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

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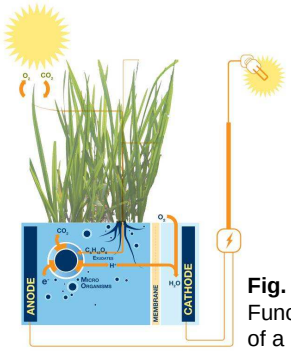

Fig. 1: Functional principle of a plant-MFC

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.

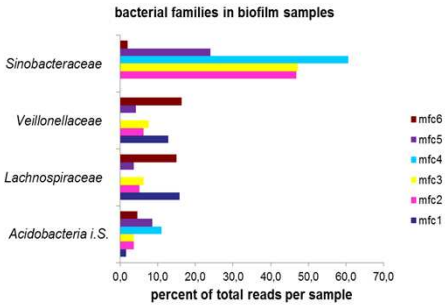

Fig. 2: Phylogenetic allocation of 454 reads.

How does a plant -MFC work?

- CO_2 fixation in the plant, exudation of small carbohydrates
- Degradation by microorganism to CO_2 (↑atomsphere), 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 O_2 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)

		inoculum	catholyte						biofilm					
Phylum	Family		mfc1	mfc2	mfc3	mfc4	mfc5	mfc6	mfc1	mfc2	mfc3	mfc4	mfc5	mfc6
		%	%	%	%	%	%	%	%	%	%	%	%	%
Acidobacteria	*Incertae Sedis*	1,1	1,6	4,6	1,9	3,1	6,9	11,3	1,5	3,6	3,6	11,1	8,5	4,6
Actinobacteria	*Micrococcaceae*	0	0,6	1,3	0	0,8	8,6	3,2	0,0	0,0	0,0	0,0	0,0	0,0
Actinobacteria	*Nocardiaceae*	0	12,3	3,3	10,7	5,0	4,6	10,3	7,3	0,6	0,4	0,0	3,2	2,0
Chlorobi	*OPB56*	0	0,9	6,7	1,5	15,6	4,8	4,7	0,3	3,0	3,2	3,8	2,0	2,3
Chloroflexi	*Anaerolineaceae*	3,4	0,7	0,9	0	4,4	0,7	24,6	0,0	0,5	0,4	8,6	0,3	6,8
Deinococcus-Thermus	*Trueperaceae*	0	4,3	0	0	0,9	0,2	0,3	1,5	0,6	0,7	1,6	0,3	0,3
Firmicutes	*Lachnospiraceae*	1,1	0	8,7	0	6,2	6,1	0	15,8	5,2	6,2	0,1	3,7	15,0
Firmicutes	*Ruminococcaceae*	0,4	0	8,4	0	3,6	4,4	0	7,2	4,4	4,2	0,0	2,6	8,7
Firmicutes	*Veillonellaceae*	0,4	0	8,7	0	8,0	5,4	0	12,9	6,3	7,6	0,0	4,2	16,3
Proteobacteria	*Alpha- Bradyrhizobiaceae*	0	1,9	0,3	4,4	0,6	1,7	0,7	1,6	0,2	0,3	0,3	1,9	0,6
Proteobacteria	*Alpha- Incertae Sedis*	0	0,8	1,3	0,3	1,0	0,9	0,7	1,6	0,6	0,6	0,0	0,2	1,5
Proteobacteria	*Alpha- Rhodobiaceae*	0,9	1,0	2,7	6,2	4,3	1,3	0,6	0,8	1,0	0,0	0,0	2,2	0,8
Proteobacteria	*Alpha- wr0007*	0	1,2	0	0	0	0,3	1,1	0,0	0,4	0,6	0,8	0,0	0,9
Proteobacteria	*Beta- Alcaligenaceae*	6,4	0,6	0	0	0,3	0	0	0,0	0,4	0,0	0,0	0,0	0,7
Proteobacteria	*Beta- Comamonadaceae*	41,0	44,0	20,5	52,4	13,7	21,9	16,4	19,5	7,9	5,9	1,9	24,6	5,3
Proteobacteria	*Beta- Rhodocyclaceae*	10,7	1,6	10,5	5,3	11,1	11,3	3,6	7,2	9,1	10,4	2,5	10,1	9,0
Proteobacteria	*Gamma- Sinobacteraceae*	0,4	0	0	0,8	0	0,4	0	0,0	46,8	47,2	60,7	24,0	2,0
Proteobacteria	*Gamma- Xanthomonadaceae*	1,3	21,0	4,5	6,3	3,6	3,8	7,2	0,9	1,9	1,8	2,2	3,6	1,8

Tab. 1: Table of the 454 sequencing results with phylum and family affiliations given in percent of total reads per sample with catholyte and biofilm samples in comparison. The dominant bacterial families are circled in green.

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).
- *Sinobacteraceae* 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 *Sinobacteraceae* the sequences showed highest similarity to *Steroidobacter denitrificans*, but the entire family is highly divers 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 *Sinobacteraceae* could be a formerly unknown important driver in biocathode performance of a plant-MFC.

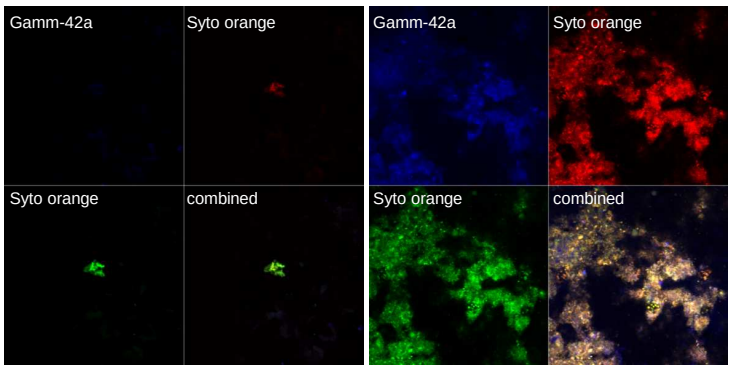

Fig. 3: Confocal laser scanning micrographs of graphite samples taken from the biocathodes of a MFC with low performance (left) and one with high performance (right). FISH probe Gamma-42a (*γ-Proteobacteria*) is depicted in blue, Syto orange (all bacteria) in red and green.

AMP Department Microbe-Plant Interactions
DES Department of Environmental Sciences
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