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Analysis of functional genomes from metagenomes: Revealing the accelerated electron transfer in microbial fuel cell with rhamnolipid addition

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A B S T R A C T

Extracellular electron transfer is the predominant electricity generation process in microbial fuel cells (MFCs). Our pervious study have proved that the anodic adsorption of rhamnolipid resulted in the Frumkin effect, which enhanced anodic microorganism attachment and accelerated anodic electron transfer. In this study, an in-depth research on the influence of rhamnolipid on functional genes of anodic biofilms metagenomes was carried out to explain its mechanism at the gene level. The result showed that the composition and distribution of functional genes in each dominant genus were different. The category of signal transduction mechanisms was the dominant function category in exoelectrogens, and its relative abundance in the metagenome significantly increased from 4.56 to 5.86% from rhamnolipid addition. Additionally, the metabolic pathway and electron flow analysis revealed that electron flows tend to choose direct electron transfer in the presence of rhamnolipids, and resulting in the increase of Coulombic efficiency from 19.10 ± 0.79% to 27.39 ± 1.07%.

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1. Introduction

Microbial fuel cells (MFCs) are designed to generate current by using bacteria as catalysts to oxidize organic and inorganic substrates. Electrons that are produced by bacteria from substrate oxidation are transferred to the anode, and then pass to the cathode through the external circuit. The extracellular electron transfer (EET) from bacteria to the anode is a complex process that is rate-limiting for the whole electron transfer. As discovered earlier, the EET-capable microbes (exoelectrogens) *Shewanella* and *Geobacter* were extensively studied in metal reduction, sulfur cycles and bio-electrochemical systems [1, 2]. Furthermore, *Pseudomonas*, *Desulfobulbus*, *Rhodopseudomonas* and other cultures have also been proven be potentially EET active [3]. The electron transfer pathway of all exoelectrogens is substantially the same and includes direct electron transfer (DET) and mediated electron transfer (MET). In DET, electrons are transferred to the anode through outer membrane-bound multiheme *c*-type cytochromes or conductive nanowires that are produced by the bacteria. In MET, electrons are transferred to the anode by soluble electron mediators as well as by other undiscovered means [4].

Substrates are the electron donors that allow exoelectrogens to achieve EET. However, most exoelectrogens have limited metabolic versatility and only can utilize low-molecular-weight dissolved organic fermentation end products [5]. Furthermore, it has been widely reported that currently operated MFC inoculated mixed cultures achieve substantially greater power output than those with inoculated pure cultures [6]. Microbial communities of mixed cultures show a great diversity because they require a diverse phenotypic microbial consortium to convert the substrates to direct electron donors. As a result, fermentative bacteria also play an important role in EET, and the interaction of exoelectrogens and fermentative bacteria is a focal point in current research.

Until now, to analyze the microbial communities of MFCs, most of the studies have focused on the 16S ribosomal RNA (rRNA) gene and its product. Regarding substrates, the anodic microbial communities have shown a great diversity of composition and relative abundance [7]. For the same anodic substrates, the anodic microbial communities are generally identical with slight changes in relative abundance that is a response to the optimized method for MFC power generation. Despite these fascinating findings, knowledge of genetic function in MFCs is still limited because approaches using 16S rRNA genes are inclined to define phylogenetic positions of microorganisms based on comparative sequence analyses rather than deciphering microorganism function [8]. As a result, it is important to understand genetic potential, functional activity and functional relationships in mixed cultures.

New sequencing technologies have brought tremendous improvements in automated sequencing and analysis of genome features. DNA shotgun metagenomics-based high-throughput genome sequencing now offers the possibility of revealing microbial structure and the

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functional potential of complex communities without prior enrichment. Until now, this technique has been successfully applied in analysis of microbial communities in marine water [9], grassland soil [10], and biofilms in membrane bioreactors [8].

Recently, metatranscriptomic analysis of microbial communities and gene expression of anode-associated biofilms has been carried out, and the result revealed that the most significant gene expression responses to applied EET stimuli occurred in only two microbial groups: Desulfobulbaceae and Desulfuromonadales. Two gene clusters that are associated with Desulfobulbaceae were determined to upregulate over 100-fold in response to higher EET rates [11]. In addition, a metagenomic analysis of MFC anodic biofilms that resulted from different anodic substrates was also carried out, and the result revealed that the abundance of genes related to cell motility, metal resistance, antibiotic resistance, and quorum sensing was significantly increased when the substrates were changed from acetate to landfill leachate [12]. Recently, Kiseleva et al. [13] analyzed the wholegenome metagenomic of anodic microbial communities from two pilot-scale MFC bioreactors fed with different distillery wastewaters operated under ambient conditions in distinct climatic zones. The results showed that Proteobacteria, Bacteroidetes and Firmicutes constituted the dominant core of the MFC microbiomes, and a range of species within the anode communities possessing the capacity for extracellular electron transfer. GeoChip-based functional gene arrays have been applied in analysis of microbial community structure, function and metabolic potential in nitrobenzene reduction biocathode [14]. However, different from metagenomic sequencing, GeoChip has limited gene probe and cannot cover the whole genes from metagenome library. Because information about genetic function in anodic microbial communities is still limited, the development of biological strategies for enhancing the power output is hindered.

As a widely used anionic biosurfactant, rhamnolipids have a significant effect on accelerating biofilm formation and enhancing the power output of MFCs [15]. Our previous study revealed that the adsorption of rhamnolipids on anode surface will cause the Frumkin effect, and resulting in an decrease of equilibrium potential as well as an increase of exchange current density [16]. On the other hand, an in-depth study on the effect of rhamnolipid on microbial communities and functional genes of anodic biofilms metagenomes can explain its mechanism at the gene level. Therefore, metagenomic analyses of anodic biofilms with/without rhamnolipids addition were carried out. The primary aims of this study included determining the following: (1) brief description of the key results in our previous study; (2) taxonomic complexity of microbial community in anodic biofilms; (3) Functional genome analysis of the dominant genera; (4) Function analysis of the metagenomes and electron flow.

2. Methods and materials

2.1. Construction and operation of air-cathode single-chambered MFCs

Air-cathode single-chambered MFCs were fabricated with plexiglass with outer dimensions of 5 (length), 5 (width) and 4 cm (height) and an effective working volume of 28 cm^3 (built-in cylinder, length 4 cm, diameter 3 cm) for each MFC [17]. Anodes were made of carbon fiber with a titanium wire as described by Logan et al. [18]; the outer diameter and length of MFCs were 3 cm. Cathodes of MFCs consisted of carbon cloth that was modified with 0.40 mg/m^2 of Pt catalyst. All MFC cascades were operated with an external resistance of 1000 Ω at 25 °C in a thermostatic room. Rhamnolipids (purchased from Victex Company, Daqing, China) that were applied in this study were characterized as a mixture of mono-rhamnolipid congeners and di-rhamnolipid congeners, and the purity of rhamnolipids was 90 ± 5%. The anolyte contained 1 g/L glucose, 17.1 mg/L $Na_2HPO_4 \cdot 12H_2O$, 3.0 mg/L KH_2PO_4, 0.3 mg/L NaCl, 0.494 mg/L $MgSO_4 \cdot 7H_2O$, 0.01 mg/L $CaCl_2$ and 10 mL of trace elements as described by Rabaey et al. [19]. The pH of the electrolyte was kept at 7.0–7.2.

Two groups of MFCs were run in triplicates. Each MFC was identically constructed, inoculated with 10% surplus sludge supernatant (centrifuged at 3500 rpm); this inoculant resulted in high resistance to process disturbance, a high versatility of substrates, and high power output. The sludge was anaerobic sludge that had been collected from the secondary settling tank of Wenchang Wastewater Treatment Plant, Harbin, China. Subsequently, in one group of the MFCs that was used as a control (MFC_{NR}), rhamnolipids were not added; another group of MFCs were dosed rhamnolipids on the 7th day after inoculation (MFC_R), and the concentration of rhamnolipids was 40 mg/L [16].

2.2. Electrochemical analyses and calculations

Real-time whole-cell voltage was determined using a data acquisition board (PISO-813, PCI-DAS Co., Ltd.) that was connected to a computer, and this voltage was recorded every thirty minutes. The anode and cathode potentials were monitored relative to a Ag/AgCl reference electrode (+0.197 V vs. standard hydrogen electrode). Polarization curves were measured using an electrochemical workstation (CHI 660e, Chenhua, Shanghai). Polarization curves were calculated using a linear sweep voltammetry (LSV) analysis. In LSV experiments, the working electrode was the anode, and the counter and reference electrodes were cathodes. The voltage range in LSV was swept from −1.2 V to 0 V at the scan rate of 1 mV/s. Current density (I) and power ($P = I \times V$) were calculated from the polarization curve data. Coulombic efficiency (CE), which is the ratio of charge that is calculated by integrating the current over time (C_p, Eq. (2)) to the theoretical charge based on the COD (chemical oxygen demand) removal (C_{th}, Eq. (3)) in MFC [20], was calculated as in Eq. (1)

$$CE = \frac{C_p}{C_{th}} \times 100\% \tag{1}$$

$$C_p = \int idt \tag{2}$$

$$C_{th} = \frac{FZ_{O_2}\Delta COD}{M_{O_2}} \tag{3}$$

where i (C/s) was the real-time current of the MFC, F ($F = 96{,}485$ °C/mol) was Faraday's constant, Zo_2 ($Zo_2 = 4$) was the number of electrons used in oxygen reduction, ΔCOD (in g) was mass of COD that was removed from glucose-anolyte through the treatment of MFC, and Mo_2 ($Mo_2 = 32$ g/mol) was the molecular weight of oxygen.

The t-test was adopted for comparing power density and CE between the two groups. The t-test was analyzed using OriginPro 2015. If $P < 0.01$, there is significant difference between the two groups. If $0.01 < P < 0.05$, the difference between the two groups is not very significant. If $P > 0.05$, there is no significant difference between the two groups.

2.3. Microscopic examination of anodic biofilms

SEM (Quanta 200, FEI, U.S.A.) was used to image the biofilms of carbon fiber brush anodes. The pretreatment of anodic biofilm samples was performed as described previously [21]. All samples were fixed with 2.5% glutaraldehyde at 4 °C for 2 h, dehydrated with a gradient of ethanol concentrations (20, 50, 70, 85, 95, and 100%) and isoamyl acetate (100%), then dried in a vacuum oven at room temperature for 4 days. After samples were dried, they were placed on aluminum foil and sprayed with alloy. The attached biomass was determined using modified bicinchoninic acid (BCA) protein assay kit (Sangon Biotech, Shanghai, China). The biomass samples were collected from the anode carbon fibers, which were cut off about 5 mm and rinsed with sterile distilled

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