Diversity and proteomic characterization of saturated and unsaturated LCFA anaerobic degrading communities

Andreia F. Salvador[1], Ariane Bize[2], M. Madalena Alves[1], Théodore Bouchez[2], Diana Z. Sousa[1]

[1]*IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal*

[2]*Irstea, UR HBAN, 1 rue Pierre-Gilles de Gennes, 92761 Antony Cedex, France*

Long-chain fatty acids (LCFA) are a suitable substrate for methane production. Previous studies suggest possible differences between degradation of saturated and unsaturated-LCFA. In this work, we investigated differences in microbial diversity and in protein expression of anaerobic communities degrading C18 saturated and unsaturated-LCFA.

Anaerobic sludge incubations with stearate (saturated-LCFA (C18:0)) and with oleate (unsaturated-LCFA (18:1)) were done in separate vials. LCFA, volatile fatty acids and methane concentrations were monitored overtime and biomass samples for DNA and protein extraction collected at day 5 of incubation. 16S rRNA gene was pyrosequenced and results compared and analyzed using Qiime software package. In parallel, 16S rRNA gene was analyzed by fingerprint analysis (DGGE, Denaturing Gradient Gel Electrophoresis). Proteins were separated by SDS-PAGE and digested in gel for further LC-MS/MS analysis. Spectra files were matched against model spectra created from each peptide in the UniProtKB protein database by using X! Tandem. Scaffold was used to validate MS/MS based peptide and protein identification and to compare protein expression between samples.

Bacterial and archaeal DGGE profiles showed differences between oleate and stearate incubations (43% similatrity). Bacterial community was rather diverse and distributed among 13 phyla, including *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Proteobacteria*, *Spirochaetes* and *Synergistetes*. Sequences clustering within the class *Deltaproteobacteria* were more abundant in stearate sample, whereas more sequences from *Synergistia* were retrieved from oleate sample. Sequences belonging to genus *Syntrophomonas*, in which some representatives are known to consume LCFA, were present in both incubations although their abundance was less than 1%. Archaeal communities were dominated by *Methanosaeta*-like organisms (96% for stearate and 82% for oleate). *Methanobacterium*, *Methanolinea* and *Methanospirilum* were also present, but more abundantly in oleate incubation.

A total of 205 proteins (120 protein functions) were identified, 83% of which were related to archaeal methane metabolism (Methyl-coenzyme-M reductase, Coenzyme F420-reduncing hydrogenase, coenzyme-B sulfoethylthiotransferase, etc). Proteins related to regulation, protein folding, RNA processing, transport, ATPase activity, nitrogen regulation and stress response were also detected. Most of the proteins were the closest to *Methanosaeta concilii* proteins, but resemblance with proteins from microorganisms within *Methanobacterium*, *Methanospirillum* and *Methanoregula* genera were also identified.

Bacterial proteins related with several functions and metabolic cycles could be identified (*e.g.* glycolysis, TCA cycle, transport, oxidative phosphorilation, fatty-acids metabolism, etc.). Several aldehyde and alcohol dehydrogenases were detected, which could be related to fatty-acid metabolism. Differences in protein expression between oleate and stearate samples could not be correlated with specific protein functions. Proteins with identical function could be retrieved from oleate and stearate samples but deriving from different groups of microorganisms. *Deltaproteobacteria*, *Gamaproteobacteria*, *Bacteroidales* and *Synergistetes* were the predominant groups identified according to protein analysis.

This study reports novel data on the metaproteome of anaerobic LCFA-degrading ecosystems. However, a direct link between specific LCFA degradation patterns and protein expression

could not be established mainly due to the lack of genomic and proteomic information in databases, specifically regarding LCFA degradation by anaerobic microorganisms.

Acknowledgements:
FEDER-COMPETE and FCT for Project FCOMP-01-0124-FEDER-014784 and POPH-QREN and FCT for PhD grant SFRH/BD/48960/2008.