Phylogenetic Diversity of Dominant Bacterial and Archaeal Communities in Plant-Microbial Fuel Cells Using Rice Plants

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Introduction

A sediment microbial fuel cell (MFC) utilizes naturally occurring electropotential differences in freshwater or marine sediments for electricity generation [15]. In a sediment MFC, the anode is buried in the sediment (anaerobic zone) and the cathode is placed in the overlying body of water (aerobic zone). The electrons in inorganic or organic compounds are transferred abiotically or by anode-respiring microorganisms to the anode and flow through an electrical circuit to the cathode and the final electron acceptors such as oxygen, the reduction of which is catalyzed by chemical catalysts such as platinum [26] or by microorganisms [12, 38]. Aside from electrical power generation, sediment MFCs can be used for the removal of unwanted contaminants in sediments, including methane [4, 8, 28, 37], because the anodes, as new electron acceptors, can stimulate the oxidation of reduced compounds. Another advantage of sediment MFCs is that they can be used without a proton exchange membrane and a reactor, which are the primary costs in the construction of other types of MFCs [16].

A plant-MFC (P-MFC) is a type of sediment MFC, in which root exudates produced by aquatic plants such as rice are used as electron donors for anode-respiring microorganisms. Although many studies were performed to characterize P-MFCs and improve the performance, the power output of P-MFCs reported to date ranged from 4 to 26 mW/m² of geometric photosynthetic surface area when O₂ was used as an electron acceptor without catalysts [37].

In this study, the phylogenetic diversities of bacterial and archaeal communities in a plant-microbial fuel cell (P-MFC) were investigated together with the environmental parameters, affecting its performance by using rice as a model plant. The beneficial effect of the plant appeared only during a certain period of the rice-growing season, at which point the maximum power density was approximately 3-fold higher with rice plants. The temperature, electrical conductivity (EC), and pH in the cathodic and anodic compartments changed considerably during the rice-growing season, and a higher temperature, reduced difference in pH between the cathodic and anodic compartments, and higher EC were advantageous to the performance of the P-MFC. A 16S rRNA pyrosequencing analysis showed that the 16S rRNAs of Deltaproteobacteria and those of Gammaproteobacteria were enriched on the anodes and the cathodes, respectively, when the electrical circuit was connected. At the species level, the operational taxonomic units (OTUs) related to Rhizobiales, Geobacter, Myxococcus, Deferrisoma, and Desulfobulbus were enriched on the anodes, while an OTU related to Acidiferrobacter thiooxydans occupied the highest proportion on the cathodes and occurred only when the circuit was connected. Furthermore, the connection of the electrical circuit decreased the abundance of 16S rRNAs of acetotrophic methanogens and increased that of hydrogenotrophic methanogens. The control of these physicochemical and microbiological factors is expected to be able to improve the performance of P-MFCs.

Keywords: Plant-microbial fuel cell, bacterial community, archaeal community, 16S rRNA
These values were significantly lower compared with those obtained using wind turbines (5–7.7 W/m\(^2\)) or solar panels (4.5–7.5 W/m\(^2\)) [51].

To improve the power generation of P-MFCs, both physicochemical and biological aspects must be considered. Effects of plant species [5], anode distance from roots [9], type of material for the anode [3] or cathode [56], and P-MFC configuration [22, 53] on the power generation of P-MFCs have been investigated. However, the basic parameters affecting the performance of all types of MFCs such as temperature, electrical conductivity (EC), the concentration of electron donors, and pH [19, 26, 50] have not been monitored in most of the studies on P-MFCs [29, 46, 49]. Information about these parameters together with performance data of P-MFCs is needed to identify the most important physicochemical factors limiting the performance of P-MFCs.

To identify the microbial communities associated with power generation in P-MFCs, analyses of the microbial communities on electrodes in P-MFCs were conducted in previous studies. De Schamphelaire et al. [14] reported that the anode in a P-MFC with rice plant was enriched with Desulfobulbus-like species, members of Geobacteraceae, and unclassified archaea, based on denaturing gradient gel electrophoresis, terminal restriction fragment length polymorphism, and clone library analyses. Kouzuma et al. [34] showed that Geobacteraceae bacteria were associated with the anodes in rice field MFCs, and acetate- and glucose-fed MFCs, and the rice field MFCs and glucose-fed MFCs shared the same phylogenetic groups of Geobacteraceae based on pyrosequencing analysis of the 16S rRNA genes and Illumina shotgun metagenomic analysis. However, most studies to date have not performed an analysis of the microbial communities on cathodes, which may be as important as those on anodes for power generation in MFCs [21].

In this study, a more extensive experiment was performed to characterize the physicochemical and microbiological aspects of a P-MFC, including an analysis of the cathodic bacteria. The variations in physicochemical parameters and the abundances of metabolically active bacterial and archaeal groups were monitored along with the power output of the MFCs during the rice-growing season. This study is expected to help identify the important physicochemical and microbiological factors affecting the performance of P-MFCs.

**Materials and Methods**

**Experimental Setup**

The P-MFC experiment was performed in a greenhouse at the Rural Development Administration, Suwon, Korea from April 30 to October 22 in 2013. Each of 12 plastic pots (25 cm diameter; 30 cm height) was filled with 5.39 kg of soil, which was collected from nearby rice fields, and air-dried and sieved. Urea, fused phosphate, potassium chloride, and rice straw compost were applied at respective amounts of 0.22 g, 0.21 g, 0.09 g, and 11.13 g per kg soil. Anodes and cathodes made of graphite felt (6.5 mm in thickness, SQ10001-01-02; CeTech, Taiwan) were soaked in ethanol for 1 day, 0.1 N HCl for 1 day, and distilled water until the experiment. A cylinder-shaped anode was made by rolling up a rectangular piece of graphite felt (45 cm × 15 cm) and placed 2.5 cm below the surface of the soil. The soil was flooded with pond water to create a layer of overlying water of 5 cm, which was maintained during the experimental period using the same water. A doughnut-shaped cathode (20 cm outer diameter; 10 cm inner diameter) was floated on the water surface by attaching pieces of polyethylene foam to the bottom of it. The anodes and cathodes were connected with a copper wire and the connections were sealed using silver epoxy covered by non-conductive epoxy. The circuit was completed using an external load of 100 Ω. A schematic diagram of the microbial fuel cell used in this study is shown in Fig. S1.

The experiment used four types of MFCs: MFC with an unconnected circuit and no rice plant (UN), with a connected circuit and no rice plant (CN), with an unconnected circuit and rice plant (UP), and with a connected circuit and rice plant (CP). Each of the four treatments consisted of triplicate pots, and the 12 pots were placed randomly considering differences in solar radiation according to the location in the greenhouse (Fig. S2). In the unconnected treatments (UN and UP), the anodes and cathodes were connected once a month for at least 4 h, whereas in the connected treatments (CN and CP), they were connected constantly. All four treatments were incubated without plants until May 28, at which point a seedling of Samgwang rice (Oryza sativa L. var. japonica) was transplanted into each pot of the UP and CP treatments. Rice was selected as the model plant because it is the most important food crop in Korea. In the greenhouse, the temperature was maintained above 25°C until May 13, after which it was not controlled until the end of the experiment.

**Analysis of Electrical and Chemical Properties**

The voltages across the resistance were measured every hour using an Agilent 34970A Data Acquisition Unit (Agilent Technologies, USA). The temperature in the sediment was measured every hour using a T-type thermocouple (Miraetech, Korea) in only one pot among the three replicate pots of each treatment. Polarization curves and power density curves were constructed using different external resistances (20–10,000 Ω) on October 18, as described by Logan et al. [40]. The voltages were recorded after a stable one was obtained, which took at least 30 min. The current and power were normalized to the projected anode area (675 cm\(^2\)).

The EC and pH in the anodic and cathodic compartments were measured using the water samples collected at intervals of
approximately 1 week. Water samples from the anodic compartment (the pore water) were collected using a plastic pipe, which had a length of 10 cm and a diameter of 1 cm, and was mounted vertically 2.5 cm below the surface of the sediment and 1.0 cm from the outside of the anode. The side of the pipe was perforated along its length and covered with filter cloth to prevent infiltration of soil (Fig. S1). The water samples in the cathodic compartment were collected from the overlying water.

The effect of the root exudates was evaluated by filtering the water sample collected from the anodic compartment with a syringe filter with a pore size of 0.4 µm and determining the soluble total organic carbon (TOC) using a TOC analyzer (Vario TOC cube; Elementar, Germany).

Analysis of Microbial Communities

Electrode samples for microbial characterization were collected on August 28, 2013. Three cubes (0.6 cm x 0.6 cm x 0.6 cm) were cut from different sites of each electrode with an ethanol-sterilized blade and transferred to a sterile 2 ml tube. RNA was extracted using the RNA PowerSoil Total RNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA), according to the manufacturer’s instructions. Aliquots of RNA extracts were treated with RQI RNase-Free DNase (Promega, Madison, USA) and purified using the RNeasy MinElute Cleanup kit (Qiagen, Hilden, Germany). cDNA was synthesized using the GoScript reverse transcription system and random hexamers (Promega) according to the manufacturer’s instructions.

The phylogenetic diversities of the bacterial and archaeal 16S rRNAs were examined using pyrosequencing analysis. Mix I contained 5 µl of 10 mg/ml of bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA), 1 µl of deoxynucleoside triphosphates (dNTPs, each 10 mM), 1 µl of forward primer (20 µM), 1 µl of reverse primer (20 µM), and 1 µl of cDNA in a total volume of 25 µl. Mix II contained 5 µl of 10x PCR buffer (Roche, Mannheim, Germany) and 0.25 µl of Taq DNA polymerase (5 U/µl; Roche) in a total volume of 25 µl. Mix I was incubated at 70°C for 5 min, combined with Mix II, and immediately subjected to thermal cycling. The bacterial 16S rRNA genes were amplified using the primers V1-9F (5'-AC-GAGTTTGATCMTGGCTGAG-3') and V3-541R (5'-X-AC-WTTACCGCGCTGCTGG-3') [10] and the archaeal 16S rRNA genes were amplified using the primers 4F (5'-AG-GGTGTGATCCTGCGG-3') [31] and AV3-519R (5'-X-AG-GGTTTACCCGGCGCGKGTG-3') [27], where X denotes a 7–11 nucleotide-long barcode followed by a common dinucleotide linker: AC for bacteria or AG for archaea. Thermal cycling was performed using the following PCR protocol: for bacteria, initial denaturation at 94°C for 2 min, followed by 35 cycles at 94°C for 15 sec, 45°C for 30 sec, and 72°C for 60 sec, and final extension at 72°C for 7 min. For archaea: initial denaturation at 94°C for 2 min, followed by 40 cycles at 94°C for 15 sec, 59°C for 30 sec, and 72°C for 60 sec, and final extension at 72°C for 7 min. The PCR products were gel-purified with the QIAquick Gel Extraction Kit (Qiagen) and analyzed by pyrosequencing, which was performed by the National Instrumentation Center for Environmental Management (Seoul, Korea) using a 454 GS FLX Titanium Sequencing System (Roche), according to the manufacturer’s instructions.

Pyrosequencing data analysis and the construction of phylogenetic trees were performed as described by Ahn et al. [1].

Statistical Analysis

The differences among the different treatments were determined with one-way analysis of variance followed by Tukey’s pairwise comparison. P values less than 0.05 were considered significant. All analyses were performed using the R software package (ver. 3.1.0; http://www.r-project.org).

DNA Sequence Data

The raw pyrosequencing data are available in the NCBI Sequence Read Archive under the accession number PRJNA255941.

Results and Discussion

Effect of Rice Plants, Temperature, EC, and pH

Fig. 1 shows the variations in voltage and temperature for the CN and CP treatments during the rice-growing season. During the entire experimental period, the voltage showed diurnal oscillation, which coincided with temperature variation, and this occurred irrespective of the presence of rice plants (Figs. 1 and S5). Kaku et al. [29] and Kouzuma et al. [34] attributed the diurnal oscillation of voltage in a P-MFC under field conditions to the difference in the amount of root exudates between the daylight and dark. Furthermore, Bombelli et al. [5] showed that the diurnal oscillation occurred only in the presence of plants, and did not occur in the absence of plants at a constant temperature. Our results show that diurnal oscillation can occur without plants, and the primary reason for this phenomenon appears to be the temperature variation.

No distinct difference in voltage was observed between the CN and CP treatments until July 8. Between July 9 and July 25, the voltage in the CP treatment was lower than that in the CN treatment. This is likely due to the decrease in conductivity in the pore water in the CP treatment during this period (Fig. 3A), because the decrease in conductivity increases the internal resistance of the MFC, resulting in power reduction [26, 36]. We attribute this trend to the absorption of ions by rice plants during this period. From that point until the end of the experiment, however, the voltage in the CP treatment was maintained higher than that in the CN treatment. When the polarization and power density curves were constructed at the end of the experiment (October 18) (Fig. 2), the maximum power density was 2.4 mW/m² for the CN treatment and 7.3 mW/m².
for the CP treatment, an approximately 3-fold difference. The maximum power density obtained for the CP treatment is slightly higher than the 5.9 mW/m$^2$ reported by Kaku et al. [29], which was determined through a field experiment on August 7, 2008. This result indicates that the presence of rice roots can increase the electrical power of MFCs, as reported by De Schamphelaire et al. [13].

When we removed the rice plants and anodes from the pots at the end of the experiment, we could observe that the roots of rice plants penetrated through the anodes (Fig. S3), suggesting that the root exudates might be used by anode-respiring bacteria. However, the difference in soluble TOC in the pore water between the CN and CP treatments was not observed after June 25 (Fig. S4). It is possible that the root exudates were rapidly degraded by microorganisms and thus were undetectable in the pore water.

The pH values in the cathodic and anodic compartments increased rapidly when the temperature was maintained above 25°C (until May 14, Fig. 3B). After that point, the pH in the cathodic compartment increased gradually, while that in the anodic compartment decreased gradually, and at the end of the experiment, this difference was above 2.0 for the CN and CP treatments while it ranged from 1.2 to 1.6 for the UN and UP treatments (Fig. 3C). Previous studies have shown that the operation of MFCs causes the

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**Fig. 1.** Variations in voltage across an external load of 100 Ω (A) and the temperature in the sediment (B) in the CN and CP treatments. Data are the averages of triplicates. Some data points are missing owing to technical problems. Dates for rice transplanting and electrode sampling are indicated by arrows.

**Fig. 2.** Polarization curves (triangles) and power density curves (squares) for the CN treatment (open symbols) and CP treatment (closed symbols) determined on October 18. Data are the averages of triplicates.
difference in pH between the cathodic and anodic compartments and attributed this to the insufficient transfer of protons from the anodic compartment to the cathodic compartment [19, 44]. However, because the difference in pH between the cathodic and anodic compartments also increased when the circuit was not connected (the UN and UP treatments), it is likely that processes other than current generation are involved in this phenomenon. It was reported that a low pH in the anodic compartment inhibits proton transport from the anodic biofilm [18, 54], and a high pH in the cathodic compartment adversely affects the reduction of oxygen on the cathode [43, 44, 50], both resulting in the reduction of power generation. At the end of the experiment, we temporarily decreased the pH of the cathodic compartment from 7.3 to 6.0 by adding 1 N HCl and observed that the voltage increased from 0.1 to 0.2 V in the CP treatment (Fig. S6). Thus, efforts to reduce the difference in pH between the cathodic and anodic compartments will be needed to increase the power production of P-MFCs.

**Prokaryotic Community Analysis**

**Overview of the prokaryotic communities.** The bacterial and archaeal 16S rRNAs obtained from the electrodes were analyzed using pyrosequencing, and the data are summarized in Table 1. The archaeal 16S rRNA gene was undetectable by PCR in the cathode samples in all four treatments, suggesting that archaea played a minor role in the cathodic reaction. The number of operational taxonomic units (OTUs) and richness estimators of the bacterial 16S rRNAs from the cathodes and the archaeal 16S rRNAs from the anodes decreased when the circuit was connected, although this difference was not significant. On the other hand, the diversity indices of bacterial 16S rRNAs from both anodes and cathodes decreased when the circuit was connected, although a significant difference was observed only for the inverse Simpson index between the anodes of the UN and CN treatments. Overall, this result indicates that the connection of the circuit decreased the prokaryotic diversity of the electrodes, perhaps through the enrichment of microbes with selective advantages under this condition. The voltages produced in the UN and UP treatments, which were measured once a month for at least 4 h, were on average 50% and 61% of those of the corresponding connected treatments, respectively (data not shown). This result indicates that the microorganisms involved in power generation were more enriched in the connected treatments than in the unconnected ones. The decrease in diversity of

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**Fig. 3.** Variations in electrical conductivity (A), pH (B), and difference in pH between the cathodic and anodic compartments (C) in the four types of sediment MFCs. Symbols: anodic compartment (straight line) and cathodic compartment (dotted line), unconnected (open symbols) and connected (closed symbols), without rice plant (circles) and with rice plant (triangles). Values are the means ± standard deviations, $n = 3$. 
bacterial communities on electrodes in closed circuits was previously observed in sediment MFCs [24] and single-chamber, air-cathode MFCs [47].

The abundances of the dominant OTUs (those having average relative abundances more than 1% in any of the four treatments) are presented in Table S1. Several OTUs showed dramatic differences in abundance among the replicates of the same treatments. For example, the otu0004 in Table S1A occupied 15.4% in one replicate of the CP treatment, but it occupied only 0.9% in the other replicates. This result is probably due to the uneven distribution of electron donors and different physicochemical conditions in the sediment; thus, more extensive sampling will be required for further precise analysis of microbial communities on electrodes.

**Bacterial communities on anodes.** The phylum distributions of bacterial 16S rRNAs (up to the class level for Proteobacteria) on the anodes in the different treatments are shown in Fig. 4A. Among them, Deltaproteobacteria occupied the highest proportions in all four treatments. The abundance of Deltaproteobacteria was higher in the CN and CP treatments (29.9% and 32.2%, respectively) than in the UN and UP treatments (13.1% and 26.5%, respectively), and this difference was significant between the UN and CN treatments (Fig. 4A). A clear dominance of Deltaproteobacteria on the anodes was observed in previous studies on marine or freshwater sediment MFCs [6, 14, 24, 47], and the strictly anaerobic condition in sediment MFCs compared with other types of MFCs was suggested to be one of the reasons for this dominance [41]. No significant difference between the unconnected and connected treatments was observed for the other taxonomic groups.

The phylogenetic positions of the OTUs that were dominant (≥1%) and more abundant in the connected treatments (CN and CP) than in the corresponding unconnected treatments (UN and UP) on the anodes (denoted by the prefix “Anode”) are indicated in Fig. 5A; their relative abundances are indicated as heat maps. These OTUs were assigned to Alpha- and Deltaproteobacteria, Chloroflexi, and an unclassified group.

**Anode_otu0004**, which was assigned to Alphaproteobacteria, was the most abundant OTU (5.7%) in the CP treatment, whereas it occupied only 0.77% in the UP treatment and was not detected in the UN and CN treatments. This OTU was classified as Rhizobiales and showed the highest 16S rRNA identity (95.6%) with *Filomicrobium insigne* SLG5B-T among the type species. One isolate affiliated with the order Rhizobiales, *Rhizobium electricum* Mfc52-T, was reported to generate electricity in a cellulose-fed MFC [33]. However, the similarity of the 16S rRNA gene betweenAnode_otu0004 and strain Mfc52-T is only 84.7%; therefore, the involvement of Anode_otu0004 in electricity generation requires further investigation.

### Table 1. Summary of 16S rRNA pyrosequencing data obtained from bacterial and archaeal biofilms on the electrodes in the microbial fuel cells.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of reads</th>
<th>Number of OTUs†</th>
<th>Good’s coverage†</th>
<th>Richness estimator‡</th>
<th>Diversity index‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
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<tr>
<td>on anodes</td>
<td></td>
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</tr>
<tr>
<td>UN</td>
<td>1,771</td>
<td>812 ± 82</td>
<td>0.74 ± 0.03</td>
<td>1,401 ± 158</td>
<td>6.3 ± 0.2</td>
</tr>
<tr>
<td>CN</td>
<td>1,771</td>
<td>721 ± 68</td>
<td>0.75 ± 0.03</td>
<td>1,389 ± 117</td>
<td>5.7 ± 0.3</td>
</tr>
<tr>
<td>UP</td>
<td>1,771</td>
<td>639 ± 77</td>
<td>0.79 ± 0.03</td>
<td>1,233 ± 98</td>
<td>5.7 ± 0.2</td>
</tr>
<tr>
<td>CP</td>
<td>1,771</td>
<td>662 ± 124</td>
<td>0.77 ± 0.06</td>
<td>1,356 ± 422</td>
<td>5.6 ± 0.3</td>
</tr>
<tr>
<td>Bacteria</td>
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<td>on cathodes</td>
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</tr>
<tr>
<td>UN</td>
<td>1,771</td>
<td>561 ± 39</td>
<td>0.83 ± 0.02</td>
<td>963 ± 114</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td>CN</td>
<td>1,771</td>
<td>459 ± 46</td>
<td>0.89 ± 0.02</td>
<td>685 ± 92</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td>CP</td>
<td>1,771</td>
<td>486 ± 79</td>
<td>0.86 ± 0.03</td>
<td>900 ± 206</td>
<td>5.0 ± 0.4</td>
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<tr>
<td>Archaea</td>
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<tr>
<td>UN</td>
<td>381</td>
<td>50 ± 12</td>
<td>0.94 ± 0.02</td>
<td>76 ± 18</td>
<td>2.7 ± 0.3</td>
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<tr>
<td>CN</td>
<td>381</td>
<td>46 ± 12</td>
<td>0.95 ± 0.01</td>
<td>73 ± 7</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>CP</td>
<td>381</td>
<td>49 ± 26</td>
<td>0.95 ± 0.05</td>
<td>67 ± 45</td>
<td>2.8 ± 0.3</td>
</tr>
</tbody>
</table>

Archaear 16S rRNAs were obtained only from the anodes. Values are the means ± standard deviations of triplicates.

†Calculated at a 97% 16S rRNA similarity cut-off.

‡The values sharing the same letter among the four treatments have no significant difference (*p* > 0.05).
Most of the OTUs that were dominant and more abundant in the connected treatments on anodes (7 of 10 OTUs) were assigned to Deltaproteobacteria (Fig. 5A) and they formed four distinct clusters within Deltaproteobacteria. Among them, Anode_otu0024 occupied 2.2% of bacterial 16S rRNAs in the CN treatment, whereas Anode_0069 and Anode_0074 occupied approximately 1.0% in the CP treatment. These three OTUs formed a cluster with type species of the genus *Geobacter* (Fig. 5A). *Geobacter* spp. have been identified as the dominant members on anode biofilms in many bioelectrochemical systems [32], and *G. sulfurreducens* is one of the most intensively studied bacterial species as an exoelectrogenic bacterium [39]. Phylogenetically, the above three OTUs were distributed within *Geobacter* subsurface clades and were not clustered with *G. sulfurreducens* and *G. metallireducens*. The predominance of *Geobacter* subsurface clades in diverse Fe(III)-reducing subsurface environments [25] and anodes of P-MFCs using rice plants [34] was previously reported. The electrochemical properties of subsurface *Geobacter* species have not been thoroughly studied compared with *G. sulfurreducens* and *G. metallireducens*. In addition, significant differences were found between the genomes of a subsurface *Geobacter* species and non-subsurface *Geobacter* species [2]. Thus, the isolation and characterization of subsurface *Geobacter* species are expected to help understand and improve electricity generation in P-MFCs.

Anode_otu0010 occupied 5.2% of 16S rRNAs on the anode of the CP treatment, the second most abundant OTU in the CP treatment, which was not detected in the other treatments. Phylogenetically, it was deeply branched between aerobic, fruiting-body-producing Myxobacteria and facultatively anaerobic, non-fruiting-body-producing *Anaeromyxobacter dehalogenans* [45] within the class Deltaproteobacteria (Fig. 5A). Given the current data, the involvement of Anode_otu0010 in electricity generation is difficult to ascertain.

Anode_otu0019 occupied 2.2% and 1.0% in the CN and CP treatments, respectively, while it was recovered at the rates of 0% and 0.5% in the UN and UP treatments, respectively. Anode_otu0019 formed a cluster with *Deferrisoma camini* S3R1 (with 16S rRNA identity of 88.6%), a moderately thermophilic, strictly anaerobic, Fe(III)-reducing bacterium isolated from a deep-sea hydrothermal vent chimney [48]. It is currently unknown whether this strain can use an electrode as an electron acceptor.

Anode_otu0003 occupied the highest proportion (8.0%) of the bacterial 16S rRNAs in the CN treatment and was recovered at below 0.5% in the other treatments. Anode_otu0012 occupied 3.2% and 1.7% in the CN and CP treatments, respectively, while it was recovered at below 0.02% in the unconnected treatments. Taxonomic analysis classified these two OTUs into the family Desulfobulbaceae (Fig. 5A), which comprises strictly anaerobic, sulfate-reducing bacteria [35]. In previous studies, the sequences affiliated with Desulfobulbaceae were highly enriched on anodes in marine sediment MFCs [24] and P-MFCs using rice plants [14]. A strain affiliated with the family Desulfobulbaceae, *Desulfovibrio propionicus* DSM 20322, was shown to use a graphite electrode as an electron acceptor and lactate,
Based on our results and the previous studies, this group of bacteria seems to play an important role in electricity generation in sediment MFCs.

**Bacterial communities on cathodes.** Alphaproteobacteria occupied the highest proportions (22.9–25.5%) on the cathodes in all four treatments with no significant differences among the treatments (Fig. 4B). The abundance of Alphaproteobacteria was the highest among the bacterial communities found in the cathodes. This suggests that Alphaproteobacteria play a crucial role in the electricity generation process in cathodes of sediment MFCs.
of Gammaproteobacteria was higher in the CN and CP treatments (9.4% and 21.2%, respectively) than in the UN and UP treatments (2.6% and 6.8%, respectively), and this difference was significant between the UP and CP treatments. The other taxonomic groups did not show such a trend.

The phylogenetic positions of the OTUs that were dominant (≥1%) and more abundant in the connected treatments (CN and CP) than in the corresponding unconnected treatments (UN and UP) on the cathodes (denoted by the prefix “Cathode”) are indicated in Fig. 5A. These OTUs were distributed within Alpha-, Beta-, and Gammaproteobacteria, Bacteroidetes, and Planctomycetes.

Among them, Cathode_otu0001 occupied the highest proportions in the CN and CP treatments (5.3% and 14.8%, respectively) and was not detected in the UN and UP treatments (Fig. 5A). Phylogenetically, Cathode_otu0001 formed a deeply branched cluster with uncultured bacterial sequences among the orders Thiotrichales, Chromatiales, Legionellales, Methylcoccales, and Acidithiobacillales within Gammaproteobacteria (Fig. S7). Among the related bacterial strains, many can oxidize sulfur compounds such as sulfide and thiosulfate (indicated by dots). Among them, Acidiferrobacter thiooxydans was clustered with Cathode_otu0001 and Cathode_otu0045 with a bootstrap value of 60% (Fig. S7). Cathode_otu0045 also occupied 0.2% and 1.6% in the CN and CP treatments, respectively, and was not detected in the unconnected treatments (Fig. 5A). Acidiferrobacter thiooxydans is a facultatively anaerobic, acidophilic, and chemolithoautotrophic bacterium that obtains energy from the oxidation of ferrous iron and reduced sulfur compounds [20]. Another gammaproteobacterium with similar physiology, Acidithiobacillus ferrooxidans, is able to grow by catalyzing oxygen reduction on electrodes under acidic conditions [7, 42]. Kato et al. [30] showed that acetate oxidation by Geobacter sulfurreducens and nitrate reduction by Thiobacillus denitrificans can be coupled with electron transfer through conductive minerals such as magnetite. In addition, three uncultured bacterial sequences found to be enriched on a biocathode in previous studies (GenBank accession numbers JN541149, JN802222, and GU129136 in Fig. S7) [12, 52, 55] formed a cluster with Cathode_otu0001 and Cathode_otu0045. Taken together, these observations suggest that Cathode_otu0001 may be an aerobic chemolithoautotroph that can use sulfur compounds as electron donors and may be involved in oxygen reduction on the cathodes. Isolation of bacteria corresponding to Cathode_otu0001 may help to improve the rate of oxygen reduction on cathodes and thus the performance of P-MFCs.

Archaeal communities on anodes. The Miscellaneous
Crenarchaeota Group (MCG) occupied the highest proportions of the archaeal 16S rRNAs (47.7–70.3%) on the anodes in all four treatments (Fig. 4C). The abundances of Methanosarcinales and Methanocellales were lower in the connected treatments than in the unconnected treatments, whereas that of Thermoplasmatales was higher in the connected treatments than in the unconnected treatments. However, a significant difference was observed only for the abundance of Methanocellales between the UN and CN treatments. In contrast, the abundance of Methanomicrobiales was higher in the planted treatments, suggesting that the presence of rice plants stimulated this group of archaea.

The phylogenetic positions of the archaeal OTUs that were dominant (≥1% in any of the four treatments) and showed consistent trends in abundance between the unconnected and connected treatments are indicated in Fig. 5B. The dominant OTUs assigned to the phylum Thaumarchaeota were clustered with clones that were obtained primarily from anaerobic environments such as sediment or anaerobic digesters using culture-independent studies. One of them, otu001, was the most abundant OTU in three of the four treatments, with no significant difference among the treatments.

Among the dominant OTUs assigned to the phylum Euryarchaeota, otu003 and otu018 were clustered with Methanosaeta spp. and otu010 was clustered with Methanoseta spp. These three OTUs showed higher abundances in the unconnected treatments than in the connected treatments. Meanwhile, otu009 showed high 16S rRNA gene similarities (97.1%) to Methanomassiliicoccus luminyensis, a methanogen of the class Thermoplasmata, and was more abundant in the connected treatments than in the unconnected treatments. These trends were the same as that observed at the order level (Fig. 4C).

Methanosarcina and Methanosaeta are the only two genera of methanogens that are able to catabolize acetate [11], whereas Methanomassiliicoccus luminyensis uses H2/CO2 as a substrate and does not use acetate [17]. The decrease in abundance of acetotrophic methanogens and the increase in abundance of hydrogenotrophic methanogens in MFCs have frequently been observed [4, 14, 47]; these trends were attributed to a competition for acetate between the acetotrophic methanogens and anode-respiring bacteria that utilize acetate as an electron donor.

This study showed that the presence of rice plants could increase the power generation of sediment MFCs only after a certain time point. This is likely related to the period during which the root and the anode have physical contact. Thus, to increase this period will be important for an effective use of P-MFCs. The power generation of the P-MFC in the current study was affected by temperature, pH, and EC, similarly to other types of MFCs. Because the power output of the P-MFC (also the sediment MFC) responded immediately to temperature fluctuations, it would be advantageous to install P-MFCs in places where temperature can be maintained high, such as in a greenhouse. The anodic compartment of the P-MFC was maintained anaerobic without the use of a membrane, probably because aerobic bacteria at the soil surface served as an oxygen barrier. However, the large difference in pH between the cathodic and anodic compartments indicates a high resistance to proton transport between the two compartments. Thus, it will be important to facilitate proton transport to improve the performance of P-MFCs.

The abundances of 16S rRNAs of the bacteria related to Rhizobiales, Geobacter, Myxococcus, Deferrisoma, and Desulfobulbus, were enriched on the anodes, while that of bacteria related to Acidiferrobracter thiocydans was enriched on the cathodes. Because these 16S rRNA sequences showed low similarities (<97%) to the previously characterized bacteria, the isolation of these bacteria and characterization of their electrochemical properties will help to optimize the performance of P-MFCs.

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References


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