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Bacterial Communities in Microbial Fuel Cells Enriched with High Concentrations of Glucose and Glutamate

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Abstract In this study, glucose and glutamate (copiotrophic conditions) were used to enrich electrochemically active bacteria (EAB) in a microbial fuel cell (MFC). The enriched population consisted primarily of γ-*Proteobacteria* (36.5%), followed by *Firmicutes* (27%) and δ-*Proteobacteria* (15%). Accordingly, we compared our own enrichments done under many different conditions with those reported from the literature, all of which support the notion that electrochemically active bacteria are taxonomically very diverse. Enrichments with different types and levels of energy sources (fuels) have clearly yielded many different groups of bacteria.

Key words: Microbial fuel cell (MFC), mediator-less, copiotrophic, glucose and glutamate, bacterial community

A microbial fuel cell (MFC) is a device designed to convert chemical energy that a microbe can harvest into electrical energy via microbial activities [6]. This energy can be derived from carbon compounds, which are the main components found in activated sludge. A mediatorless MFC can be operated because electrochemically active bacteria (EAB) are capable of transferring electrons directly to the electrode [6]. EAB have been routinely enriched using a fuel cell-type electrochemical device [1, 4, 7, 9, 10, 13]. It has been found that both the nature and the concentration of the fuel sources used in MFCs are crucial to the types of bacteria that eventually populate the electrode. Study of the microbial population of sludge wastewater is necessary to identify the microorganisms involved in recycling processes.

In this study, microbial communities were examined on the electrodes of MFCs fed with artificial wastewater (AW) incorporated with glucose and glutamate. It was imperative to study not only the range of compounds utilized and the performance of the MFCs, but also the microbial populations dominating and contributing to this energy conversion. Also reported here is a comparison of this enrichment with others in the literature [1, 4, 7, 9, 10, 13, 14].

Copiotrophic mediator-less MFCs, which were set up and run as continuous BOD sensors (previously described by Chang *et al.* [3]), were used for this study. A copiotrophic environment [8] was made with artificial wastewater components, as described above [3], using glucose and glutamate modified to a final concentration of 200 mg/l as BOD. Three MFCs were run under the same conditions.

A DNA library was developed using the same method as described in our previous studies [9, 13]. The community composition was assessed by comparing the sequences of amplified 16S rDNA genes [9, 13]. A phylogenetic tree was constructed using the neighbor-joining method by a MEGA version 3.1 program (http://www.megasoftware.net). All the 16S rDNA gene sequences were submitted to the GenBank database with assigned accession numbers ranging from AY563443 to AY563472.

The performance, characteristics, and reproducible data of these mediator-less MFCs have been extensively studied in our laboratory by Chang *et al.* [3] and Moon *et al.* [11]. Figure 1 is a representative tree produced through neighbor-joining and shows the five major groupings of the clones. Table 1 shows the population of the copiotrophically enriched MFC as measured by clone abundance. Twenty-seven different clone types were identified in 74 random clones sequenced. Five phyla were categorized among 27 clone types. Among the clone types identified, the γ-*Proteobacteria* (class) were the most abundant, comprising 24 of the 74 clones picked. They were also the most diverse, comprising 6 of the 27 clone types identified. Other abundant

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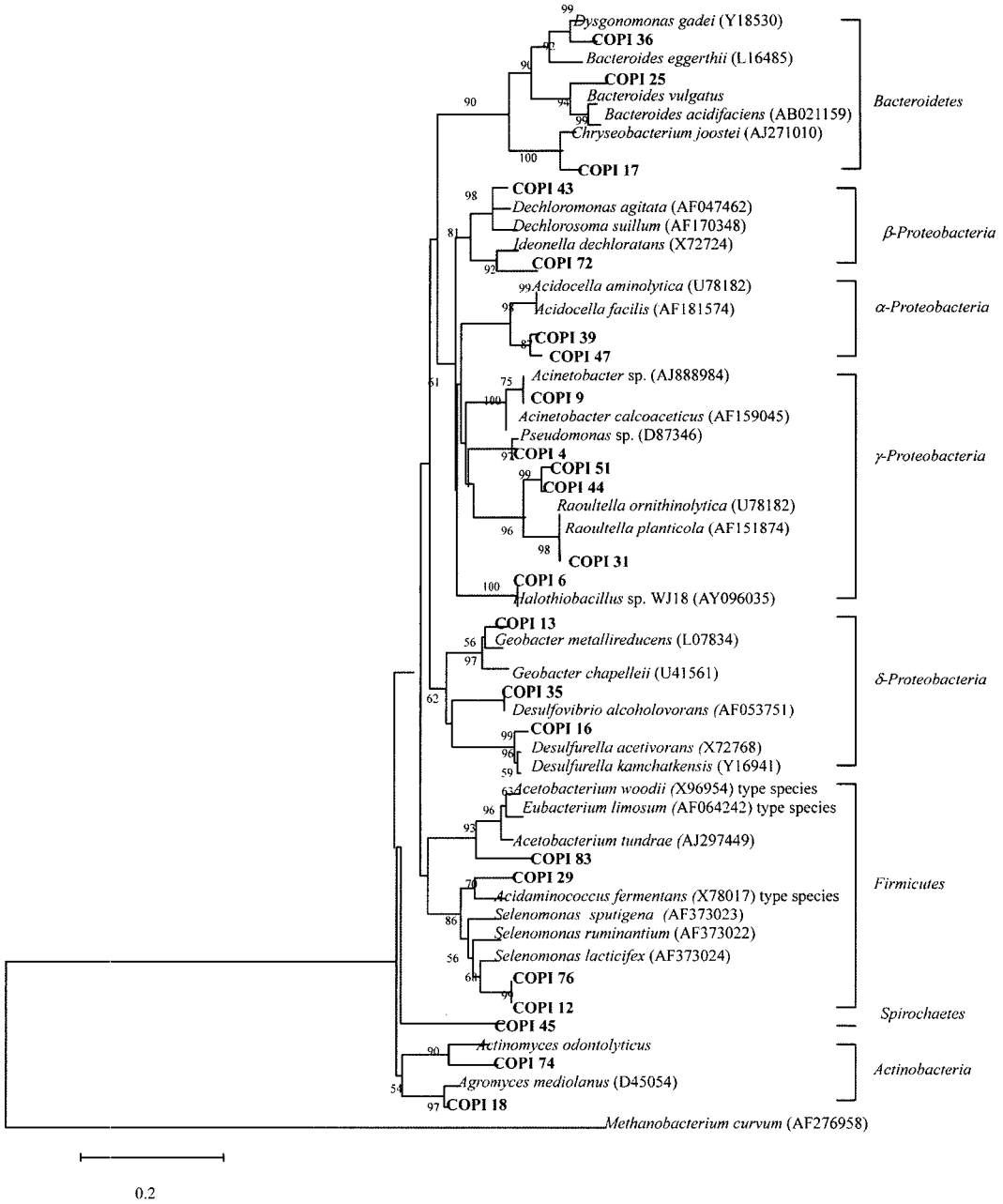

Fig. 1. Phylogenetic tree of 16S rDNA sequences.
Aligned by Clustal-W using the neighbor-joining method and kimura-2-parameter model. Bootstrap values are shown on or below branches of 1,000 replicates receiving >50% bootstrap support. The scale indicates 0.2% sequence divergence. The tree was rooted by outgrouping sequence AF276958.

phyla were the *Firmicutes* (18/74 clones picked and 5/27 clone types identified), and δ-*Proteobacteria* (11/74 clones picked and 4/27 clone types identified). Other phyla (*Bacteroidetes*, *Actinobacteria*, *Cyanobacteria*, *Spirochaetes*, β-*Proteobacteria*, and α-*Proteobacteria* {class}) were present, but as minor components of the community. Of the 27 clone types identified, 16 clones showed less than 97% similarity with relatives in GenBank, suggesting that many of the bacteria enriched in the MFC could be novel species.

The small ribosomal RNA genes have been amplified and sequenced from the MFCs enriched with different fuels in this laboratory [7, 9, 13]. Comparison showed that the population of bacteria that dominates the electrode is dependent on both the type and concentration of the electron donor used as fuel (Table 2). When artificial wastewater was used, γ-*Proteobacteria* and *Firmicutes* bacteria were most abundant under copiotrophic conditions; however, oligotrophic MFC was dominated by α-*Proteobacteria*.

Table 1. Bacterial clones retrieved from an MFC enriched with artificial wastewater.

Clones	Similar relatives (RFLP types)[a]	Homology (%)	Phylum (class)
COPI 1	*Burkholderia multivorans* (2)	96	*Proteobacteria* (β-*Proteobacteria*)
COP1 3	*Eubacterium pyruvivorans* (1)	90	*Firmicutes*
COPI 4	*Pseudomonas aeruginosa* (16)	98	*Proteobacteria* (γ-*Proteobacteria*)
COPI 6	*Halothiobacillus* sp. WJ18 (1)	96	*Proteobacteria* (γ-*Proteobacteria*)
COPI 9	*Acinetobacter* sp. 16S rRNA gene (4)	98	*Proteobacteria* (γ-*Proteobacteria*)
COPI 12	*Selenomonas*-like sp. oral strain GAA14 (6)	92	*Firmicutes*
COPI 13	*Geobacter metallireducens* (4)	94	*Proteobacteria* (δ-*Proteobacteria*)
COPI 16	*Desulfurella acetivorans* (4)	95	*Proteobacteria* (δ-*Proteobacteria*)
COPI 17	*Chryseobacterium joostei* (1)	97	*Bacteroidetes*
COPI 18	*Agromyces mediolanus* (1)	91	*Actinobacteria*
COPI 20	*Pseudanabaena* PCC7408 gene (1)	86	*Cyanobacteria*
COPI 25	*Bacteroides thetaiotaomicron* VPI-5482 (2)	93	*Bacteroidetes*
COPI 29	*Acidaminococcus fermentans* (1)	92	*Firmicutes*
COPI 31	*Raoultella ornithinolytica* (1)	98	*Proteobacteria* (γ-*Proteobacteria*)
COPI 35	*Desulfovibrio alcoholovorans* (1)	98	*Proteobacteria* (δ-*Proteobacteria*)
COPI 36	*Dysgonomonas gadei* (2)	93	*Bacteroidetes*
COPI 39	*Acidocella* sp. WJB-3 (1)	97	*Proteobacteria* (α-*Proteobacteria*)
COPI 43	*Dechloromonas* sp.LT-1 (1)	96	*Proteobacteria* (β-*Proteobacteria*)
COPI 44	*Citrobacter amalonaticus* (2)	97	*Proteobacteria* (δ-*Proteobacteria*)
COPI 45	Uncultured *Spirochaete gene* (7)	98	*Spirochaetes*
COPI 47	*Xanthomonas axonopodis nv. Allii* (1)	97	*Proteobacteria* (γ-*Proteobacteria*)
COPI 51	*Salmonella enterica* subsp. (1)	92	*Proteobacteria* (γ-*Proteobacteria*)
COPI 60	*Ideonella* sp.B513 (1)	98	*Proteobacteria* (β-*Proteobacteria*)
COPI 72	*Brachymonas denitrifican* (1)	89	*Proteobacteria* (β-*Proteobacteria*)
COPI 74	*Actinomyces canis* (1)	91	*Actinobacteria*
COPI 76	*Centipeda periodontii* (1)	89	*Firmicutes*
COPI 83	*Acetobacterium paludosum* (9)	90	*Firmicutes*

[a]Clone abundancy.

On the other hand, oligotrophic MFC enriched using river water contained a bacterial population dominated by β-*Proteobacteria*. Acetate and propionate, both non-fermentable electron donors, were occupied by different bacterial populations with around 70% of δ-*Proteobacteria* and 41% of other classes respectively. These differences were further substantiated by a denaturing gradient gel electrophoresis (DGGE) method to examine the small ribosomal RNA genes of the MFCs (Fig. 2). These observations confirm the findings of Chang *et al.* [2] that an MFC system can be a tool for selecting EAB consortia.

The differences in bacterial populations between the enriched cultures may also be due to the types of fuel cells used for the enrichment studies. For example, an NCBE-type fuel cell with a high membrane/electrode ratio was used for the starch processing wastewater [7], whereas a

Table 2. Comparison of bacterial communities in MFCs enriched with different fuels.

Fuel (value as COD)[a]	Class (%)						Ref.
	α-*Proteobacteria*	β-*Proteobacteria*	γ-*Proteobacteria*	δ-*Proteobacteria*	*Firmicutes*	Others	
Glucose/glutamate (copiotrophic, 200)	1.4	6.8	**36.5**	14.9	27.0	13.4	This study
Glucose/glutamate (oligotrophic, 10)	**64.4**	21.1	3.3	0	0	11.1	[12]
SPW[b] (400)	27.2	**40.9**	0	0	4.5	27.1	[6]
River water (≈5)	10.8	**46.2**	12.9	12.9	0	17.2	[12]
Acetate (300)	7.0	1.7	17.3	**68.8**	1.0	3.8	[12]
Propionate (100)	0	19.4	22.4	10.2	0	**41.8**	In preparation

[a]COD unit was measured in mg/l.
[b]SPW: starch processing wastewater.

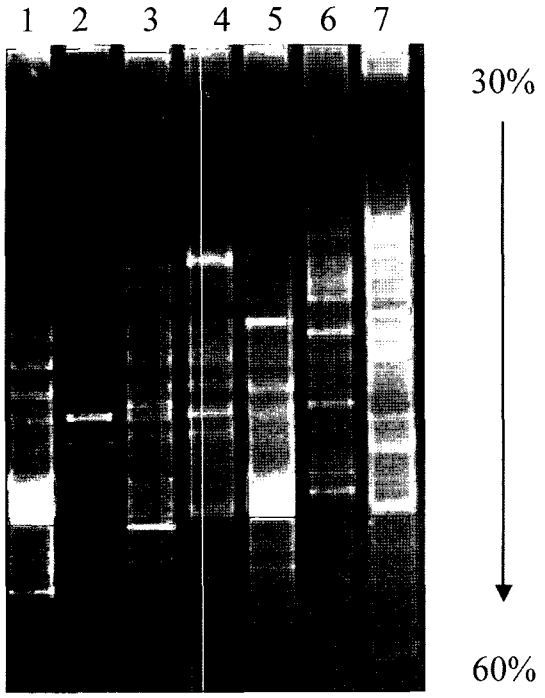

Fig. 2. Comparison of DGGE patterns of microbial communities of various MFCs.

Denaturing gradients used were from 30 to 60%. Lane 1, Sludge (inoculum); lane 2, Acetate enriched MFC; lane 3, Artificial wastewater (copiotrophic) enriched MFC; lane 4, Starch wastewater enriched MFC; lane 5, Starch wastewater; lane 6, Acetate control MFC; lane 7, Artificial wastewater (oligotrophic) enriched MFC.

sensor-type MFC with a low membrane/electrode ratio was used for the AW enrichment [9, 13]. It is known that more oxygen diffuses into the anode compartment in the NCBE-type than in the sensor-type [3, 5, 12]. The bacterial population enriched with the NCBE-type may contain more aerotolerant strains than that enriched in a sensor-type fuel cell.

In conclusion, diverse bacterial populations in MFCs enriched under different conditions show that electrochemical activity is not restricted to a few phyla of bacteria. With a deeper understanding of these EAB, we could manipulate them to play an important role in biogeochemical recycling in the future. Furthermore, MFCs have the potential to be selective devices for culturing microorganisms, especially EAB, by determining the type and concentration of electron donor and acceptor and the type of MFC to be used.

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