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Production of electrically-conductive nanoscale filaments by sulfate-reducing bacteria in the microbial fuel cell

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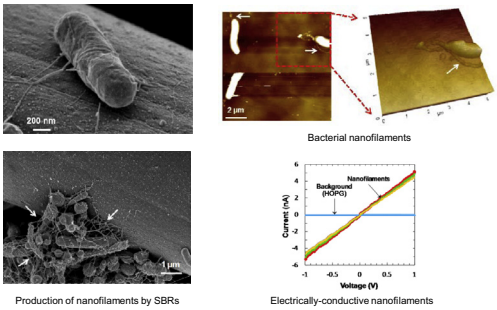
HIGHLIGHTS

- Sulfate-reducing bacteria produce nanoscale filaments for extracellular electron transfer.
- These nanofilaments were electrically-conductive.
- Nanofilaments can transfer electrons directly to insoluble extracellular electron acceptors.
- Bacterial nanofilament is an alternative strategy to use insoluble electron acceptors.

GRAPHICAL ABSTRACT



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ABSTRACT

This study reports that the obligate anaerobic microorganism, *Desulfovibrio desulfuricans*, a predominant sulfate-reducing bacterium (SRB) in soils and sediments, can produce nanoscale bacterial appendages for extracellular electron transfer. These nanofilaments were electrically-conductive (5.81 $S \cdot m^{-1}$) and allowed SRBs to directly colonize the surface of insoluble or solid electron acceptors. Thus, the direct extracellular electron transfer to the insoluble electrode in the microbial fuel cell (MFC) was possible without inorganic electron-shuttling mediators. The production of nanofilaments was stimulated when only insoluble electron acceptors were available for cellular respiration. These results suggest that when availability of a soluble electron acceptor for SRBs (SO_4^{2-}) is limited, *D. desulfuricans* initiates the production of conductive nanofilaments as an alternative strategy to transfer electrons to insoluble electron acceptors. The findings of this study contribute to understanding of the role of SRBs in the biotransformation of various substances in soils and sediments and in the MFC.

1. Introduction

The utilization of inorganic ions such as NO_3^-, Fe^{3+}/Mn^{4+}, and SO_4^{2-} as electron acceptors by anaerobic microorganisms is necessary for the microbial transformation of organic and inorganic substances present in the anoxic subsurface environment (Ishii et al., 2013; Lovley, 1991). On the other hand, the dissimilatory reduction of solid electron acceptors such as iron and manganese oxides has received little attention, although they are abundantly present in soils and sediments. Only a few mechanisms have been proposed hitherto: e.g., electron transfer via mediators or electron shuttles (Lovley et al., 1996) and electron transfer via a *c*-type cytochrome in the outer membrane of the cells (Bond and Lovley, 2003). It has been suggested that dissimilatory iron reducers such as those from the genera *Geobacter* and *Shewanella* transfer electrons directly to insoluble electron acceptors via bacterial nanowires and that cells need to be in direct contact with insoluble electron acceptors to reduce them (El-Naggar et al., 2010; Gorby et al., 2006; Reguera

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et al., 2005). Evidence supporting the necessity of direct contact between bacterial cells and insoluble electron acceptors forms the basis for an understanding of the mechanism of extracellular electron transport to insoluble electron acceptors in nature. So does the role of the iron reducer's nanoscale bacterial appendages in transferring electrons directly to solid electron acceptors such as Fe(III) oxides.

The microbial fuel cell (MFC) is a novel device that uses electrically-catalytic microorganisms to convert organic or inorganic substances into electricity (Logan et al., 2006). Thus, this device has been considered as a useful means of evaluating extracellular electron transfer reactions near electrode surfaces, acting as a natural insoluble or solid-phase electron acceptor in the environment (Kiely et al., 2011). Several strategies have been suggested to explain extracellular electron transfer in MFCs, including (i) indirect electron transfer by mediators (sulfate, iron oxides, phenazines, or quinones) that function as electron shuttles from cells to electrodes (Newman and Kolter, 2000); (ii) direct electron transfer from the cell to the electrode via the *c*-type cytochrome associated with the outer membrane (Chaudhuri and Lovley, 2003); and, (iii) direct electron transfer through bacterial nanowires or other filamentous structures (Gorby et al., 2006). In mediatorless MFCs, microorganisms transfer electrons directly to electrodes without mediators and use the electrode as the sole electron acceptor (Chaudhuri and Lovley, 2003; Gregory et al., 2004). Many microorganisms are capable of donating electrons to the anode of MFC and producing electricity; however, to date, only a few species of iron reducers such as *Geobacter sulfurreducens* and *Shewanella oneidensis* MR-1 have been found to produce nanowires for use as mediators during the transfer of electrons directly to the electrode (El-Naggar et al., 2010; Gorby et al., 2006).

Sulfate-reducing bacteria (SRBs) are ubiquitously present in highly reducing soil and sediment environments. SRBs typically use soluble sulfate as the terminal electron acceptor in the respiration process for their growth (Heidelberg et al., 2004). Through this process, SRBs contribute greatly to the carbon and sulfur cycles and bioremediation of contaminated subsurface systems (Martins et al., 2009). Some SRBs such as *Desulfobulbus propionicus* and *Desulfovibrio desulfuricans* use electron acceptors other than sulfate, including Mn^{4+} (Myers and Nealson, 1988), Fe^{3+} (Lovley et al., 1993), fumarate (Tomei et al., 1995), and NO^{3-} (Marietou et al., 2009). Moreover, SRBs can be used as the electricity-generating microbes in MFCs (Cordas et al., 2008; Zhao et al., 2008). To date, several strategies have been suggested for electron transfer from SRBs to solid electrodes in the MFC system. Some studies have shown that SRBs including *D. desulfuricans* can transfer electrons indirectly to electrodes via inorganic electron mediators or shuttles such as sulfate/sulfide, thereby generating electricity (Zhao et al., 2008). Another study reported that when SRBs are employed as a microbial catalyst in a mediatorless MFC, electrons are transferred to the electrode via contact between microbes and electrodes through a *c*-type cytochrome in the outer cell membrane (Cordas et al., 2008). No prior studies, however, have addressed direct electron transfer by SRBs via microbial appendages, as demonstrated previously for iron reducers (El-Naggar et al., 2010; Gorby et al., 2006; Reguera et al., 2005).

Therefore, in this study, the utility of nanoscale bacterial appendages (filaments) produced by SRBs for direct electron transfer to insoluble electron acceptors was assessed. Because *D. desulfuricans* is a predominant SRB in diverse anoxic environments and has a well-characterized genome (Devereux and Mundfrom, 1994), this bacterium was chosen as a representative SRB. It was hypothesized that *D. desulfuricans* produces nanofilaments when only insoluble electron acceptors are available for extracellular electron transfer instead of the soluble electron acceptor, SO_4^{2-} Using an MFC inoculated with SRBs, including *D. desulfuricans*, the morphological features and functions of the nanofilaments produced by SRBs were characterized.

2. Methods

2.1. Bacterial strain and culture conditions

D. desulfuricans (ATCC 27774) was obtained from the American Type Culture Collection. The cells were preharvested in the growth medium at 37 °C under strict anaerobic conditions. The growth medium contained the following components (per liter): 0.5 g of K_2HPO_4, 1.0 g of NH_4Cl, 1.0 g of Na_2SO_4, 0.1 g of $CaCl_2{\cdot}H_2O$, 2.0 g of $MgSO_4{\cdot}7H_2O$, 2.0 g of sodium lactate, 1.0 g of yeast extract, 1.0 mg of resazurin, 0.5 g of $FeSO_4{\cdot}7H_2O$, 0.1 g of sodium thioglycolate, 0.1 g of ascorbic acid, and 1 mL of a trace element solution. The medium was adjusted to pH 7 with 0.1 M NaOH, followed by sterilization using autoclaving at 121 °C for 15 min. The cells were harvested by centrifugation for 20 min at 10,000*g* and 4 °C, washed twice with a 50 mM phosphate buffer solution (pH 7), and then seeded in the anodic chamber of the MFC. A mixed culture of SRBs was isolated from anaerobic sewage digestion of sludge collected from a domestic wastewater treatment plant by subculturing the bacteria in an SRB-selective medium. The medium (pH 7) composed of 2 g/L of sodium lactate, 0.3 g/L of sodium citrate, 0.1 g/L of yeast extract, 4.5 g/L of Na_2SO_4, 0.06 g/L of $CaCl_2{\cdot}2H_2O$, 1 g/L of NH_4Cl, 0.5 g/L of KH_2PO_4, 2 g/L of $MgSO_4{\cdot}4H_2O$, 0.5 g/L of $FeSO_4{\cdot}7H_2O$, 0.3 g/L of disodium ethylenediamine tetraacetate, and 0.2 g/L of K_2CrO_4. The mixed SBR culture was kept under strict anaerobic conditions for a week, and then subcultured. These microbial inocula were then washed with a 50 mM phosphate buffer solution (pH 7). A mixed culture of SRBs was seeded in the anodic chamber of MFC.

2.2. Production of bacterial nanofilaments

The cells were grown in the growth medium at 37 °C anaerobically. Sulfate (50 mM) and Fe(III) oxide (α-Fe_2O_3; 50 mM) were provided as soluble and insoluble electron acceptors, respectively. In a control experiment, the cells were grown in the growth medium without an electron acceptor.

2.3. MFC preparation and operation

Studies using electrodes as solid electron acceptors were performed in a dual-chamber MFC with a working volume of 150 mL (Fig. S1). The two chambers were separated by a Nafion 117 proton exchange membrane (PEM; Dupont, USA), with the PEM pretreated as described previously (Tang et al., 2011). The cathode chamber was filled with an electrolyte solution containing a 30 mM Tris buffer solution (pH 7.0), which was continuously purged with water-saturated air. The pure culture of *D. desulfuricans* was seeded in the anode chamber. The mixed culture of SRBs was also added in a separate MFC experiment. Organic substrate was supplied as an electron donor (fuel), and neither an electron acceptor nor an electron-shuttling mediator was provided, except for the electrode. The medium of the anode chamber was purged with the mixture of N_2 and CO_2 (8:2, v/v) to maintain anaerobic conditions. Graphite felt (surface area 35 cm^2) served as cathodic and anodic electrodes (GF series, Electrosynthesis, USA), and both electrodes were connected to a platinum wire through an external resistance of 100 Ω. Voltage was monitored using a digital multimeter (Model 2700, Keithley Instruments, USA). The recorded voltage was converted into current density using Ohm's law ($I = V/R{\cdot}A$), where I = current (amperes), V = voltage (volts), R = resistance (Ω), and A = electrode surface area (m^2). The coulombic efficiency (CE)