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Electricity generation by *Shewanella* sp. HN-41 in microbial fuel cells

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ABSTRACT

Microbial fuel cells (MFCs) provide new opportunities for energy generation through conversion of organic matter to electricity by electricity-generating bacteria. In this study, *Shewanella* sp. strain HN-41 was described as an exoelectrogen that had the ability of extracellular electron transfer in MFCs fed with lactate or glucose. The maximum power density produced by the strain HN-41 in lactate- and glucose-fed single-chamber MFCs reached 71.6 and 18.2 mW m^{-2}, respectively. The strain showed strong capability to reduce Fe(III) with lactate or glucose as electron donor during the initial incubation period, and secreted flavin mononucleotide (FMN), riboflavin, and traces of flavin adenine dinucleotide in MFCs. Addition of riboflavin and FMN as electron mediators contributed to 2–5 folds increase in power density. These findings on the ability of *Shewanella* sp. HN-41 to couple oxidation of glucose contributed to the expansion of our knowledge on utilization of carbon source by *Shewanella* sp.

1. Introduction

As a bioelectrochemical device, microbial fuel cells (MFCs) can efficiently convert organic compounds, wastewater, and renewable biomass to electricity. Bacteria that are capable of transferring extracellular electrons oxidize organic matter and transport the electrons to the anode in MFCs [1]. *Shewanella* sp. is the most-studied exoelectrogenic bacterium used for electricity production in MFCs [2,3]. These bacteria can respire a diverse range of organic substrates and reduce soluble or insoluble metal complexes during their catabolic metabolism. Although *Shewanella oneidensis* MR-1, a typical exoelectrogen, is not capable of metabolizing saccharides as a carbon source for extracellular electron transfer, later studies have demonstrated that several *Shewanella* sp., including *S. baltica*, *S. frigidimarina*, and *S. japonica* could oxidize glucose under aerobic conditions [4,5]. Nevertheless, electricity generation by *Shewanella* sp. in MFCs using glucose as the sole electron donor has been rarely reported [5].

The major mechanisms of extracellular electron transfer by exoelectrogens in MFCs are as follows. (1) The electrons are directly transferred from the outer surface of c-type cytochromes to the electrode [6,7]. (2) Some microorganisms could excrete soluble redox molecules that serve as "electron shuttles" to mediate electron transfer between the bacterial cells and electrode [8–11]. (3) The electrically

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conductive pili or nanowires promote electron transfer across the multilayer biofilms on anodes [12,13]. As an electron shuttle, flavins can mediate extracellular electron transfer [8], and some microorganisms have been found to have the ability to use flavins for extracellular electron transfer [14]. *Shewanella*, as an exoelectrogen, can not only secrete flavins, but can also produce higher power output by utilizing exogenous flavins.

In the present study, electricity generation and iron reduction by an electricity-producing bacterium, *Shewanella* sp. HN-41, using glucose or lactate as the sole electron donor were investigated. Flavins secreted by strain HN-41 were measured under different culture conditions, and the effect of exogenous flavins as electron shuttle on extracellular electron transfer by strain HN-41 was examined.

2. Materials and methods

2.1. Strain and culture medium

Shewanella sp. HN-41 was obtained from Applied and Environmental Microbiology Laboratory of Gwangju Institute of Science and Technology. The strain was cultured aerobically at 30 °C in Luria–Bertani (LB) medium (pH 7.0) in a shake flask. Prior to inoculation into MFCs, the cells were harvested by centrifugation and washed thrice in 50 mM PIPES–NaOH buffer (pH 7.0). The washed cells were resuspended in *Shewanella* medium to the desired cell concentration (OD_{600} of 0.6). The *Shewanella* medium consisted of the following (per liter): 10 mM glucose or 10 mM lactate, 15.1 g of PIPES, 1.5 g of NH_4Cl, 0.6 g of NaH_2PO_4, 3 g of NaOH, 0.1 g of KCl, 5.8 g of NaCl, 10 ml of trace mineral mix, and 10 ml of vitamin mix (pH 7.0).

2.2. MFC configuration and operation

Electricity generation by strain HN-41 was evaluated by using single-chamber air-cathode and two-chamber MFCs equipped with a carbon cloth anode (7 cm^2). The air-cathode single-chamber MFCs (4 cm long, 3 cm diameter, 25 mL volume) were used as previously described [15]. The air-cathode was a (30% wet-proofed) carbon cloth (type B, E-TEK, 7 cm^2) coated with 0.5 mg cm^{-2} of Pt (20 wt% Pt/C) and a Nafion (5%, Dupont) binder on the water-facing side. The two-chamber reactors were constructed using two 12.5-ml cubic cells connected by a cation exchange membrane CMI-7000S (Membranes International, Glen Rock, NJ, USA), which separated the liquid in the anode and cathode chambers of the MFCs, but allowed the protons to pass between the chambers [16]. The anode chamber was filled with *Shewanella* medium with 50 mM PIPES–NaOH solution. The cathode chamber contained 50 mM $K_3Fe(CN)_6$ in 50 mM phosphate buffer (2.77 g L^{-1} of $NaH_2PO_4 \cdot 2H_2O$, 11.55 g L^{-1} of $Na_2HPO_4 \cdot 12H_2O$, 0.31 g L^{-1} of NH_4Cl, and 0.13 g L^{-1} of KCl). Three duplicate MFCs for each substrate and each type of reactor were operated for 20 days. The cell voltage (V) across a 1000-Ω external resistor in the MFCs circuit was monitored every 30 min using a multimeter, and was automatically recorded in a personal computer by a data acquisition system (model 2700 with 7702 module; Keithley Instruments Inc.). The reactors were sterilized before the operation in an autoclave at 121 °C for 15 min.

2.3. Electrochemical analysis

The anode potential was successively poised at +200 mV against the Ag/AgCl reference electrode by connecting to the working electrode of a potentiostat (Model WMPG1000 Multichannel Potentiostat/Galvanostat, Korea), while the cathode was used as a counter electrode, with each potential operated for two consecutive cycles. One cycle period was defined as 24 h. Power densities of MFCs were determined based on linear sweep voltammetry (LSV) using a potentiostat (Model WMPG1000 Multichannel Potentiostat/Galvanostat, Korea) from −0.6 to −0.05 V at a rate of 0.1 mV s^{-1}, which allowed obtaining a peak curve of power density.

2.4. Chemical analysis

For the determination of protein concentrations, the bacterial cells were harvested by centrifugation at 12,000 rpm for 10 min and washed twice with 50 mM PBS (pH 7.0). The washed cells were resuspended in 0.1 M NaOH and heated in boiling water for 10 min to obtain cell lysate. This lysate was centrifuged at 12,000 rpm for 5 min and the suspension was used for protein determination [17]. Protein concentrations in the planktonic cells in MFCs and pure culture were monitored using the Modified BCA Protein Assay Kit (Sangon Biotech, Shanghai, China), according to the manufacturer's instructions. Furthermore, the protein concentrations in the tested samples were calculated according to the protein standard solutions ranging from 0 to 1.0 g L^{-1}.

The suspension solution was filtered through a 0.22-μm membrane filter, and cell-free samples were analyzed for flavins using reversed-phase high-performance liquid chromatography (RP-HPLC; LC-10A, Shimadzu Corporation, Japan) with a UV–VIS detector (SPD-10A, Shimadzu, Japan), as previously described [9]. The concentrations of Fe(II) and Fe(III) were measured according to the ferrozine assay, as previously described [18]. Fe(III) was reduced to Fe(II) by using hydroxylamine hydrochloride, and then determined as described earlier.

3. Results and discussion

3.1. Cell growth and iron reduction using lactate or glucose as the sole electron donor

Strain HN-41 was found to have the ability to oxidize lactate, glucose, and yeast extract and simultaneously secrete flavins with oxygen as the electron acceptor (Fig. S1, Supporting Information). In order to measure the iron reduction capacity, strain HN-41 was grown at 30 °C in anaerobic culture tubes containing *Shewanella* medium consisting of lactate (10 mM), glucose (10 mM), or yeast extract (1.8 g/L) and ferric citrate (10 mM). The Fe(III) reduction rate showed a quick increase with lactate or glucose as the electron donor during the initial incubation period (0–20 h), followed by a slow increase after 24 h. The reduction rate with glucose as the electron donor