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Diversities of Electricigens in Microbial Fuel Cells and Biohydrogen-Producing Bioelectrochemical Cells

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The generation of electricity in a microbial fuel cell (MFC) is based on the fact that electrochemically active bacteria derive energy from the oxidation of organic matter linked to the reduction of another compound, typically oxygen [1]. The MFC generates electricity, but also can be modified to produce hydrogen instead using a two-chambered bioelectrochemical cell (BEC) in which a cathode was kept free of oxygen and the thermodynamic barrier overcome by augmenting the circuit with the addition of a small external voltage. Regardless of MFC or BEC, the primary biocatalysts are the electrochemically active bacteria in the anode chamber diversely referred to as electricigens [1], anodophilic bacteria [2], and exoelectrogens [3]. They can completely oxidize organic compounds to carbon dioxide with an insoluble electrode serving as the sole electron acceptor. Better understanding of the bacterial community and dominant species in the anode biofilm are strongly required, even though the optimization of MFC configuration or architecture to reduce the internal resistance has been considered to be the most critical way to increase the performance.

Therefore, comparative studies on the bacterial diversities in the electricity-generating MFC as well as in the hydrogen-producing BEC were performed. In addition, metabolically viable cells contributing to electron transfer on to the anode was investigated by examining cell membrane integrity.

Bacterial Community in the MFC

The microbial communities in the anode biofilms of the acetate-fed MFC were dominated by *Proteobacteria*, especially the β-subclasses. 51.2% of the sequences obtained from an anode biofilm were β-*Proteobacteria*, and 66.8% of these were in the genus *Thauera* with a >95% similarity to *Thauera aromatica* LG356. The next most frequently detected bacteria was δ-*Proteobacteria* (34.1%), with a predominance of *Geobacter* related species, followed by others (12.2%) and α-*Proteobacteria* (2.4%). In

addition, when the influences of different electron donors, such as acetate, butyrate, propionate, and glucose, on the bacterial communities in the MFC were examined, a relative abundance of β-*Proteobacteria* but an absence of γ-*Proteobacteria* was observed in all MFCs except for the propionate-fed system. A propionate-fed MFC was dominated by Fermicutes (59.3% of clones), next mostly by γ-*Proteobacteria* (18.5%).

Bacterial Community in the BEC

Biohydrogen gas was efficiently generated, with the hydrogen yield of 2.1 mol H_2/mol acetate, through the biocatalyzed electrolysis in the BEC at applied voltages above 0.30 V. The bacterial community of the hydrogen producing BEC showed a remarkable difference compared with that of the MFC. There were substantially fewer bacterial species in the communities of the anode biofilms from the BEC than observed in the MFC. Moreover, *Pelobacter propionicus*-like species were remarkably dominant. *Geobacter* sp. were the important species in the MFC and were still an integral member of the bacterial community for the BEC.

Our bacterial community assay employing universal bacterial primers was mainly focused on the electrochemically active bacteria which are very diverse in their origin but mostly belong to the domain of bacteria rather than archaea. Consequently, methoanogens were not included in this community assay. We expected that the proliferation of electrochemically active bacteria, in contrast gradual die out of methanogens because they can not utilize electrode as an electron acceptor. However, their presence is supported by the sufficient production of methane in the anode compartment, and the importance of methanogens in the anodic biofilms remains largely unexplored yet. The continuous augmentation of the circuit by an external voltage had no harmful effect on the bacterial viability but resulted in a remarkable change in the bacterial community as the MFC was changed to the BEC run for the production of hydrogen. We now know the dominant species in different MFC-based systems. Therefore, further improvement in performance could be achieved by adapting proper bacterial species or via metabolic engineering approach.

References

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