

# Metaproteomics of anaerobic microbial communities degrading long-chain fatty acids

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## Abstract

The anaerobic conversion of long-chain fatty acids (LCFA), and specifically the difference between the degradation of unsaturated- and saturated-LCFA, is not fully understood. In this work, syntrophic degradation of stearate (C18:0) and oleate (C18:1) was studied. A comparative metaproteomics approach, in which proteins were analyzed by LC-MS/MS, was combined with 16S rRNA gene pyrosequencing. Saturated- and unsaturated-LCFA were converted to methane by the anaerobic consortia. 16S rRNA gene pyrosequencing revealed differences in the microbial composition of sludges incubated with stearate and oleate, separately. Abundance of microorganisms within *Deltaproteobacteria* and within *Synergistia* taxa was higher in stearate and oleate incubations, respectively. *Methanosaeta* was the most abundant methanogen in both conditions. Metaproteomics results were similar and comparable distributions of COG functional categories were found for both samples. Archaeal proteomes were much better identified than bacterial ones, with five times more proteins retrieved. Most of the proteins identified belong to *Methanosaeta concilli* and *Syntrophobacter fumaroxidans*, two organisms that have their genome sequenced. *Syntrophobacter* belongs to *Deltaproteobacteria*, however this group was not dominant in oleate incubation as determined by pyrosequencing results. Studying metaproteomes of complex microbial communities is still a big challenge especially because most of the genomes are not sequenced which hinders protein identification.

## Keywords

Long-chain fatty acids; Metaproteomics; 16S rRNA gene; Methanogenic communities.

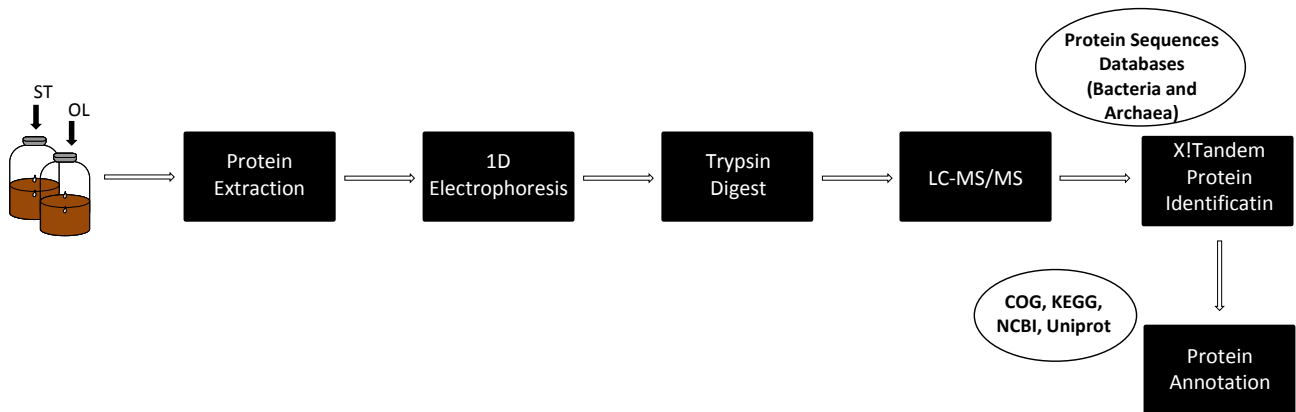
## INTRODUCTION

Long-chain fatty acids (LCFA) can be anaerobically converted to CH<sub>4</sub> and CO<sub>2</sub>. However, the mechanisms and microbiology underlying the conversion of saturated- and unsaturated-LCFA are not fully understood. Previous studies suggest that distinct groups of microorganisms might be involved on the degradation of saturated- and unsaturated- LCFA (Sousa et al. 2007, Sousa et al. 2009). However, these studies were based on the analysis of 16S rRNA gene sequences, which provides useful information on microbial composition but fails in directly linking microorganisms to their function and activity. In contrast, by analyzing the metaproteome of a community it is possible to identify the proteins that are expressed by the microorganisms and, in this way, infer about specific functions present in the ecosystem. Metaproteomics has recently been applied to environmental samples (e.g. Kolmeder et al. 2012, Wilmes et al. 2008). In this work, we used a comparative metaproteomics approach to study the proteins expressed by methanogenic anaerobic mixed cultures degrading both saturated- (stearate) and unsaturated-LCFA (oleate).

## MATERIAL & METHODS

Anaerobic sludge was incubated in batch vials with 2 mM stearate - C18:0 (ST) or oleate - C18:1 (OL) separately. LCFA, VFA and methane were monitored during LCFA conversion. Samples for protein extraction were withdrawn after 5 days of incubation, subsequent to the onset of LCFA degradation. Proteins were extracted from sludge samples as described by Wilmes and Bond (2004)

and processed as described by Ibrahim et al. (2007) (Figure 1). Tandem mass spectra were matched towards bacterial and archaeal protein databases (UniprotKB). Scaffold software was used to group, visualize and validate the identified proteins. Peptide and protein probability thresholds were set to higher than 90% and 95%, respectively, with at least 2 unique peptides identified per protein. Functional annotation of proteins was obtained by scanning protein sequences against the Cluster of Orthologous Groups database (COG database) (Marchler-Bauer et al. 2011). In parallel, sludge samples from incubations OL and ST were the subject of taxonomic characterization. For this propose, DNA was extracted using UltraClean® Soil DNA Isolation Kit (MO BIO Laboratories, Inc.), and 16S rRNA gene was pyrosequenced (using the primer sets 28F/519R and Arch349F/Arch806R for *Bacteria* and *Archaea*, respectively) by Research and Testing Laboratory (Texas, USA). The obtained 16S rRNA gene sequences were analyzed with QIIME software (Caporaso et al 2010).

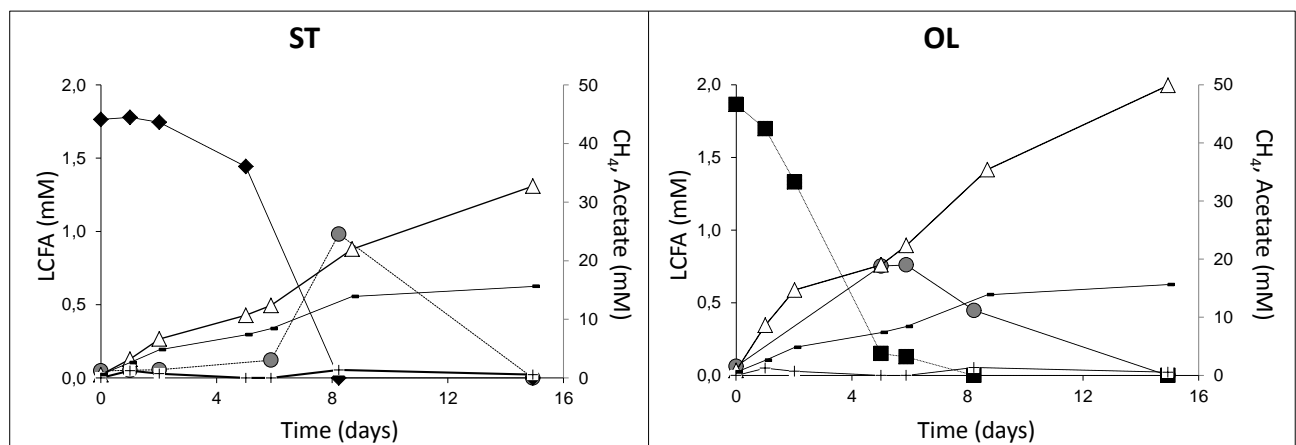


**Figure 1.** Experimental workflow for the functional analysis of metaproteomes from anaerobic sludge samples incubated with LCFA.

## RESULTS AND DISCUSSION

### LCFA degradation and production of VFA and methane

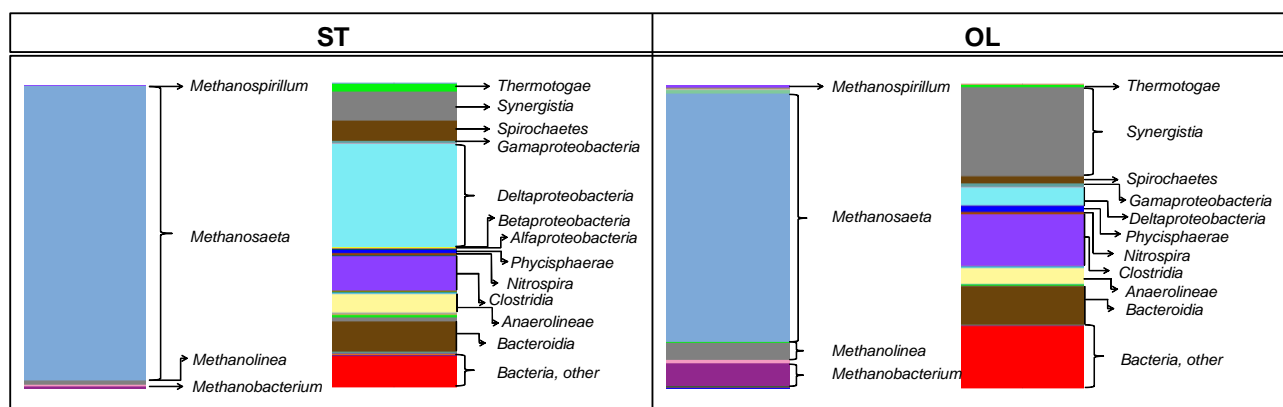
LCFA were successfully converted to methane by the anaerobic consortia (Figure 2). Palmitate was the main intermediate detected during degradation of oleate and stearate. Residual amounts of acetate were also detected (lower than 1.4 mM) in OL and ST incubations.



**Figure 2.** Stearate (◆) and oleate (■) conversion in ST and OL batch assays. Products detected include methane (Δ), acetate (+) and palmitate (●). Methane production in the blank assay (-) in which no LCFA was added.

### Taxonomic characterization of ST and OL microbial communities

Taxonomic composition of ST and OL microbial communities was assessed by tag pyrosequencing of the 16S rRNA genes. Acetoclastic archaea belonging to the *Methanosaeta* genus were the most abundant methanogens, according to pyrosequencing results (Figure 3). Sequences related to the hydrogenotrophic archaeal species *Methanospirillum*, *Methanolinea* and *Methanobacterium* were more abundant in OL than in ST incubation. The same taxonomic groups of Bacteria were identified in both samples, with the exception of alpha- and beta-*Proteobacteria* that apparently are only present in ST incubation. The proportion of most taxonomic groups in the two samples was different with some being more dominant in ST (e.g. delta-*Proteobacteria*) or conversely in OL incubations (e.g. *Synergistia*). A significant percentage of sequences (10% in ST and 20% in OL) could only possibly be classified at the level of the domain *Bacteria*, which suggests that important microorganisms related to LCFA degradation might not be known so far.

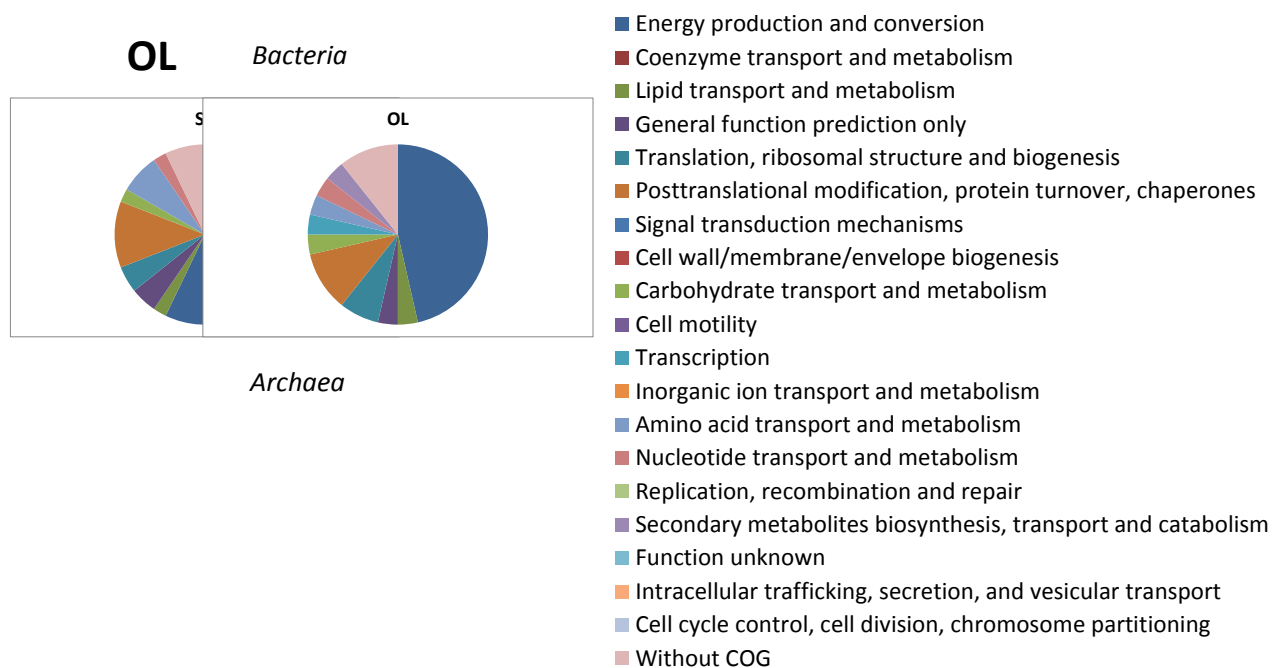


**Figure 3.** Representation of archaeal and bacterial taxonomic groups detected in ST and OL incubations based on pyrosequencing analysis of 16S rRNA genes.

### Functional metaproteomics of LCFA degrading consortia

Metaproteome analysis of an anaerobic consortia incubated with LCFA resulted in the identification of 53 bacterial and 265 archaeal proteins corresponding to 10 and 19 distinct COG categories, respectively (Figure 4). Both archaeal and bacterial proteins identified in OL and ST assays were assigned to similar COGs. A significant part of the proteins identified were classified into COGs: Energy production and conversion, coenzyme transport and metabolism, chaperones, lipid transport and metabolism and amino acid transport and metabolism, among others. Some of the identified proteins could not be assigned to a specific COG (Figure 4).

The level of identification of bacterial proteins was very low, and no proteins directly related to fatty acid or lipid metabolism could be retrieved. This is a consequence of the low amount of genomic information directly obtained from LCFA-degrading bacteria and available in public databases. In contrast, methanogenic proteins are better represented, which can be explained by the taxonomic similarity between methanogens, whose genomes are sequenced and available, and those that are present in ST and OL incubations. Above 80% of archaeal proteins corresponded to *Methanosaeta* proteins (data not shown), the most abundant methanogen in ST and OL incubations according to pyrosequencing results (Figure 3). It is important to increase the number of genome sequences of syntrophic LCFA-degraders in public databases in order to fully explore the metaproteome of LCFA-degrading microbial communities.



**Figure 4.** Comparison of COG functional categories distribution for the metaproteome of anaerobic LCFA-degrading microbial communities. COGs identified for bacterial and archaeal domains are represented separately.

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