Assessment of the microbial community in the cathode compartment of a plant microbial fuel cell

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Introduction: In plant microbial fuel cells (plant-MFCs) living plants and microorganisms form an electrochemical unit able to produce clean and sustainable electricity from solar energy. It is reasonable to assume that besides the bacteria in the anode compartment also the cathode compartment plays a crucial role for a stable high current producing plant-MFC. In this study we aim to identify dominant bacterial species in the cathode compartment of the plant-MFC.

How does a plant-MFC work?
- CO₂ fixation in the plant, exudation of small carbohydrates
- Degradation by microorganisms to CO₂ (atmosphere), H⁺, e⁻
- e⁻ donation at anode for metabolic energy, flow via electrical circuit to cathode
- H⁺ transport through a separating membrane for electroneutrality
- reduction of diffused O₂ with H⁺ and e⁻ to water

Experimental set-up
- Preparation of DNA samples from catholyte and biofilm of the cathode of six differently performing MFCs (0 to -850 mA/m²)
- Fixation of biofilm samples from all six MFCs for fluorescent in situ hybridization (FISH).
- Amplification of bacterial 16S-rDNA via PCR from all samples (650bp)
- 454-pyrosequencing of these amplicon libraries to identify the microbial key players in the cathode compartment
- Assembly with 98% similarity by the Newbler software and phylogenetic allocation of the 16S-rDNA sequences by the ARB software package
- Selection for specific 16S-rRNA targeted oligonucleotide probes for FISH to verify the sequencing results and localize dominant bacterial species via confocal laser scanning microscopy (CLSM)

Results and discussion
- In the catholyte samples we found the genus Brachymonas (β-Proteobacteria) to be the most abundant in four out of six samples with the highest similarity to Brachymonas denitrificans.
- More striking was the abundance of a yet unknown member of the family Sinobacteriaceae (γ-Proteobacteria) in the biofilm samples from the cathode with a relative abundance of up to 30% of all sequences (see fig. 2).
- Sinobacteriaceae was the most dominant family in all MFCs with a good performance (4 MFCs) and was not significantly detectable in the low performing MFCs (2 MFCs).
- Within the Sinobacteriaceae the sequences showed highest similarity to Steroidobacter denitrificans, but the entire family is highly diverse and not many species are described so far.
- With CLSM a biofilm of up to 17µm thickness with many active γ-Proteobacteria was observed on graphite samples from high performing MFCs, while no biofilm or only without active γ-Proteobacteria was present in low performing MFCs (s. fig.3).
- FISH supports data obtained by 454-sequencing.

Conclusion: Members of the family of Sinobacteriaceae could be a formerly unknown important driver in biocathode performance of a plant-MFC.

![Fig. 1: Functional principle of a plant-MFC](image)

![Fig. 2: Phylogenetic allocation of 454 reads.](image)

![Fig. 3: Confocal laser scanning micrographs of graphite samples taken from the biocathodes of a MFC with low performance (left) and one with high performance (right). FISH probe Gamma-42a (γ-Proteobacteria) is depicted in blue, Syto orange (all bacteria) in red and green.](image)