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Modifying the endogenous electron fluxes of *Rhodobacter sphaeroides* 2.4.1 for improved electricity generation

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ABSTRACT

The purple bacteria *Rhodobacter sphaeroides* serve as a promising biocatalyst in the photo-microbial fuel cell system (photo-MFC). This gram-negative species performs highly efficient anoxygenic photosynthesis that ensures an anaerobic environment in the anode compartment. Previous studies incorporating *R. sphaeroides* into photo-MFC were conducted using platinum as the anode electrode. In this study, we detected a steady current generation of *R. sphaeroides* in a bioelectrochemical system where glassy carbon was the working electrode and a typical growth medium was the electrolyte. The bioelectricity generation synchronized with the supplementation of reduced carbon source and showed immediate response to illumination, which strongly indicated the correlation between the observed current and the cytoplasmic quinone activity. Modifications of the endogenous electron flows mediated by quinone pool are shown to have significantly enhanced the bioelectricity generation. We anticipate that the findings in this study would advance future optimization of *R. sphaeroides* as an anode strain, as well as facilitate the study of bioenergetics in photosynthetic bacteria.

1. Introduction

Solar powered microbial fuel cells (photo-MFC) are potential candidate for harnessing solar energy as a clean and environmental-friendly energy source. In a conventional MFC, the generation of bioelectricity is achieved by using microorganisms in the anode as a cheap and regenerative biocatalyst [1]. By applying phototrophic inoculum, solar energy can be incorporated into central metabolism of anode microbes and contribute directly or indirectly to the bioelectricity generation. Photo-bioelectricity generation takes many forms: the synergistic relationship between photoautotrophic species and electroactive heterotrophic consortium [2–4]; solely using phototrophic species through either electron shuttle mediation [5–8] or direct electron transfer [9,10]. Among various photosynthetic microorganisms, cyanobacteria have raised most attention owing to their remarkable light harvesting capability and self-sustainability by fixing atmospheric CO_2 into carbohydrate, which in turn is transformed to bioelectricity through cellular metabolism [11,12]. However, cyanobacteria generates oxygen as a photosynthetic byproduct, which scavenges electrons and severely reduces the current collected via external circuit [13]. Consequently, it is of great interests to search for alternative photosynthetic species that produces bioelectricity in an anoxygenic manner.

Rhodobacter sphaeroides, a purple non-sulfur bacterium that performs highly efficient anoxygenic photosynthesis, could serve as a potential biocatalyst in photo-MFC. Under high oxygen tension, *R. sphaeroides* carries out chemoheterotrophic growth using oxygen as the terminal electron acceptor. Yet this species is most adapted to anaerobic photoheterotrophic growth when a series of organic compounds are available for generating energy. The well-established culturing methods and genetic manipulation tools have paved ways for the study of *R. sphaeroides* as a model organism in bacterial photosynthesis. Recently, several models have also been developed to investigate metabolic networks in *R. sphaeroides*. This increasing availability of genetic and physiological resources have prompted researchers to utilize *R. sphaeroides* as a platform for various applications, such as bio-hydrogen production [14–16], polyhydroxybutyrate (PHB) production [17,18], membrane protein production [19,20] and bio-remediation [21].

Previously, bioelectricity generation has been achieved using *R. sphaeroides* as the anode microbe through its hydrogen

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Table 1
Primers used in this study for plasmic construction.

Purpose	Primer	Sequences (5′-3′)
Amplifying *ubiG* for Gibson assembly	UF	GAGAAATTAACCATGGAATCGTCCAGCACCATCGAC
	UR	TAATTAAGCTTAGCTGCGCCGCACGCTCGCGGTAAC
Amplifying *sdh* for Gibson assembly	SF	AATTAACCATGGCTGAAACGAAGCGGGTCGAG
	SR	CTAATTAAGCTTAGATGACGCGTTCGACCATCATC
Amplifying pIND4 for Gibson assembly to *ubiG*	pIND-UF	GTGCGCGCAGCTAAGCTTAATTAGCTGAGCTTGGAC
	pIND-UR	GGACGATTCCATGGTTAATTTCTCCTCTTTAATTC
Amplifying pIND4 for Gibson assembly to *sdh*	pIND-SF	CGCGTCATCTGAGAGGATCCAGATCTCATCACCATC
	pIND-SR	CGCGTCATCTAAGCTTAATTAGCTGAGCTTGGAC

producing mechanism. When *R. sphaeroides* is grown under photoheterotrophic conditions with a limited nitrogen source, a molybdenum containing nitrogenase can functionally catalyze the formation of hydrogen from protons to dissipate excessive electrons from the ubiquinone pool [22]. The *in-situ* oxidation of hydrogen at the anode subsequently results in the bioelectricity generation. However, these photo-MFCs require expensive catalysts for hydrogen oxidation, such as platinum, which is prone to inactivation under dirty microbial environment. Additionally, redox active polymers have been introduced to facilitate the electrochemical communication between a phylogenetically close species, *Rhodobacter capsulatus*, and carbon electrode without involving hydrogen [23]. However, this experiment was conducted in dark and aerobic condition, and the performance of this system under anaerobic light condition was undocumented.

In this study, we report a steady current generation of wild type *R. sphaeroides* during chronoamperometric measurement without involving the *in-situ* oxidation of hydrogen. Using glassy carbon as the anode and Sistrom's minimal medium as the electrolyte in a three electrode system, the bioelectricity generation synchronized with the supplementation of reduced carbon source and showed immediate response to illumination. Our results show that the genetic manipulation of endogenous electron transfer pathways in *R. sphaeroides* has significantly affected the bioelectricity output, which strongly supports a correlation between observed current and cytoplasmic Quinone pool (Q)—mediated electron chains. This study is to our knowledge the first report to utilize genetic engineering tools for improving efficiency of a photo-MFC, which motivates further investigations into anode strain optimization. The observed correlation between current output and electron transport chain manipulation should facilitate study of bioenergetics in photosynthetic bacteria.

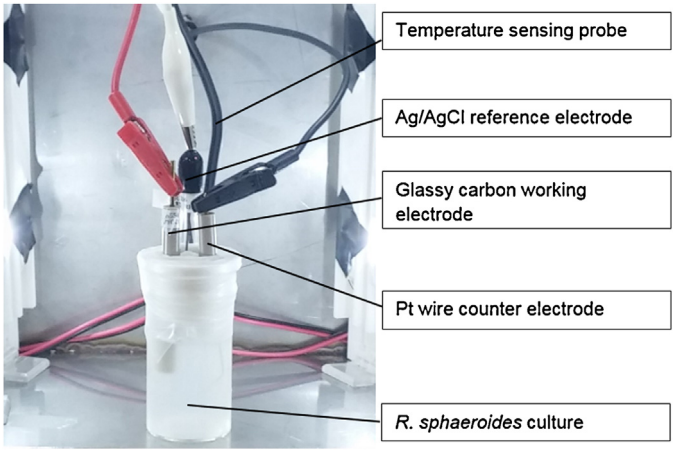

Fig. 1. Light Chamber for chronoamperometry current measurement of *R. sphaeroides*. 8 White LED light bulbs (BackLED S Plus, Osram) were used as the light source in this study.

2. Materials and methods

2.1. Organisms and growth conditions

R. sphaeroides 2.4.1 was purchased from American Type Culture Collection (ATCC). The mutant HPC was kindly donated by Prof. Mark Gomelsky [15]. *R. sphaeroides* and mutants strains (HPC, SDH, UbiG) were grown aerobically or photoheterotrophically in Sistrom's succinate minimal medium [24] at 30 °C under a shaking speed of 250 rpm. For photoheterotrophic growth, cultures were illuminated by LED light (White LED bulbs, BackLED S Plus, Osram) of 17 Wm^{-2}. *Escherichia coli* S17-1 (ATCC 47055) was grown in LB medium at 37 °C. Antibiotics were used at the following concentrations for *E. coli* culture, kanamycin (Km), 30 μg/mL; for *R. sphaeroides* culture, kanamycin (Km), 25 μg/mL; tellurite, 50 μg/mL.

2.2. Plasmid construction and conjugation

To overexpress the 3-demethyl ubiquinone-9, 3-methyltransferase (UbiG) in strain RSUBIG, *ubiG* (GenBank Accession No.: NC_007493) was amplified with primers ubiG_F/R (Table 1). The expression plasmid pIND4 was linearized using PCR with primers pIND (ubiG) _F/R, followed by purification and DpnI digestion. The linearized vector and the gene were assembled using Gibson Assembly kit (NEB) to produce pIND-UbiG. pIND-ubiG was further transformed into *E. coli* strain S17-1 for conjugation.

For strain RSSDH, where succinate dehydrogenase is overexpressed, a 3980 bp polycistronic gene cluster encoding the *R. sphaeroides* succinate dehydrogenase (GenBank Accession No.: NC_007493 region: 2374936.2738916) was amplified and assembled into pIND4 using primers sdh_F/R, and pIND(sdh) _F/R. The resultant plasmid pIND-sdh was then transformed into strain S17-1 for conjugation.

Conjugation was used to transfer the recombinant plasmids into *R. sphaeroides* as described previously [20]. Both donor cells (*E. coli* S17-1 containing target vector) and recipient cells (*R. sphaeroides*) were grown to an early log phase (OD_{600} ~0.3). 20 mL of donor cells and recipient cells were collected and gently resuspended in 500 μL LB medium. 100 μL of the cell mixture of mating ratio ~1:1 was transferred on a pre-dried LB-agar plate and incubated at 30 °C overnight. The plate was then flushed with LB medium to collect the biofilm on the surface. Cell suspension was plated on Sistrom's agar with potassium tellurite (50 μg/mL) and respective antibiotics for the target vector. Plates were incubated at 30 °C for at least 3 days. Positive exconjugants were inoculated into Sistrom's medium supplemented with antibiotics.

2.3. Bioelectrochemical current measurements

The bioelectrochemial current measurement was carried out by means of Chronoamperometry (CA) under potentiostatic control (CBP bipotentiostat system/Pine Research Instrumentation) using a three-electrode configuration with a glassy carbon working