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Electrochemical communication between heterotrophically grown *Rhodobacter capsulatus* with electrodes mediated by an osmium redox polymer

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ABSTRACT

The metabolically versatile purple bacteria *Rhodobacter capsulatus* was investigated to check its possible applicability in biofuel cells and electrochemical microbial biosensors. The wild type strain ATCC 17015 and mutant strain 37b4 lacking the lipopolysaccharide capsule was compared for their ability to communicate with electrodes modified with an osmium redox polymer. In this work, aerobic heterotrophically grown *R. capsulatus* were used to screen for efficient cell − electrode communication for later implementation using photoheterotrophically grown bacteria. The bacterial cells embedded in the osmium polymer matrix demonstrated efficient electrical "wiring" with the electrodes and were able to generate a noticeable current with succinate as substrate. Interestingly, at 2 mM succinate the wild type strain showed much better bioelectrocatalytic current generation (4.25 μA/cm^2) than the strain lacking capsule (1.55 μA/cm^2). The wild type strain also exhibited a stable current response for longer time, demonstrating that the bacterial lipopolysaccharide in fact enhances the stability of the polymer matrix layer of the modified electrode. Control experiments with *R. capsulatus* without any mediator did not show any current irrespective of the capsule presence. This demonstrates that development of photosensors and other light driven bioelectrochemical devices could be feasible using *R. capsulatus* and will be at focus for future studies.

1. Introduction

The growing interest in microbial fuel cells (MFCs), for both electricity generation and a wide range of other applications [1] and electrochemical microbial biosensors [2], has during the last decade led to an increased attention in "wiring" microbial cells to electrodes to facilitate the electrochemical communication [3–7]. The bacteria are able to transfer electrons to an electrode mainly by the use of various redox mediators (artificial or produced by bacteria) or using direct electron transfer (DET) via cytochrome rich membranes, electrically conductive pili, or nanowires produced by the bacteria [8,9]. Limited bacterial groups such as *Geobacteraceae* [10,11] and *Shewanellaceae* [12–14] possess the ability to communicate electrochemically with the electrodes without any mediator, thanks to the metabolism and electron conducting structures evolved for use in their natural habitat. These bacteria may, however, not be the most suitable for many applications. Consequently, bacteria that are unable to communicate with the electrodes by themselves have been the focus of research during the last decade [2,7,15,16]. Many attempts have been made to enhance the electron transfer by exploring genetic engineering to provide the bacteria with better electron conducting structures or other enhanced metabolic features [6,17–19]. In addition, research on various other influential factors like anode materials, inoculum sources, substrates, separators, cathode, design, operational parameters etc. have resulted into several advancements, increased performance and widened application range for MFC technology [20–25].

In addition to pure microbial cultures MFC research mainly employs electrochemically enriched biofilms of mixed culture or *G. sulferreducens* from different inoculum sources [5,11,26]. Amongst the widely used bacteria for MFC research, very few like *Shewanella* sp. possess metabolic diversity and facultative oxidant tolerance [27,28]. Furthermore, although research has been conducted with different mixed microbial inoculum sources and pure cultures, very few reports introduced new metabolically versatile microbial catalysts for MFCs and whole cell biosensors [5,26]. Moreover, most of the organisms investigated in MFCs so far, are strictly anaerobic [5], which limits their applicability. Therefore, to speed up the development of MFCs and whole cell electrochemical biosensors, there is a necessity to explore alternative versatile bacteria that might increase the types of microorganisms that can be used as possible catalysts at the bioanode or as biocathode [4].

In this work we have investigated the metabolically versatile α-proteobacterium *Rhodobacter capsulatus* [29,30] and tested its capability as a microbial catalyst for MFC and microbial biosensor. *R. capsulatus* is amongst the most nutritionally versatile non-sulfur purple bacteria [31], which have the capacity to grow rapidly under

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either anaerobic photosynthetic conditions or aerobic dark conditions [30,32]. *R. capsulatus* has previously been utilized for anoxygenic hydrogen production [33,34], but this bacterium has never been tried in any electrochemical application. In this study, we used heterotrophically grown *R. capsulatus* under aerobic conditions and tested its ability to communicate electrochemically with osmium (Os) redox polymer modified electrodes. *R. capsulatus* is a Gram-negative organism with an outer and an inner membrane, with a thin peptidoglycan cell wall in between. In addition, wild type *R. capsulatus* possesses an outer capsule of slimy lipopolysaccharides [35,36]. The exact composition of the lipopolysaccharide layer varies among different *R. capsulatus* strains [37], but the capsule presence as such may interfere with the polymer used for communication with the electrode and increase the distance from the outer membrane surface to the electrode surface. Therefore, the mutant strain 37b4 that does not produce any lipopolysaccharide capsule [38] was compared to the wild type *R. capsulatus*.

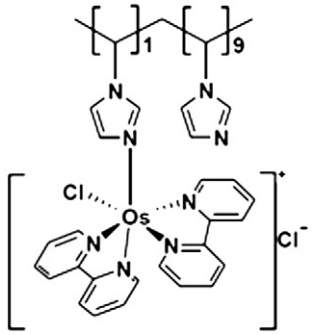
The use of flexible Os redox polymers in biofuel cells (BFCs) or biosensors has gained attention due to the efficient electron shuttling properties, polymeric structure, a stable adsorption in addition to the possibility to lead to the formation of multiple layers of both immobilized enzymes and microbial cells on the electrode surface [6,39–41]. Our earlier studies with such polymeric mediators showed an enhanced electron transfer between different microbial cells and electrodes [7,15,17,18]. Therefore, the current research is a logical continuation of previous work from our group involving the electrochemical communication between different bacteria and electrodes using Os polymers. For this study, an Os polymer with a high positive redox potential (0.176 V vs. SCE; 0.221 V vs. Ag|AgCl, sat. KCl) was chosen, because of its proven ability of efficient wiring of enzymes to electrodes [42,43]. This Os polymer has a high redox potential and a short length of the side chains, where the $Os^{3+/2+}$ functionalities are located at their ends. In addition, the advantage of using high redox potential polymers is that they efficiently compete with O_2 as electron acceptor [18].

Herein, the electrochemical communication of the wild type and capsule lacking mutant strains of *R. capsulatus* with Os redox polymer modified electrodes was investigated using cyclic voltammetry (CV) measurements. Additionally, the current response characteristics of the bacterial cell modified Os polymer graphite electrodes were evaluated for succinate as substrate in flow analysis mode. Succinate was chosen as this substrate can be used both for heterotrophic and phototrophic *R. capsulatus* cells. The succinate:quinone oxidoreductase enzyme has not been biochemically characterized in *R. capsulatus*, but primary sequence comparison reveal it to be similar to that of *Paracoccus denitrificans* [44] and thus belonging to class 1 [45]. Furthermore, the ability of *R. capsulatus* to communicate with the electrode by itself through a DET mode was also investigated in potentiostatically controlled half-cell set-ups. Besides, the electron transfer efficiency of the Os polymer was compared with that of a soluble mediator, hexacyanoferrate. The stability of the current response for *R. capsulatus* with Os polymer modified electrodes was carefully considered throughout the experiments. Both gold electrodes protected by a thiol based self assembled monolayer as well as graphite electrodes were used in line with previous investigations [7,18].

2. Experimental

2.1. Chemicals

[Os(2,2′-bipyridine)$_2$-poly(N-vinylimidazole)$_{10}$Cl]$^{2+/+}$ (poly[Os(bpy)$_2$(PVI)$_{10}$Cl]$^{2+/+}$) of E° equal to +176 mV vs. SCE (sat. KCl)/+221 mV vs. Ag|AgCl (sat. KCl) ("Scheme 1") was synthesized as reported in [46]. The weight-average molecular weight of the poly(vinylimidazole) as determined by viscometry in ethanol was 10,000 g/mol [47]. All chemicals were purchased from Sigma–Aldrich/Merck and were of either research or analytical grade. All aqueous solutions were prepared by using water purified and deionized (18 MΩ) with a Milli-Q system (Millipore, Bedford, MA, USA).



Scheme 1. Structural representation of the osmium redox polymer.

2.2. Microbial growth conditions and inoculum preparation

R. capsulatus strains, ATCC 17015 (wild type) and 37b4 (capsule lacking mutant) were purchased from DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). These strains were grown and maintained on minimal peptone yeast extract medium (MPYE) agar plates [29]. A single, well isolated colony was used for inoculum preparation throughout the study. For inoculum preparation, the bacterial cells were grown aerobically in 10 mL of MPYE broth (in 50 mL baffled E-flasks at 200 rpm) for 20 h at 30 ± 2 °C by transferring 1 mL of cell suspension prepared by suspending an isolated colony into the saline (0.85% NaCl). The cells grown in MPYE were harvested in the early stationary growth phase by centrifuging the broth at 4000 rpm for 10 min. Further, the cells were washed once in 20 mM MOPS (3-morpholinopropanesulfonic acid) buffer (pH 7.4), centrifuged again as before, resuspended in the same buffer to adjust cell density to 1 g/mL (wet weight) [48] and used immediately for electrochemical experiments (CV and amperometric flow mode measurements).

For batch mode chronoamperometry (CA) experiments the cells harvested from the growth medium were transferred to 200 mL of synthetic medium composed of PIPES buffer 15.1 g/L; NaOH 3.0 g/L; NH_4Cl 1.5 g/L; KCl 0.1 g/L; $NaH_2PO_4 \cdot H_2O$ 0.6 g/L; NaCl 5.8 g/L; mineral solution 10 mL/L; vitamin solution 10 mL/L; amino acid solution 10 mL/L [49]. The substrate used was 10 mM succinate (disodium salt). After incubation of flasks at 30 ± 2 °C (500 mL baffled E-flasks, aerobically while shaking at 200 rpm for 72 h) the cells were harvested and used as an inoculum in potentiostatic half-cell set ups hosting a similar synthetic medium. These experiments were performed to check the capability of *R. capsulatus* to communicate with an electrode without any mediator.

2.3. Preparation of the electrodes modified with bacteria

Either gold (BAS, West Lafayette, IN, USA) or spectrographic graphite rods (Ringsdorff Werke GmbH, Bonn, Germany, type RW001, 3.05 mm diameter and 13% porosity) were used as working electrodes. The procedure explained by Coman et al. [18] was followed to prepare the modified working electrodes. Polycrystalline gold electrodes (projected surface area; 0.02 cm^2) were electrochemically cleaned by cycling in 0.1 M NaOH between 0 and −1000 mV vs. NHE, followed by mechanical polishing on Microcloth (Buehler, Lake Bluff, IL) in an aqueous alumina UF slurry (1 and 0.1 μm, Struers, Copenhagen, Denmark). The electrodes were rinsed with water, ultrasonicated for 5 min in Milli-Q water, followed by cycling in 0.5 M H_2SO_4 between 0 and +1950 mV vs. NHE, and finally rinsed with Milli-Q water. Formation of a self-assembled monolayer (SAM) of aldrithiol at the electrode surface was done by immersing the electrode in a saturated aqueous solution of