Meta-analysis of amplicon sequencing datasets to understand the ecology of Chloroflexi in methanogenic full scale reactors

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Abstract: The phylum Chloroflexi is frequently found in high abundance in methanogenic reactors but their role is still unclear as most of them remain uncultured and understudied. The present meta-analysis focused on the phylum Chloroflexi in full-scale methanogenic reactors. We combined high-throughput sequencing of 16S ribosomal RNA (rRNA) gene datasets from 25 full-scale methanogenic reactors fed with different industrial wastewaters studied worldwide. 16S rRNA gene amplicon sequencing revealed diverse Chloroflexi populations, dominated by the class Anaerolineae. Other less abundant classes were Dehalococcoidia and SJA-15. According to the phylogenetic analysis the predominant Chloroflexi OTUs form a cluster within the Anaerolineae class with no cultured representatives. While members of the Anaerolineae class were predominant, their diversity is still poorly described in anaerobic reactors.

Keywords: Chloroflexi; metagenomics, full scale methanogenic reactor.

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Introduction

Anaerobic digestion is composed by a series of biological reactions performed by microorganisms which can be used as a form of biological treatment of organic waste and wastewater and can produce energy in the form of methane.

At least four microbial trophic groups contribute to the organic waste degradation forming CH4 and CO2 as final products: hydrolytic bacteria, fermenting bacteria, syntrophic bacteria and methanogenic archaea (Amani et al. 2010). Different configurations of methanogenic reactors are currently used for the treatment of wastewater such as Up-flow anaerobic sludge blanket (UASB), Internal Circulation (IC) and Expanded Granular Sludge Bed (EGSB) (Jeison et al., 1999, Lettinga 1995; Pereboom et al., 1994). In some cases, sludge granulation is a necessary phenomenon in reactors as it provides appropriate settling ability, high biomass retention time and increases the resistance to high organic loading rates and toxic shocks (Speece 1996). Sludge granulation favors the syntrophic process between the fermentative bacteria and methanogenic archaea (Ariesyady et al. 2007).

Chloroflexi has been reported as one of the most predominant phyla present in anaerobic wastewater treatment systems and has even been defined as part of the core group (Shu et al. 2015). Within the Chloroflexi phylum, members of the Anaerolineae class are predominant in anaerobic reactors but their role in these systems has not been completely addressed (Bovio et al., 2018; Yamada and Sekiguchi 2009). This is mainly due to the difficulties to cultivate and isolate members from this class. The cultivated members are slow growing fermenting bacteria with filamentous morphology. It has been postulated that they are involved in granule formation
in UASB reactors due to their filamentous morphology (Sekiguchi et al. 2001). But, in a previous study on the performance of an UASB reactor installed in our country, the overgrowth of filamentous Chloroflexi was related to an unusual anaerobic bulking process that caused sedimentation problems (Borzacconi et al. 2008). The conditions favoring the overgrowth of these bacteria remain unknown. Increasing the knowledge about this group is essential to control their growth, sustain granule formation and prevent bulking episodes.

In order to contribute to the understanding of the ecology of Chloroflexi in methanogenic reactors, we conducted a meta-analysis of publicly available microbial datasets utilizing high-throughput sequencing (454, Ion Torrent and Illumina platforms) in 25 full scale methanogenic reactors treating different industrial wastewaters to investigate their taxonomic composition, diversity.

Material and Methods
Assembly of data sets. This meta-analysis includes sequences from published papers (detailed in Table 1), all of which used bacterial 16S rRNA gene sequencing to survey the microbial community in anaerobic reactors. Eight studies were selected that comprised 25 full-scale methanogenic reactors in either one of the following configurations (Table 1): UASB, IC, EGSB, UBF or AnaEG. PCR-generated amplicons from bacterial 16S rRNA genes were sequenced using a diversity of sequencing platforms across the studies (Illumina, Ion Torrent and Pyrosequencing) and primers that targeted different regions of the 16S rRNA gene of Bacteria (Table 1).

The data sets were retrieved from the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI, USA) or raw data was requested from the authors directly. Raw sequences from each study were processed and quality filtered using QIIME version 1.9.1 (Caporaso et al. 2010). Chimeric sequences were remove with UCHIME from USEARCH v.6.0.203 (Edgar 2010).

Clustering and taxonomic assignments. The sequences from all studies were grouped into ‘species-level’ operational taxonomic units (OTUs) by using Silva database release 132. Sequences classified into Chloroflexi phylum were retained for further analysis.

Table 1 Studies included in these meta-analyses and datasets information

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of reactor</th>
<th>Sample name</th>
<th>Water source</th>
<th>Regional location</th>
<th>platform</th>
<th>Primers</th>
<th>Raw data sources</th>
<th>Demultiplex reads</th>
<th>Relative abundance</th>
</tr>
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<tbody>
<tr>
<td>Bovio et al., 2017</td>
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Results and Conclusions
Sequence analysis at phylum level showed that the phylum Chloroflexi had a relative abundance between 2 and 35% in the reactors studied (Figure 1). Other abundant phyla were Bacteroidetes, Firmicutes, Proteobacteria and Synergistetes. Chloroflexi as well as the previously mentioned groups formed the core group of bacteria in all reactors.
The analysis of sequences belonging to the *Chloroflexi* phylum at class level showed that *Anaerolinea* was the predominant group presenting a relative abundance between 32 and 99% (Figure 2). Other abundant classes were *Dehalococcoidia* and SJA-15.

A phylogenetic tree was constructed to determine the phylogenetic position of the OTU sequences belonging to the *Chloroflexi* phylum (Figure 3).

**Figure 1** Taxonomic distribution according to the 16S rRNA gene amplicon sequencing analysis at phylum level.

**Figure 2** Taxonomic distribution according to the 16S rRNA gene amplicon sequencing analysis. The results were presented at class level.

**Figure 3** Phylogenetic distance tree of the bacterial phylum *Chloroflexi* constructed based on 16S rRNA gene sequencing. Only OTUs higher than 10% was used in the analysis.
For this analysis, OTUs with a relative abundance greater than 10% and sequences of culture representatives were selected. The majority of the OTUs formed a branch without any cultured representatives within the *Anaerolinea* class. The rest of the OTUs grouped with sequences of the *Ardenticaenia, Caldilinea*, and *Dehalococcoides* classes.

In conclusion, the phylum *Chloroflexi* was abundant in all studied methanogenic reactors. Members of the *Anaerolineae* class were predominant and were not closely related to any species from cultured representatives indicating that the diversity is still poorly described in methanogenic reactors.

**References**


