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Functional analysis of syntrophic LCFA-degrading microbial ecosystems

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Introduction and Aim

Long-chain fatty acids (LCFA) are present in lipid rich wastewater and can be converted to methane anaerobically, coupling wastewater treatment to bioenergy production. Differences in the degradation of saturated and unsaturated long-chain fatty acids (LCFA) by anaerobic consortia are not completely understood. Previous studies showed a segregation on the microbial community composition when the same inoculum sludge was incubated with saturated- or with unsaturated-LCFA (Sousa et al., 2007), suggesting differences in their degradation pathways. In order to get more insights on this and aiming linking microbial community structure to function, a comparative shotgun metaproteomics study of a mesophilic anaerobic sludge incubated with saturated- and unsaturated-LCFA was conducted. Additionally, the metaproteome of a defined co-culture of *Syntrophomonas zehnderi* and *Methanobacterium formicicum* growing on saturated- and unsaturated-LCFA to methane was also analyzed.

Methods

Anaerobic digester sludge was incubated separately with 2 mM of palmitate (C16:0), stearate (C18:0) or oleate (C18:1) as the only carbon and energy source. Samples were collected over time for LCFA, volatile fatty acids (VFA) and methane quantification, as well as for DNA and protein extraction. The diversity and similarity between the anaerobic cultures were estimated by automated ribosomal intergenic spacer analysis (ARISA) and microbial composition was assessed by 454-pyrosequencing of 16S rRNA genes following the protocols previously described by Smith et al. (2010). Proteins were extracted as detailed by Wilmes and Bond (2004), separated by SDS-PAGE electrophoresis and digested with trypsin prior to LC-MS/MS analysis. Proteins were identified by using X!Tandem and Scaffold software (Craig and Beavis, 2004; Searle, 2010), and functional annotation was obtained by scanning protein sequences against the Cluster of Orthologous Groups database (COG database) (Marchler-Bauer et al., 2011). The same procedure was used for the identification of proteins expressed by the defined co-culture of *S. zehnderi* and *M. formicium* when growing on 1 mM of oleate or stearate.

Results and Conclusions

Functional analysis of complex microbial communities degrading LCFA

Saturated- and unsaturated-LCFA were converted to methane in approximately 15 days. Proteins identified during conversion of palmitate, stearate and oleate were assigned to the same COGs functional categories. Most abundant categories were related with energy production and conversion, post-translational modification and lipid metabolism. As it was observed in previous studies (Sousa et al., 2007), microbial communities degrading saturated- and unsaturated-LCFA diverged. ARISA fingerprinting profiles

were similar between palmitate and stearate incubation but the same was not verified for oleate incubation. 16S rRNA gene pyrosequencing also revealed a different microbial community composition during stearate and oleate incubations. Microorganisms related to *Methanosaeta concilii* were the most abundant methanogens detected by 16S rRNA gene pyrosequencing and the majority of the proteins identified were most similar to proteins of *Methanosaeta concilli*. Hydrogenothrophic methanogens related to *Methanobacterium*, *Methanolinea* and *Methanospirillum* genera were apparently more abundant and active during oleate degradation. More than 40 % of the bacterial proteins were assigned to syntrophic bacteria, emphasizing the importance of syntrophic relationships in LCFA-degrading environments. Proteins assigned to those of *Syntrophobacter fumaroxidans*, *Pelobacter propionicus* and *Pelotomaculum thermopropionicum* were the most abundant bacterial proteins, although microorganisms closely-related to these bacteria were not detected by 16S rRNA gene 454-pyrosequencing. Metaproteomic studies of complex microbial communities are still a big challenge mostly because the genomes of abundant and active microorganisms are not all sequenced, which difficult proteins identification.

Functional analysis of S. zehnderi co-culture degrading LCFA

S. zehnderi and *M. formicicum* co-culture converted oleate and stearate to methane. The protein pool expressed by *S. zehnderi* and *M. formicicum* was distributed by 9 and 19 different COG functional categories, respectively. Key enzymes involved in the utilization of fatty acids and methane production could be identified. Proteins assigned to coenzyme transport and metabolism functional category, which comprises several proteins related to methanogenesis, counted for 13-17% of the spectra used for *Methanobacterium* proteins identification. Although formate was never detected during LCFA degradation in previous studies (Sousa et al., 2007), formate dehydrogenases were highly expressed by *M. formicicum* and were also expressed by *S. zehnderi*, which suggests that formate might be an important interspecies electron carrier between *S. zehnderi* and *M. formicicum* when converting LCFA to methane.

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