun sequencing

Results

Class level overview of the bacterial population

<table>
<thead>
<tr>
<th>Class level</th>
<th>Mean frequency (V4-V9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorobia</td>
<td>9.1</td>
</tr>
<tr>
<td>Bacteroida</td>
<td>10.7</td>
</tr>
<tr>
<td>Acidobacteria</td>
<td>8.7</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>8.8</td>
</tr>
<tr>
<td>Planctomycetes</td>
<td>5.6</td>
</tr>
<tr>
<td>Alphaproteobacteria</td>
<td>2.2</td>
</tr>
<tr>
<td>Betaproteobacteria</td>
<td>1.5</td>
</tr>
<tr>
<td>Desulfobacterota</td>
<td>1.5</td>
</tr>
<tr>
<td>Thermotogae</td>
<td>1.0</td>
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<tr>
<td>Archaeanota</td>
<td>1.0</td>
</tr>
<tr>
<td>Planctomycetes</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Effect of bead beating on DNA integrity. At high bead beating durations the DNA is fragmented.

Effect of primer set on the observed community structure. DNA extractions were done with FastDNA®, V4-V9 primer set and high bacterial and archaeal diversity was observed. The V3-V4 (Sundberg et al. 2013) primer set seems promising for capturing the overall community composition of both bacteria and archaea.

Conclusions

- PCR independent validation is needed when conducting amplicon based studies!
- Four times the standard bead beating is recommended (160 s) in order to capture the microorganisms with relatively tough cell walls.
- The Sundberg et al. (2013) primer set seems promising for capturing the overall community composition of both bacteria and archaea.
- Every step of the protocol introduces variance, particularly the DNA extraction. However, the workflow gives good reproducibility.

Fluorescence in situ hybridisation with archaea specific probes maps the archaea. Methanolinea is commonly observed in high abundance in thermophilic anaerobic digester, typically forming microcolonies, while Methanomicrobiia are often present in abundance in mesophilic reactors as short filaments. Ammonia oxidising archaea and methanogenic digester samples is consistent with these observations.

www.cmc.aau.dk  rhk@bio.aau.dk