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Application of molecular techniques to monitor biohydrogen production by different bacterial consortia (*Clostridium* spp.) in a Bioreactor

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Our current dependence on fossil fuels as the primary energy source contributes to global climate change, environmental degradation and health problems. Hydrogen offers a tremendous potential as a clean, renewable energy currency and it is compatible with electrochemical and combustion processes for energy conversion without producing carbon-based emissions. Many microorganisms, especially photosynthetic as well as facultative and anaerobic bacteria have been reported to produce large amounts of hydrogen from soluble and insoluble biomass. *Clostridia,* being obligate anaerobes, are capable of biogas production during ‘dark fermentation’ of a wide range of carbohydrates. Hydrogen is produced during a reversible reduction of protons accumulated during the fermentation process to dihydrogen, a reaction which is catalyzed by hydrogenases. These proteins are especially abundant in *Clostridia.*
In this project, entitled Micro-H_2 we have focused on a new direction in bio-hydrogen production systems which is the use of mixed cultures of microorganisms (consortia). We have expected that the combination of complementary metabolisms would significantly increase the efficiencies of mixed systems compared to monocultures. However, a few fundamental studies needed to be carried out in order to investigate and improve the stability of microbial populations involved in the processes. It is now recognized that molecular microbial ecology tools provide the scientific basis for the

processes used in environmental biotechnology. To characterize the bacterial communities from the diversity point of view, quantitative techniques such as Real-Time Quantitative PCR and FISH (Fluorescence in situ hybridization) and semi-quantitative DGGE (Denaturing Gradient Gel Electrophoresis) have been optimized and applied on different bioreactor samples. This approach enabled for the temporal monitoring of the evolution of bacterial consortia both in terms of species dominance and their metabolic activity. Molecular analysis of bacterial consortia allowed for careful examination of interactions between different bacterial species within a consortium, which is crucial in the stabilization of the hydrogen production process. Better understanding of bacterial hydrogen metabolism is essential for its sustainable production.