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Engineering of bacterial electrochemical activity with global regulator manipulation

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ABSTRACT

The electrochemical activity of electroactive bacteria is unique and essential for bioelectrochemical systems (BES) with promising energy and environment applications. However, the strategies for bacterial electrochemical activity modulation are still limited. Herein, a novel approach for engineering of bacterial electrochemical activity with global regulator (sigma factor RpoS) manipulation was developed. By knockout of *rpoS* in *Pseudomonas aeruginosa* PAO1, the biofilm formation on the electrode was enhanced and the bacterial cells were adapted to a new electron shuttle, which promoted the extracellular electron transfer and improved the performance of BES. This work demonstrated that global regulator manipulation could be efficient for electrochemical activity engineering and offered new opportunities for BES applications.

1. Introduction

The electroactive bacteria are a group of microorganisms that are able to exchange electrons between bacteria cells and solid surface, known as extracellular electron transfer (EET) [1]. These electroactive bacteria could be applied in bioelectrochemical systems (BES) for versatile applications including bioelectricity generation, biosensing, biodegradation and bioproduction [1–3]. However, the low electron transfer efficiency accounts one of the major limitations for most BES. Thus, exploring new strategies and techniques to improve the electron transfer efficiency between bacteria and electrode is urgently required [4–6].

Although most previous efforts focused on electrode material fabrication and modification [7–9], there are emerging interests to engineer electroactive bacteria with genetic tools to improve their electroactivity [10–13]. Despite various strategies developed in the previous works, these attempts principally aimed to endow the engineered electroactive bacteria with the single and well-defined phenotype. However, the electroactivity of bacteria is determined by a series of phenotypes. Thus, it is quite challenging to improve the electroactivity *via* genetic engineering of single or several specific function genes, and engineer the electroactive bacteria from the view of global gene regulation would be a more efficient strategy to be explored.

Pseudomonas aeruginosa is a well-recognized electroactive bacterial species due to its unique capability to produce phenazine derivatives to facilitate the EET [14,15]. The gene expression of *P. aeruginosa* is globally regulated by a series of global regulators including one of best-characterized sigma factor RpoS (σ^S) regulon [16]. Transcriptional profiling analyses revealed that more than 700 genes are regulated by RpoS in the stationary stage, which including the gene coding phenotypes that are critical for the electroactivity of *P. aeruginosa* like phenazine synthesis, motility and biofilm formation, rhamnolipid synthesis and microbial secretion [17,18]. Furthermore, sigma factors are group of regulators for general stress response in various bacteria, including *Geobacter* and *Shewanella* species [19,20], and are believed to play essential roles in energy metabolism of *Geobacter sulfurreducens* [21]. Thus, RpoS manipulation may not only change the phenotypes of *P. aeruginosa* and modulate its electroactivity, and more importantly, would potential expand the toolbox for genetic engineering of electroactive bacteria.

To this end, the effect of global regulator (RpoS) knockout on the electroactivity of *P. aeruginosa* was investigated in details. The electrochemical activity of *P. aeruginosa* PAO1 wild type strain and its *rpoS* mutant in microbial fuel cell (MFC, one kind of BES) were compared.

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Further analyses on phenazine production, voltammetry performance and biofilm formation were conducted and unveiled the mechanism of global regulator (RpoS) engineering for improvement on bacterial electroactivity.

2. Materials and methods

2.1. Strains and culturing

Wild type *Pseudomonas aeruginosa* PAO1 (*P. aeruginosa* PAO1) and its global regulator RpoS mutant (strain Δ*rpoS*) were used as MFC inoculum culture. The strain Δ*rpoS* was constructed as previous work [17].

Overnight bacteria medium (both wild type and the mutant) (0.5 ml) was inoculated into 50 ml LB broth (BD, USA) and aerobically cultivated in the shaker at 37 °C and 200 rpm. The bacteria were harvested by centrifuge (5000 rpm, 7 min) after 8 h' incubation. The bacteria residue was then suspended by M9 salt medium and 400 μl suspension was inoculated in the MFC anode chamber. All the chemicals were purchased from Sigma-Aldrich (USA) and Merck (USA) unless otherwise indicated.

2.2. MFC setup and electrochemical measurement

Dual-chamber MFCs with carbon cloth (2 × 3 cm) as anode and cathode were used in this work, as our previous work [12]. M9 medium with 4 g/L glucose was used as the anodic medium and 50 mM ferricyanide was used as catholyte. All the MFCs were operated anaerobically and three parallel MFCs were set up for both strains.

After inoculation, MFCs were continuously discharged with a constant load of 2 KΩ. Output potential was recorded by a digital Multimeter (ESCORT 3146A). Cyclic Voltammetry (CV) analyses were conducted on the 8th day in a three-electrode system by CHI 660D electrochemical workstation (CH Instrument, China), with a scanning rate of 50 mV/s and 1 mV/s from − 0.7 V to 0.4 V (*vs.* Saturated Calomel Electrode (SCE)).

2.3. Phenazines analyses and biofilm characterization

Phenazines were analyzed with high performance liquid chromatography (HPLC) as previous report with minor modification [22]. Briefly, filtered samples were analyzed by an Agilent HPLC system equipped with Agilent C18 analytical column and a photodiode array detector. Phenazines were separated by gradient of water-0.1% acetic acid (solution A) and acetonitrile-0.1% acetic acid (solution B) at the flow rate of 1 ml/min.

The anode biofilm morphology was observed by a JEOL field emission scanning electron microscope (FESEM, JSM-6700F-FE-SEM, Japan), as with our previous work [12].

3. Results and discussion

To study the effect of *rpoS* on bacterial electroactivity, *P. aeruginosa* PAO1 and its *rpoS* mutant (Δ*rpoS*) were used as the pure culture inoculum of MFCs and the MFC current output for the consecutive batch operation was compared (Fig. 1). The output current density of PAO1 reached a plateau of 3.0 μA/cm^2 after 103 h. Meanwhile, the steady output current of MFC with strain Δ*rpoS* was around 4.2 μA/cm^2, with a peak current density of 4.5 μA/cm^2 achieved at 50 h. Thus 50% higher current output was obtained by knockout the sigma factor RpoS of *P. aeruginosa*. Interestingly, this sigma factor manipulation also reduced the time to reach the maximum current (50 h *vs.* 103 h), which could also evidence the improved electroactivity by *rpoS* knockout [23].

Besides the previous achievements in engineering the single gene with defined phenotype change to improve the bacterial electroactivity, the global regulator manipulation (sigma factor RpoS knockout in this study) may bring more complicated influence since plural phenotypes that are keenly related with bacterial electroactivity may involve (as illustrated in Fig. A.1 in supporting information). As *P. aeruginosa* could synthesize phenazines for EET, the phenazine production was first characterized to study whether the enhanced electroactivity of Δ*rpoS* was due to improved phenazine production. It is interesting that phenazine-1-carboxylic (PCA) was detected in the supernatant of both strains after aerobic cultivation (with a retention time of 13.7 min in the HPLC profile, Fig. 2a & b), meanwhile pyocyanin (PYO) was observed only in the supernatant of strain Δ*rpoS* (with a retention time of 6.0 min). The improved PYO synthesis is reasonable as *rpoS* knockout can activate *rhl* quorum sensing system and improves PYO synthesis [24].

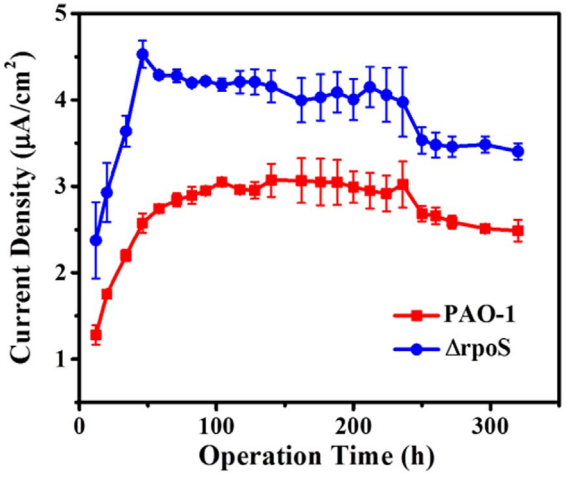

Fig. 1. V-t curves of MFC inoculated with *P. aeruginosa* PAO1 and its *rpoS* knockout strain Δ*rpoS*.

However, phenazine production in MFC anode significantly differed with that in aerobic cultivation (Fig. 2c & d). Slightly more PCA (around 50%) was produced by PAO1 than strain Δ*rpoS* in MFC anode. Meanwhile no PYO was identified for both strains. The different phenazine profiles of strain Δ*rpoS* in aerobic cultivation and MFC anode implied that sigma factor manipulation may influence the bacterial phenotypes in different ways when it is used in MFC compared with that it is cultivated in aerobic conditions.

In order to elucidate the underlying mechanism of enhanced electroactivity, CV analyses were performed when the current output reached its steady state (Fig. 3). When the CV analyses were conducted with a scanning rate of 50 mV/s, the peak current can be considered as the relative abundance of redox species at the interface between biofilm and electrode [12]. The well-defined peak belongs to PCA (at − 0.28 V) was observed both in PAO1 and Δ*rpoS* (Fig. 3a) [25]. Meanwhile the peak for PYO (− 0.23 V at neutral pH) cannot be discriminated in both strains, which was consistent with HPLC analyses. The anodic peak current densities of PCA were 24.3 and 27.3 μA/cm^2 for PAO1 and Δ*rpoS*, indicating a similar PCA electroactivity for both strains. The obvious inconsistence of PCA abundance in the supernatant (PAO1 produced more PCA) and biofilm-electrode interface implies that there may be a substantial difference between the biofilm of PAO1 and Δ*rpoS*, since biofilm could promote the phenazine accumulation at electrode surface and reduce its supernatant concentration [26].

For well-catalyzed microbial anode, turnover current becomes the major part of the response current and the shape of CV curve will transform from symmetrical peaks to sigmoid shape at a slow scanning rate, which could help to study the electroactivity of specific bacteria [27]. CV analyses with slow scanning rate of 1 mV/s were thus conducted to investigate how *P. aeruginosa* strains could utilize the specific redox species for bioelectricity generation (Fig. 3b). It was found that the peak corresponding to PCA (R_1 in Fig. 3b) reached a plateau at