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Bioelectricity enhancement via overexpression of quorum sensing system in *Pseudomonas aeruginosa*-inoculated microbial fuel cells

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ABSTRACT

Low electron transfer efficiency from bacteria to electrodes remains one of the major bottlenecks that limit industrial applications of microbial fuel cells (MFCs). Elucidating biological mechanism of the electron transfer processes is of great help in improving the efficiency of MFCs. Here, we reported that *Pseudomonas aeruginosa* could use different electron shuttles in a MFC under different quorum sensing (QS) expression patterns. An electron shuttle (rather than phenazines) with a high mid-point potential of 0.20 V (vs. Ag/AgCl–KCl saturated electrode) was found to be the dominating shuttle in a wild-type *P. aeruginosa* strain. Strikingly, upon genetic overexpression of *rhl* QS system in this wild-type strain, the electron shuttle was substituted by phenazines (pyocyanin and phenazine-1-carboxylate, with a low mid-point potential of −0.17 V and −0.28 V, respectively), which directly resulted in an increase of about 1.6 times of the maximum current of the *rhl* overexpressed strain over the wild-type strain. Our result implied that manipulating electron transfer pathways to improve MFCs' efficiency could be achieved by rewiring gene regulatory circuits, thus synthetic biology strategies would be adopted.

1. Introduction

Microbial fuel cells (MFCs) are promising for simultaneous clean energy generation and pollutants treatment (Angenent et al., 2004; Du et al., 2007; Logan, 2009; Logan et al., 2006; Lovley, 2008; Osman et al., 2010; Qiao et al., 2010; Rabaey and Verstraete, 2005; Zhao et al., 2009). Microbes and microbial consortia in the anode of MFCs could oxidize a diverse array of organic substrates (including organic pollutants in wastewater) to generate and transfer electrons to inertial solid electrodes, by which the conversion of chemical energy to electricity is achieved (Angenent et al., 2004; Du et al., 2007; Lovley, 2008; Qiao et al., 2010; Rabaey and Verstraete, 2005). A number of electrochemically active bacteria (EAB) capable of transferring electrons to electrodes have been identified, including *Geobacter*, *Shewanella*, *Pseudomonas* and *Escherichia coli*, etc. (Bond et al., 2002; Li et al., 2010; Logan, 2009; Qiao et al., 2008; Rabaey et al., 2005). Two categories of electron transfer strategy were established, *i.e.*, direct electron transfer by redox outer membrane proteins (c-type cytochromes) or conductive nano-wire, and mediated electron transfer through exogenous or endogenous electron shuttles (Jiang et al., 2010; Logan, 2009; Osman et al., 2010; Reguera et al., 2005; Schaetzle et al., 2008; Schröder, 2007; Watanabe et al., 2009).

Bacteria may adopt different electron transfer systems under different conditions (Busalmen et al., 2008; Peng et al., 2010; Rabaey et al., 2004; Wagner et al., 2010). Thus, manipulating bacteria to adopt the optimal electron transfer system that can lead to reinforced current output is a feasible strategy for the optimization of MFCs (Busalmen et al., 2008; Peng et al., 2010; Wagner et al., 2010). Recent findings suggested that bacteria could use different electron transfer systems when the anode was set to different potentials, and significant current enhancement may be achieved by optimizing the anode potential (Busalmen et al., 2008; Peng et al., 2010; Wagner et al., 2010). For example, by poising the anode potential at 0.1 V (vs. Ag/AgCl–KCl saturated electrode (Ag/AgCl)) in the *Geobacter*-based MFC, a c-type cytochrome with a formal potential at −0.08 V was identified to be the major electron transfer agent for current generation. However, another c-type cytochrome with the potential centered at 0.48 V was detected by poising the anode potential at 0.6 V, which led to a doubled current output in comparison to that of poising the electrode potential at 0.1 V (Busalmen et al., 2008). Peng et al. (2010) observed that *Shewanella* adopted a redox pair of c-type cytochrome for its electron transfer when the anode potential was poised at 0 V (vs. saturated calomel electrode (SCE)). However, such active cytochrome vanished and the electron transfer agent was substituted by a diffusive electron shuttle (flavin) when the electrode was poised at −0.24 V, which

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led to a much higher current density output than that of poising the anode potential at 0 V (Peng et al., 2010). However, such current increase resulted from posing anode potential may not necessarily lead to an enhancement in the output power, since output power is potential difference × current. Alteration of electron transfer systems (either c-type cytochromes or electron shuttles) could be achieved via manipulating electrode potentials, which essentially altered genetic regulatory networks in an indirect fashion. Therefore, direct manipulating genetic networks would be expected to be an alternative way for the optimization of bacterial electron transfer systems.

Quorum sensing (QS) is a wide-spread cell-cell communication system, controlling diverse phenotypes in numerous microorganisms (Atkinson and Williams, 2009; Fuqua et al., 2001). *Pseudomonas aeruginosa*, a most adaptable EAB with the advantage of its capability of using a wide-spectrum of substrates for electricity generation in MFCs (Rabaey et al., 2005; Venkataraman et al., 2010), is one of the most intensively studied model strains for QS (Juhas et al., 2005). QS systems regulated the transcription of over 300 genes in *P. aeruginosa*, representing about 6% of its genome (Atkinson and Williams, 2009; Schuster et al., 2003). The *rhl* QS system is one of the main QS system in *P. aeruginosa* (Juhas et al., 2005), which is comprised of the transcriptional activator RhlR and the *N*-butyryl homoserine lactone (BHL) synthase RhlI. It involved in many important biological processes, such as biofilm formation and virulence factor production (Juhas et al., 2005). However, the effect of *rhl* QS system on the behaviors of *P. aeruginosa* in MFCs was not studied.

Here, we studied how the genetic overexpression of the *rhl* QS cassette impacts the electrochemical activity of *P. aeruginosa* in the MFC. We found that the wild-type *P. aeruginosa* strain CGMCC1.860 used a redox compound with the high mid-point potential of 0.20 V (vs. Ag/AgCl) as its major electron shuttle, instead of phenazines. Strikingly, overexpression of *rhl* QS system eliminated this redox compound, and led to production of pyocyanin (PYO) and phenazine-1-carboxylate (PCA) (with a much lower mid-point potential of −0.17 V and −0.28 V, respectively). Such change of electron shuttles enabled the *rhl* overexpressed strain generate 1.6 times higher current output than the wild-type strain.

2. Materials and methods

2.1. MFC set-up and electrochemical measurements

A dual chamber MFC (the size of each chamber being 5.5 cm (width) × 5.5 cm (length) × 6 cm (height)) separated with nafion 117 membrane was used in this work (Qiao et al., 2008). Carbon cloth (2 cm × 4 cm) was used for both anode and cathode electrodes. An Ag/AgCl–KCl saturated electrode (CH Instrument, Shanghai, China) was inserted into each chamber as reference electrode. The anodic medium was M9 medium (Sambrook and Russell, 2001) supplemented with 4 g/l glucose as carbon source and electron donor. The cathodic solution was 50 mM $K_3[Fe(CN)_6]$ in 50 mM KCl solution. For current generation measurement, a 2 KΩ external resistor was connected into the circuit of MFC, and the potential of the MFC was recorded by a digital multimeter (ESCORT 3146A). All other electrochemical measurements were conducted using CHI 660D electrochemical working station (CH Instrument, Shanghai, China).

2.2. Bacteria and growth conditions

Considering the potential practical application of MFCs for wastewater treatment, the wild-type strain *P. aeruginosa* CGMCC 1.860, an environmental isolate with high pollutants

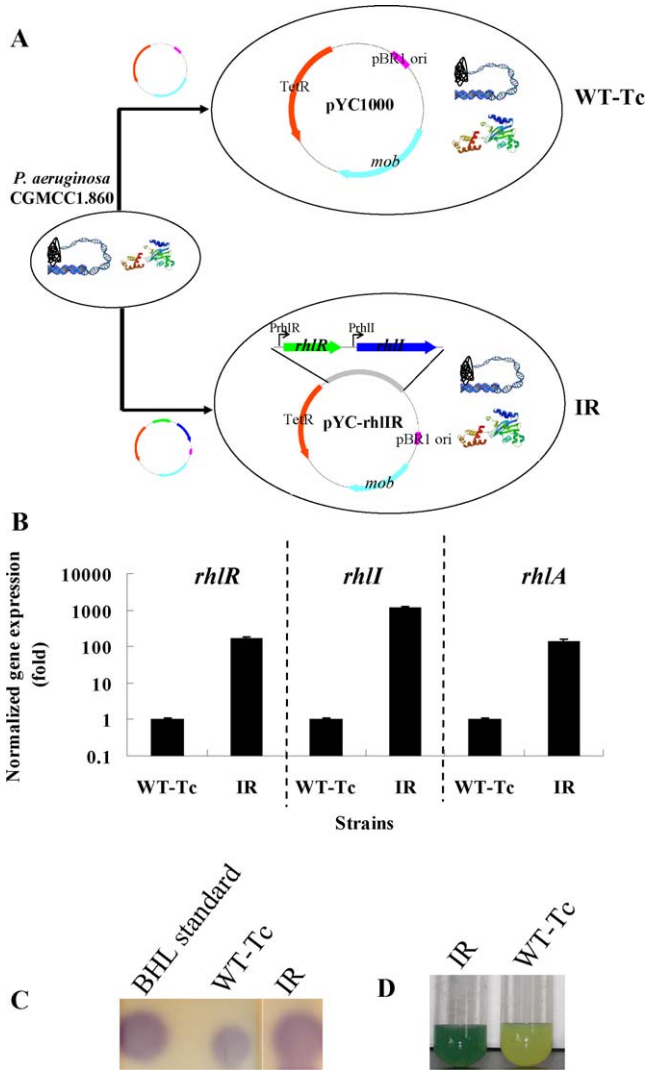

Fig. 1. Design of *P. aeruginosa* strain with overexpression of the *rhl* QS system. (A) The genetic construction (plasmid maps) of the strains WT-Tc and IR. The strain WT-Tc has a void plasmid of pYC1000; the strain IR has a multicopy plasmid that contained *rhlI* and *rhlR* genes. (B) Comparative analysis of *rhlI* and *rhlR* gene transcription (by real-time quantitative PCR) in the strains WT-Tc and IR. (C) BHL production in the strains WT-Tc and IR detected by the biosensor strain *Chromobacterium violaceum* CV026. (D) Phenazines pigment production in LB medium by the strains WT-Tc and IR.

biodegradation capabilities (Sun et al., 2006; Yong and Zhong, 2010), was used in this study. The *rhlIR* overexpressed strain (designated as IR) was constructed by transforming multi-copy broad-host plasmid pYC-rhlIR into *P. aeruginosa* CGMCC 1.860 (Fig. 1A). The control strain (designated as WT-Tc) is the wild-type strain transformed with the vacant plasmid pYC1000 (Fig. 1A). Plasmid construction (Fig. S1) and transformation were performed as described in the Supplementary Materials. Bacteria were routinely grown with shaking in LB broth at 37 °C and preserved at −70 °C in 15% glycerol. Tetracycline (250 μg/ml) was used for plasmid maintenance in *P. aeruginosa*. For the MFC inoculation, 1.5 ml of overnight *P. aeruginosa* cultures were inoculated in 150 ml LB broth, and incubated with shaking at 37 °C until the optical density at 600 nm (OD600) reached about 1.0. The cell pellets were harvested by centrifuge (5000 × *g* × 5 min) and washed with M9 medium for three times. Then, the cell pellets were resuspended with 150 ml M9 medium. The cell suspension were transferred into the anodic chamber of the MFC and purged with nitrogen gas for 30 min to remove the dissolved oxygen. The anodic chamber was