

Shotgun sequencing based detection of microbial community shifts during multi-step electrogenic bacteria selection

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Electrogenic bacteria (or electricigens) are capable of producing electrical power from organic-rich substances such as domestic and industrial wastewaters. Electricity producing species may belong to distinct microbial phyla and be differently abundant in complex electrogenic communities. Development of methods for selective enrichment and accurate identification of electricigens would facilitate synthetic design of optimal electrogenic microbial consortia for efficient wastewater treatment.

Electrogenic bacteria were selectively enriched by applying 100 mV potential to the anode of a half-MFC system inoculated with a highly diverse microbial community. After a week of a half-MFC operation, the primary anodic biofilm was transferred to fresh media within another half-MFC with applied 100 mV potential. These steps were repeated several times. Whole community shotgun sequencing was applied to track the changes in anodic communities during step-to-step selection process. MG-RAST [1] and Kaiju [2], tools that utilize different algorithmic approaches in sequencing data annotation, were used for taxonomic analysis of sampled microbial communities. The results obtained by different bioinformatics tools were similar in terms of microorganism abundance on all taxonomic levels.

In general, microbial diversity decreased over subsequent passages indicating steady selection of competitive electrogenic and associated species. Five classes of *Proteobacteria*, and the phylum *Deferribacteres* showed most significant changes in their abundances over the selection process. At deeper taxonomic levels, the replacement of *Geobacter* to *Desulfovibrio* occurred within the *Deltaproteobacteria*, the class of bacteria with most currently known electrogenic species representatives. Also, species of the electrogenic genus *Arcobacter*, within the *Epsilonproteobacteria*, demonstrated rapid accumulation on the anodic surfaces as well as in the anolyte.

Among less studied bacteria, *Denitrovibrio* affiliated with *Deferribacteres* were not abundant in the planktonic phase but were enriched in the anodic biofilms, suggesting that these bacteria play a role in electron transfer within electrogenic community. Further functional metagenomics analysis is required to understand the role of each group of microorganisms enriched under applied voltage conditions.

Overall, this study provides snapshots in the step-to-step microbial community evolution within a multi-step selection procedure, and the results provide insight to the process of electrogenic biofilm development.

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1. K.P. Keegan, E.M. Glass, F. Meyer (2016) MG-RAST, a metagenomics service for analysis of microbial community structure and function, *Methods Mol Biol*, **1399**:27-33.

2. P. Menzel, K.L. Ng, A. Krogh (2016) Fast and sensitive taxonomic classification for metagenomics with Kaiju, *Nat Commun*, **7**:11257.