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SYNTROPHIC LCFA- DEGRADING MICROBIAL ECOSYSTEMS

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KEYWORDS

LCFA, anaerobic degradation, bacterial community.

ABSTRACT

Long-chain fatty acids (LCFA) are energy-rich compounds, which are abundantly present in raw and waste materials. Thus, wastewaters that contain LCFA may yield high levels of methane in an anaerobic digestion process. Biogas formation from LCFA-containing wastewater is a sustainable technology that warrants further investigation, specifically in terms of more fundamental microbiological aspects. The aim of this work is to get more insights into the syntrophic microbial communities that degrade LCFA anaerobically. Bacterial shifts of a mesophilic sludge incubated in the presence of palmitic, stearic or oleic acid was estimated by means of automated ribosomal intergenic spacer analysis (ARISA). Slightly differences were observed between the communities incubated with saturated LCFA (palmitic and stearic acids) and the ones of the blank assay. On the other hand, evident changes were found between ARISA profiles of the communities that were incubated with oleic acid and the ones obtained for the blank assay. These results suggest that the microbial communities that degrade saturated fatty acids are very close to each other and different from the ones that degrade unsaturated fatty acids.

INTRODUCTION

Long-chain fatty acids (LCFA) are the main products of lipids hydrolysis and are frequently found in different types of wastewaters, such as the ones from dairy industry, slaughterhouses, wool scouring facilities, and edible oil processing facilities. Treatment of these wastewaters in anaerobic bioreactors may result in the production of large amounts of methane-rich biogas, as LCFA hold a rather high energetic potential. Anaerobic treatment of LCFA-based effluents has been, for the past decade, one of the main research subjects at our research group (Laboratory of Environmental Biotechnology, Centre of Biological Engineering, University of Minho, Portugal). Results have shown that, given the appropriate conditions, LCFA can be efficiently converted to high amounts of biogas [Alves *et al*., 2001, Pereira *et al.,* 2004]. This was a breakthrough in the anaerobic digestion of LCFA-based effluents as it strongly contradicted the previously accepted theories of permanent inhibition of anaerobic microbial communities by LCFA. Additionally, it highlighted the increased importance of studying the microbial composition and function of LCFA-degrading anaerobic communities in these types of ecosystems. Sousa et al (2007) found two distinct enrichment cultures growing on saturated (C16:0) and unsaturated (C18:1) LCFAs, which suggests differences in the degradation of saturated and unsaturated LCFAs.

METHODS

Sludge samples were incubated in batch assays, at 37ºC, under anaerobic conditions, in the presence of one of the following LCFAs, palmitic acid (C16:0), stearic acid (C18:0), that are both saturated LCFAs, and oleic acid (C18:1), an unsaturated LCFA. In parallel, a blank assay was prepared in which no LCFA was added. After 5 days of incubation, when approximately 50 % of the LCFA were already consumed, sludge samples were collected and DNA extracted with UltraClean® Soil DNA Isolation Kit and further amplified with primers ITSF (GTC GTA ACA AGG TAG CCG TA) and ITSR (GCC AAG GCA TCC ACC). Bacterial community composition was assessed by ARISA, a culture-independent technique for constructing bacterial community fingerprints based on the length heterogeneity of the intergenic transcribed spacer region of bacterial rRNA operons (Roesch *et al*., 2009). PCR products were separated on a chip (Agilent DNA 7500 Kit) with an Agilent Bioanalyzer. Size standards were resolved in separate wells to estimate the size of each PCR product.

RESULTS & CONCLUSIONS

Figure 1 shows the ARISA fingerprint of sludge samples that were incubated with long chain fatty acids. The profiles corresponding to sludge samples incubated with saturated LCFA (palmitic and stearic acids) are very similar to each other and also similar to the blank assay in which no LCFA was added. On the other hand, ARISA profiles of sludge samples incubated with oleic acid (unsaturated LCFA) are completely different from the others. These results suggest that the degradation of unsaturated LCFA and saturated LCFA is conducted by different organisms. The presence of oleic acid caused strong changes in the bacterial community while the presence of palmitic and stearic acids do not seem to induce changes in the initial bacterial community (blank assay).

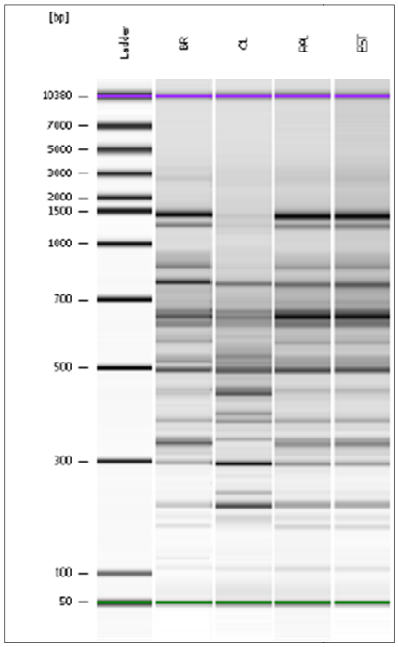

Figure 1. Gel-like image generated by the bioanalyzer. The first column is the reference DNA 7500 ladder. Base pair sizes are indicated adjacent to the ladder. Samples BR, OL, PAL and EST represent the automated ribosomal intergenic spacer analysis (ARISA) profiles for the blank, oleic acid, palmitic acid and stearic acid incubations respectively.

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