Increasing intracellular releasable electrons dramatically enhances bioelectricity output in microbial fuel cells

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Abstract

Microbial fuel cell (MFC) is a sustainable energy source that can harvest electricity energy from organic wastes. However, its low electricity output remains the bottleneck for practical applications. Herein, we report a novel approach to increase extracellular electron transfer between bacteria and anodes, thus enormously enhancing the bioelectricity output in MFCs. We find that the abolishment of the lactate synthesis pathway increases intracellular releasable electrons, which are subsequently transferred to the anode via a secreted diffusive electron shuttle. Thereby, such genetically modified strain delivers a much higher and more stable electricity output than its parental strain in MFCs.

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1. Introduction

Microbial fuel cell (MFC) is a renewable technology that can convert organic wastes into electricity [1,2]. Bacteria catalyse the decomposition of carbon sources and transfer the released electrons to MFCs’ anodes via various extracellular electron transfer (EET) mechanisms [3,4]. However, the low electricity power output remains the major bottleneck for the practical application of MFCs. One strategy to increase the electricity output of MFCs is to enhance the efficiency of EET between bacteria and anodes. Researchers developed novel anodic materials with more efficient EET to produce higher electricity power output [5–8]. Alternatively, researchers engineered bacteria to facilitate EET [9–13]. For example, a mutant Escherichia coli strain was evolved in MFCs to secrete an electron shuttle and produce higher electricity than its wild-type strain [10].

Another strategy is to increase releasable intracellular electrons in bacteria, because more electron supply offers greater potential for bacteria to generate higher current output. However, this strategy was largely ignored. To this end, we explore a rational strategy that can increase releasable intracellular electrons by disrupting reductive metabolic pathways of bacteria, thus enabling increased electricity output. Electrons transferred to solid electrodes are generated by the bacterial central carbon metabolism [1,2]. At anaerobic conditions in MFCs, bacteria store intracellular electrons generated by fermentative oxidation of carbon sources in reductive metabolites to balance their intracellular redox state (left panel, Fig. 1A) [14]. Thus, a deletion of such reductive pathways could result in an excess of reducing equivalents (in the form of NADH, the carrier of electrons) (right panel, Fig. 1A) [14]. We show here that disruption of the lactate biosynthesis pathway in E. coli (the ldhA strain) [15] can increase the ratio of NADH/ NAD +, thus the intracellular NADH availability in anaerobic cultures. Upon inoculated in MFCs, LdhA can transfer the excessive intracellular electrons to the anode via a secreted diffusive electron shuttle (a quinone derivative), leading to an enormous increase in the bioelectricity output than its parental strain (BL21).

2. Materials and methods

Dual-chamber MFCs separated by nafion membrane were used. Carbon cloth (2 cm × 4 cm) was used for both anodic and cathode electrodes. The anodic chamber contained E. coli suspension in LB medium (peptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L) [11] supplemented with 5 g/L glucose as the electron donor, and the cathodic chamber was filled with 50 mM K3[Fe(CN)6] and KCl solution. A 2 kΩ external resistor was connected into the circuit of MFCs, and the potential was recorded by a digital multimeter. Electrochemical measurements were conducted using a CHI 660D electrochemical workstation (CH Instrument, Shanghai, China) with an Ag/AgCl–KCl saturated reference electrode.

Bacteria metabolites were analysed by using high performance liquid chromatography (HPLC) (Agilent, USA) equipped with a refractive...
index detector and a HPX-87H column (Bio-Rad, USA). Intracellular NAD$^+$ and NADH were analysed using NADH/ NAD$^+$ quantification kit (Biovision, USA). The supernatants from MFCs were analysed by a UV–vis spectrophotometer (Shimadzu, Japan), and a Spectrum Gx FT-IR Spectrometer (PerkinElmer Inc, USA).

3. Results and discussion

The *E. coli* BL21 strain produced a number of reductive end-products (including succinate, ethanol and lactate) in MFCs, among which lactate is dominant (data not shown). Lactate biosynthesis is a reductive pathway catalysed by lactate dehydrogenase using pyruvate as the substrate, which is accompanied by the oxidation of NADH to NAD$^+$ (an electron consumption pathway) (left panel, Fig. 1A). Thus, the disruption of lactate biosynthesis pathway is able to redirect the increased intracellular electrons to the anode (right panel, Fig. 1A). Lactate dehydrogenase in *E. coli* BL21 is encoded by the gene *ldhA*, which was disrupted by an one-step gene inactivation method [16]. Such gene deletion was further confirmed by the PCR analysis. Upon *ldhA* deletion, no detectable lactate was observed when *ldhA*− was cultured at anaerobic conditions (Fig. 1B). These results substantiated that the gene *ldhA* was eliminated in the genome of *ldhA*−, resulting in the abolishment of lactate biosynthesis.

According to our hypothesis, upon the abolishment of lactate synthesis pathway, the releasable intracellular electrons (in the form of NADH, the electron carrier) would be increased (Fig. 1A). Since the total intracellular NAD (NADH + NAD$^+$) level remains constant, an increase in the releasable intracellular electrons is associated with an elevation in the NADH/NAD$^+$ ratio. In the anaerobic shacking culture (i.e., in the absence of EET), the NADH/NAD$^+$ ratio in *ldhA*− (1.21 ± 0.02) is ~2 times of that in BL21 (0.59 ± 0.03) (Fig. 1C). The change of the intracellular redox state was also evidenced by the elevation of the ethanol/acetate ratio [16]: 0.28 ± 0.01 for BL21, and 0.51 ± 0.01 for *ldhA*− (Fig. 1C). These results are all in good agreement with our hypothesis that the abolishment of lactate synthesis pathway significantly increases intracellular releasable electrons in anaerobic culturing conditions.

In the presence of EET in MFCs, however, we observed that the intracellular redox state of *ldhA*− (NADH/NAD$^+$ = 0.53 ± 0.04) is similar to that of BL21 (NADH/NAD$^+$ = 0.49 ± 0.03) (Fig. 1D). In addition, the ethanol/acetate ratio was also at the similar level (~0.22) for both strains in MFCs (Fig. 1D). Because MFCs differ from anaerobic cultures by the existence of EET, the excessive intracellular electrons in *ldhA*− were thus transferred to the extracellular anode via EET in MFCs. Meanwhile, such EET also decreases the excessive intracellular electrons in *ldhA*− to a similar level to BL21, resulting in similar ratios of both NADH/NAD$^+$ and ethanol/acetate for the two strains (Fig. 1D). These observations verify that *ldhA*− redirects its excessive intracellular electrons to the anode in MFCs (Fig. 1A).

Next, the electricity output of *ldhA*− and BL21 were measured in two-chamber MFCs, respectively (Fig. 2A). We observed that the MFC inoculated with *ldhA*− barely delivered current in the first 48 h, following by a slow increase to 0.51 µAM/h at 110 h, which is in agreement of the trends of slow glucose consumption (18.2 µM/h) and by-products’ production. Notably, this current density can only maintain for ~12 h and vanished at about 170 h (Fig. 2A), accompanied by a significant cell death (evidenced by measuring cell viability with LIVE/DEAD BacLight bacterial viability kit and analysed by fluorescence spectrometer) and nearly halting on glucose consumption. Such cell death may be caused by the lack of electron respiration of *ldhA*− in the anode of the MFC. However, the current output of the MFC inoculated with *ldhA*− increased steadily until 90 h, and reached its plateau at ~110 h with a maximum current density of ~3.0 µAM/cm$^2$. It is noted that *ldhA*− produced a much higher maximum current density than that of BL21 (Fig. 2A), and with ~4 times higher glucose consumption rate (83.6 µM/h) than BL21. More importantly, *ldhA*− kept this high current steadily and continuously consumed glucose and produced by-products for a much long time (> 300 h), in contrast to the faded current output of BL21 at ~170 h (Fig. 2A). This high and stable current output in the MFC suggested that *ldhA*− could efficiently transfer its intracellular electrons to the anode.

We next determine the EET mechanism of *ldhA*− in the MFC. Cyclic voltammetry (CV) of *ldhA*− shows a pair of redox waves centred at ~−0.11 V, but not for BL21 (Fig. 2B). Such contrast is further confirmed by differential pulse voltamogram (DPV) analysis (Fig. 2C). In accordance, the open circuit potential of *ldhA*−-inoculated MFC (~−0.45 V) was much lower than that of BL21 (~−0.23 V). These
evidences imply that a redox compound is involved in the EET of ldhA\textsuperscript{−}. We further observed a linear relationship between the peak current and the square root of scan rates according to the CVs at variant scan rates (Fig. 2D), which revealed that the electrochemical redox reaction of ldhA\textsuperscript{−} is a diffusion-controlled process. Moreover, such diffusive electroactive reagent was also observed in the CV of cell-free supernatant (data not shown), which eliminated the possibility of EET via c-type cytochromes. Thus, the redox compound synthesized by ldhA\textsuperscript{−} is a diffusive electroactive reagent, acting as an endogenous electron shuttle in the MFC. We further found that the CV of 2-hydroxy-1,4-naphthoquinone (HNQ) showed a typical redox pair with its mid-point potential identical to that of the electroactive compound secreted by ldhA\textsuperscript{−} (Fig. 2B). Also, in the UV-vis spectrum, the supernatant of ldhA\textsuperscript{−} displayed almost identical absorption peaks to HNQ (Fig. 3A) and other quinone derivatives[17]. The FTIR spectrum also showed the existence of several characteristic bands of quinone in the supernatant of ldhA\textsuperscript{−} (Fig. 3B). Thus, the electron shuttle secreted by ldhA\textsuperscript{−} might be a hydroxyl contained derivate of quinone.

4. Conclusions
Abolishment of the lactate biosynthesis pathway in E. coli leads to an elevation of the pool of releasable intracellular electrons, which can be redirected to the anode mediated by a self-secreted, endogenous quinone-like electron shuttle. The bioelectricity output in the MFC is thus dramatically enhanced. The central metabolic pathways are conserved in many bacteria, thus engineering intracellular metabolism to facilitate EET and bioelectricity generation in MFCs may be applicable to other microorganisms.

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References