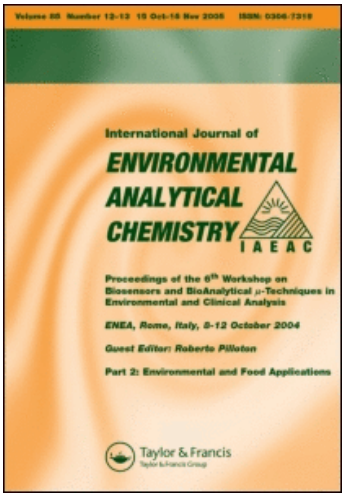

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Escherichia coli knock-out mutants with altered electron transfer activity in the Micredox® assay and in microbial fuel cells

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Electron transfer from bacteria to external electron acceptors is a biologically important phenomenon that is increasingly being harnessed as useful technology such as in the Micredox® assay and in microbial fuel cells (MFCs). Optimisation of these systems is limited by incomplete knowledge of the underlying genetics of electron transfer. The Keio collection of single gene knock-out *Escherichia coli* strains is being tested to find genes involved in electron transfer from bacteria to external electron acceptors. Initially, 21 *E. coli* strains from the Keio collection were selected and tested for altered electro-activity using the Micredox® assay. The Micredox® assay provides a rapid measurement of electron transfer from cells to a soluble electron acceptor (potassium hexacyanoferrate(III)) and was previously developed as a general test for BOD and toxicant measurement. Of the 21 Keio strains, 10 were found to have significantly reduced electron transfer and two were found to have significantly increased electron transfer. The mutant with the lowest electron transfer rate (*nuoA*) and the highest electron transfer rate (*arcA*) were then tested for electron transfer in microbial fuel cells (MFCs). The *arcA* mutant had slightly higher electron transfer rates than the wild type in mediator-less MFC while the *nuoA* mutant strain had very similar electro-activity to the wild type. However, in a mediated MFC, the mutants were consistently different from the wild type. These results demonstrate that single gene deletion strains of *E. coli* can have significantly altered electron transfer capabilities, both in the Micredox® assay and in MFCs. Importantly, the Micredox® assay was found to be a rapid and easily scaled-up method to discover genes that are important in electron transfer.

Keywords: Micredox®; microbial fuel cells; *E. coli*; electron transfer; BOD sensor

1. Introduction

Electron transfer from bacteria to external electron acceptors is a biologically important phenomenon that is increasingly being harnessed as useful technology such as in the Micredox® assay and in microbial fuel cells (MFCs). Our long-term research aim is to use genetic techniques to manipulate electron transfer from whole cells to improve the performance of these applied bio-electric systems. To do that, basic knowledge of the genetics that regulates electron transfer is needed. One approach to investigating the genetics of electron transfer is to screen gene knock-out mutant libraries for loss or gain of electron transfer ability. The Keio collection provides an impressive library of 3700 single gene, *E. coli* mutants [1]. Screening that library is a large task that requires a rapid

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assay amenable to high throughput. We propose that the Micredox® assay itself could be a suitable system to use for library screening and that the results of that assay could be generally applicable to other systems that depend upon electron transfer. For that reason we have tested 21 Keio mutants in the Micredox® assay and compared the performance of selected mutants in the Micredox® assay with their performance in MFCs.

The Micredox® assay was developed as a method for measuring BOD and toxicant concentration and is currently being commercialised for those uses [2,3]. The Micredox assay quantifies bacterial activity by measuring the rate at which the external electron acceptor potassium hexacyanoferrate (III) is reduced by bacterial respiration. The extent of potassium hexacyanoferrate (III) reduction is measured by limiting current micro-electrode amperometry. The Micredox® assay has been developed as a measure of BOD and the level of toxicants, exploiting the fact that the rate of bacterial respiration is dependent on the concentration of substrates and inhibitors. The Micredox® assay is a very general assay that robustly responds to changes in the concentration of substrates and inhibitors. However, one barrier to its broader commercial development is that the assay lacks specificity and is insensitive to toxicants at sub-inhibitory levels. In order to develop the Micredox® assay as a more sensitive and specific assay, we are investigating genes whose expression modifies the Micredox® response. By putting such genes under the regulatory control of a promoter that is inducible by specific analytes, it should be possible to develop an assay that is both more specific and more sensitive.

MFCs similarly use electron transfer from bacteria to an external electron acceptor to generate electricity. In MFCs, micro-organisms oxidise organic substrates and pass electrons to an anode electrode [4]. The electrons then pass through an external circuit to the cathode where they reduce a final electron acceptor. Where an air cathode is used, the electrons on the cathode combine with protons, produced by the micro-organisms, and with oxygen from the air. MFCs are seen as having considerable potential for generating energy from low value biomass such as domestic and industrial wastewaters. However, MFCs are not yet efficient enough for widespread, cost effective use and, to-date, MFCs have only been used in niche applications such as sediment-powered remote sensing [5–7] and for biological oxygen demand [8].

Over the past ten years, considerable progress has been made to improve the efficiency of MFCs. This research has seen the power density of MFCs increase by several orders of magnitude [9], primarily by addressing physical and chemical limitations of the system and by using mixed communities of bacteria selected at, and attached to, the surface of the anode [10,11]. In terms of understanding the microbial processes, research has focused both on identifying specific microbial community structures at the anodes and cathodes and identifying the mechanisms by which electrons are transferred from the bacteria to the anode [12–17]. Electrons can be transferred to the anode by several different pathways: (1) directly via outer surface proteins such as cytochromes or pili, or (2) indirectly, carried out either by metabolic products or by exogenously supplied mediators such as humic acids, or by endogenously produced mediators such as phenazine and quinones [16,18–26].

The genetic regulation of electron transfer to solid state anodes has received some attention, particularly in relation to the analogous process of dissimilatory metal reduction [27]. Possible electron transfer routes from electron carriers in the cell cytoplasm, through the cell membranes and periplasm directly to a solid state anode have been proposed for metal reducing bacteria [28–33]. Genetic research on components of this electron transfer route is still ongoing [34–38]. There is also continuing research into the mechanism by which endogenous and exogenous mediators shuttle electrons. The *menC* gene which

encodes o-succinylbenzoic acid synthase, an enzyme involved in the biosynthesis of menaquinone in Shewanella, appears to be involved in the production of an extracellular quinone involved in electron shuttling [26,39]. The function of endogenously produced quinones in electron transfer can be replaced by exogenous mediators, such as neutral red [40].

For MFCs using *E. coli* as the biocatalyst, it was originally proposed that electrons were transferred directly to the anode by cell contact [41]. However, the addition of exogenous mediators greatly increases the power of *E. coli* MFCs [42,43]. It seems likely that electron transfer by direct contact, if it happens at all, is not the most efficient route. Recent research suggests that electro-activated *E. coli* cells produce an endogenous mediator that is capable of shuttling electrons to the anode [44,45]. Such *E. coli* MFCs are capable of producing relatively high power densities without the addition of exogenous mediators [46]. Multiple routes of electron transfer from *E. coli* are therefore possible and we have a very incomplete understanding of the components of electron transfer in *E. coli* and their genetic regulation.

It is uncertain to what extent electron transfer in the Micredox® assay is analogous to electron transfer in other whole cell bio-electrical systems such as MFCs. In the Micredox® assay, potassium hexacyanoferrate (III) acts as a soluble mediator and is able to penetrate into the cell periplasm potentially gaining access to most of the components of the electron transport chain. As potassium hexacyanoferrate (III) has a relatively high redox potential (0.43 V at pH 7) it is probably unrestricted in accepting electrons from all components it has access to [47]. In bio-electrical systems using other mediators or not using any mediator, the Micredox® assay results may not all be useful.

Twenty-one strains from the Keio collection were initially selected on the basis of having knock-outs of genes potentially involved in electron transfer. The classes of genes included genes that: regulate entry into stationary phase (*rpoS*, *fis*, *ihfB*); are involved in NADH oxidation (*nuoA*, *nuoB*, *ndh*); are involved in catabolic pathways (*aceF*, *adhE*, *pykF*, *zwf*, *ppc*, *pgi*, *pta*, *aceE*, *ackA*, *fdhF*); regulate catabolism (*fruR*); and regulate respiration/fermentation (*arcA*, *arcB*, *fur*, *fnr*). These mutant strains were tested in the Micredox® assay and the best performing (*arcA*) and worst performing (*nuoA*) were selected for further testing in MFC assays.

2. Experimental

2.1 *MFC construction*

Four identical single-chambered MFCs with an internal volume of 19.2 cm^3 were constructed from machined polycarbonate, jointed with rubber O-rings and assembled with steel bolts. Two different MFC configurations were used: *a) MFC with Platinum (Pt) catalyst on anode*: The anode compartment and the cathode were separated by a membrane electrode assembly (MEA). The MEA was a 25 cm^2, 0.5 mg/cm^2 Pt, 3L HP-A MEA (Fuelcell store, San Diego, CA, USA) with one side of the MEA acting as an air cathode and the other in contact with one surface of a 12.25 cm^2 carbon cloth anode to provide a Pt catalyst layer to the anode. Secondly, *b) MFC without Pt catalyst on anode*: The MFC anode chamber was separated from an air cathode by either Ultrex or Nafion NRE 212 membrane (BASF Fuel Cell Inc, Somerset, NJ, USA). Where Ultrex was used, the 12 cm^2 air cathode was made of Vulcan loaded with 10% platinum (BASF Fuel Cell Inc., Somerset, NJ, USA), mixed with water and spread as a thin paste layer (at approximately

0.08 mg Pt per cm^2) on the outside surface of the Ultrex; this was then covered with a thicker, approximately 2 mm, layer of Vulcan paste. Where Nafion NRE 212 membrane was used, the air cathode was 0.5 mg/cm^2 loading of Pt using 20% Pt on Vulcan XC-72 pressed by the manufacturer (BASF Fuel Cell Inc., Somerset, NJ, USA) on one side of the Nafion. The 6.25 cm^2 carbon cloth anode was positioned 1.2 cm from the membrane and cathode assembly. For the mediated MFC, we used a MFC without Pt catalyst on anode with an Ultrex membrane, methylene blue was added to the anolyte to a concentration of 1 mM.

2.2 *MFC operation*

MFC components were routinely cleaned and soaked for at least an hour in 1% Virkon® disinfectant (Antec International, Sudbury, UK) and rinsed in sterile water. In order to avoid microbial contamination in experiments where growth medium was provided, the MFC components including the chambers, the electrodes, Ultrex membranes, media and buffer solutions were sterilised by autoclaving at 121°C for 15 minutes. The MFCs were assembled and loaded in a Class II laminar flow cabinet and sealed either with sterile tape or sterile wax.

2.3 *Electrochemical measurements and calculations*

The external circuit was through a 500 ohm resistor and the voltage across that resistor was measured using a 4 channel Quadstat potentiostat (eDAQ Pty Ltd, NSW, Australia) and continually recorded using an e-corder 1621 (eDAQ Pty Ltd, NSW, Australia) data acquisition system.

2.4 *Bacterial strains*

All bacteria used in this study were *E. coli* strains from the Keio Collection [1]. The kanamycin resistance cassette replacing the deleted gene was not removed, and all mutants are kanamycin resistant. Cells were cultured overnight at 37°C in nutrient broth. For use in the Micredox® assay and the MFC assay with Pt catalyst on the anode, cells were centrifuged at $4000 \times g$, washed twice in 0.2 M phosphate buffer pH 6.7, resuspended in phosphate buffer and adjusted to a consistent cell concentration based on OD_{660} measurement. For the MFC assay with no Pt catalyst, where the bacteria were cultivated in the MFC for several days, overnight cultures were directly introduced into the MFCs without washing or dilution and approximately one quarter of the anolyte was replaced with fresh nutrient broth each day. In all other MFC assays the anolyte was 0.2 M phosphate buffer pH 6.7 with 20 mM glucose as a carbon source.

2.5 *Micredox® assay*

The Micredox® assay was as described previously [2,3]. Cells were cultured overnight in Nutrient Broth in shaking flasks at 37°C. The following day, the cells were pelleted by centrifugation at $4000 \times g$, resuspended in 0.2 M phosphate buffer pH 6.7 and adjusted to the same OD_{660} as the least concentrated sample ($OD_{660} \sim 6$). The resuspended cells were incubated anaerobically at 37°C for two hours with 1 mM glucose as the carbon source

and 54 mM potassium hexacyanoferrate (III) as the electron acceptor for respiration. After incubation, the cells were removed by centrifugation and the microbially reduced potassium hexacyanoferrate (II) was measured by limiting current microelectrode amperometry.

3. Results

3.1 *Micredox® assay*

In four separate experiments, the 21 mutants and the wild type strain were tested for their efficiency in anaerobic respiration using the Micredox® assay and glucose as the carbon source (Figure 1). Ten of the mutants had significantly lower respiration rates than the wild type; two had significantly higher rates and no significant difference was detected in nine mutants. The greatest reduction in respiration was detected in mutants lacking either *nuoA* or *nuoB*. These particular genes encode components of the NADH dehydrogenase I complex which is known to be involved in respiration. The next greatest deficiency in respiration was in strains lacking *aceE* and *aceF* genes, which encode metabolic enzymes involved in converting pyruvate to acetyl-coA, a reaction in which NAD is reduced to NADH. Other loss-of-function mutations that decreased respiration were global regulatory genes (*fis*, *ihfB* and *fruR*) and in genes involved in glycolysis (*pgi* and *ppc*). In these experiments loss of *ndh*, the gene that encodes the alternative NADH dehydrogenase, did not significantly alter micredox activity.

A significant increase in respiration was found in the *arcA* and *ackA* mutant strains. The *arcA* gene encodes a regulatory protein that controls the switch between fermentation

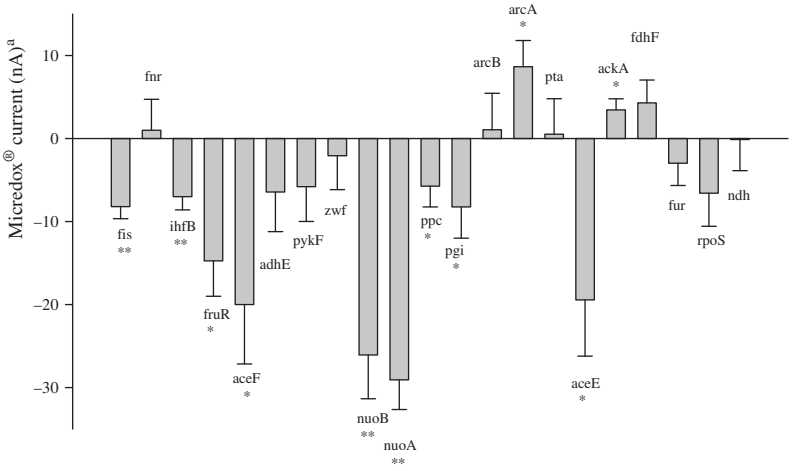

Figure 1. Respiration detected in the Micredox® assay relative to wild-type respiration. Data are the average results from four separate experiments.
The name of the deleted gene in each mutant is given above or below the corresponding data bar.
[a]: The difference in the average microelectrode limiting current (nA) recorded for each mutant subtracted from the average current recorded for the wild type strain (70.4 nA).
*: Significantly different from the wild type in paired t test at $p < 0.05$.
**: Significantly different from the wild type in a paired t test at $p < 0.01$.
$n = 4$, error bars are the standard error.

and respiration. Loss of *arcA* leads to higher activity in the TCA cycle, increasing respiration. The *ackA* gene encodes an enzyme that catalyses the formation of acetate. Loss of this gene presumably increases respiration at the expense of fermentation.

The worst performing mutant (*nuoA*) and the best performing mutant (*arcA*) were selected for further testing. As *ndh* encodes NADH dehydrogenase II, the alternative NADH dehydrogenase to the *nuoA-N* dehydrogenase, we also looked more closely at that mutant. In the initial Micredox® assay, the strains were cultured overnight in shaking flask cultures, likely to be a more aerobic and disturbed growth environment than the MFCs. To test if the growth environment would affect the Micredox® results, further experiments were carried out where the strains were cultured overnight either in shaking flask cultures or without shaking in sealed bottles without a head space. The growth conditions did not alter the Micredox® results in multiple experiments which consistently confirmed that *nuoA* was significantly impaired in the Micredox® assay compared to the wild type and *arcA* was significantly better performing. However, in these experiments we found that *ndh* was generally impaired in the Micredox® compared to the wild type and in some experiments was as poorly performing as *nuoA*.

3.2 *Mediator-less MFC with Pt catalyst on anode*

To test whether the Micredox® results could predict the relative ability of the mutants to produce power in an MFC, the worst performing mutant, *nuoA*, and the best performing mutant, *arcA*, were used as the bio-component in the anode compartment of MFCs with a carbon anode coated with platinum. Cells from overnight cultures were washed in phosphate buffer and incubated for 18 hours in MFCs with 20 mM glucose. Total Coulombs transferred and maximum power through a 500 ohm external resistor were measured. The *arcA* mutant strain marginally outperformed the wild type strain (Figure 2). The difference in output was small but statistically significant ($p < 0.05$,

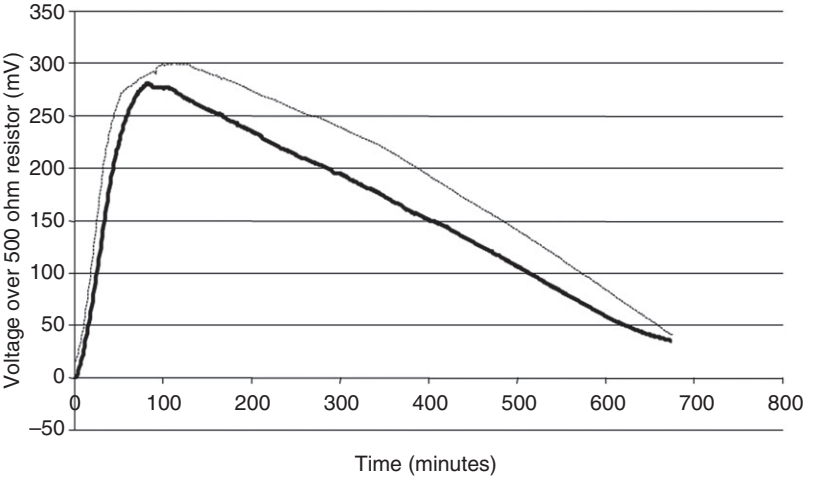

Figure 2. MFC voltage over 500 ohm external resistor. Data are the average of eight independent experiments.
Solid line: data from MFCs with the wild type strain. Dashed line: data from MFCs with the *arcA* mutant.

1-tailed t-test, $n = 8$) with a mean maximum power of 0.22 mW (wild type: 0.17 mW) and a mean Coulombic output of 16.1 C (wild type: 13.7 C). However, no difference was detected between *nuoA* and the wild type in 10 experiments.

In these experiments, the anode contained platinum and was therefore potentially capable of catalysing the oxidation of glucose and metabolic products produced by the cells. In these cases, the electron transfer process would likely be different from the Micredox® assay. To test whether that was the case, washed overnight cultures were resuspended in 50 mL buffer with 20 mM glucose and incubated for five hours in 50 mL plastic tubes. Cells were harvested by centrifugation and the supernatant was filtered with a 0.22 um filter to remove cells. The harvested cells were resuspended in 50 mL buffer without glucose. The harvested cells and the filtrate were added to the anode compartments of separate MFCs. In these MFC assays, the measurable current was predominantly produced by MFCs to which the filtrate had been added (Figure 3). Power from the filtrate alone was sufficient to account for all the output previously seen in the MFCs where cells had been incubated in the MFC with glucose. Glucose at 20 mM in buffer alone did not produce current and the power produced by the MFCs containing filtrate was dependent on the length of time the cells were incubated with the glucose prior to the filtrate being added to the MFC. Together, these experiments indicate that in these MFCs, power was most likely produced by oxidation of metabolites secreted by the cells.

Similarly, the source of the current in the Micredox® assay was tested. After 2 hours incubation in the Micredox® assay, *E. coli* cells at an OD_{660} of 0.4 produced a current of 59.9 nA ($n = 4$, standard error = 9.6 nA). Cells at the same concentration were incubated for two hours with glucose and then the cells were removed by centrifugation prior to the Micredox® assay. The supernatant, incubated in the Micredox® assay, produced a current of 1.6 nA ($n = 4$, standard error = 0.1 nA). Therefore, the Micredox® assay measures

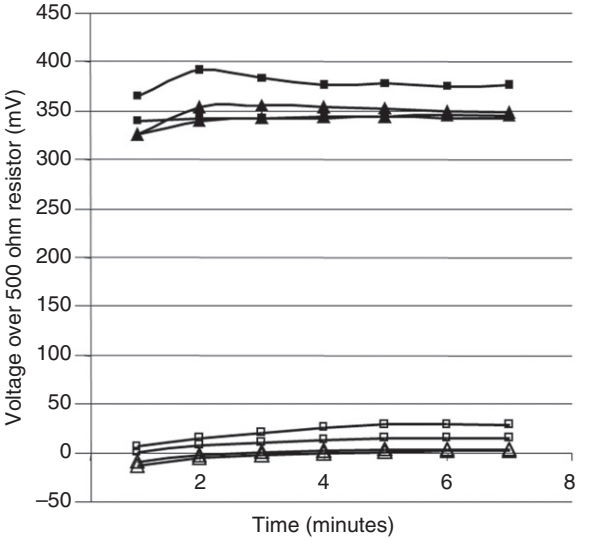

Figure 3. MFC voltage over 500 ohm external resistor. Anolyte contained either cells (open symbols) or filtrate (closed symbols) from a five-hour incubation in plastic tubes of either wild type *E. coli* (triangles) or the *nuoA* mutant (squares).

electron transfer primarily from cells and not from substances secreted from the cells. The Micredox® assay is clearly not analogous to the MFC with Pt catalyst on the anode.

3.3 *Mediator-less MFC without Pt catalyst on anode*

When *E. coli* was incubated in MFCs constructed with a carbon fibre anode without a Pt catalyst, there was initially no increase in power output when glucose was added to the MFC. After a few days of incubation in the MFC, where approximately one quarter of the anolyte was replaced with fresh growth medium each day, the MFCs became responsive to the addition of glucose, suggesting that the cells had adapted to the MFC environment. The current produced, per anode area, by these MFCs was about half that produced by MFCs with a platinum anode. Four identical MFCs were set up containing either the wild type strain or one of *nuoA*, *arcA* or *ndh* mutant strains. These MFCs were run in parallel over approximately two weeks. The experiment was repeated four times and the total Coulombs transferred through a 500 ohm external resistor were compared. Again, only the *arcA* mutant was different from the wild type ($n = 4$, $P < 0.05$, 1-tailed t test) with a mean of 39.5 C compared to 22.8 C for the wild type and 24.3 for *nuoA*. The *arcA* mutant typically grew to slightly higher cell densities, compared to the other strains, in these experiments where we could not control cell numbers, and that could account for the higher number of Coulombs for that strain.

3.4 *Mediated MFC*

The Micredox® assay measures electron transfer to a soluble electron acceptor, whereas mediator-less MFCs depend on electron transfer to solid anodes. We sought to find out if the Micredox® assay would predict the performance of the mutant strains in a mediated MFC where electron transfer is initially to a soluble electron acceptor. When the *E. coli* strains were incubated at an OD_{660} of 1.5 in mediated MFCs with a carbon anode without a platinum catalyst and with 1 mM methylene blue as a mediator, the results for the three strains tested was consistent with the results produced by the Micredox® assay. That is, the *arcA* mutant out-performed the wild type strain while the *nuoA* mutant performed poorly compared to both *arcA* and the wild type (Figure 4).

4. Discussion

The Micredox® assay was used as a rapid screen to discriminate mutants on the basis of their ability to reduce a soluble electron acceptor. Of the 21 mutants tested, 12 were found to be significantly different from the wild type. As the Keio mutants each have a specific gene replaced with a kanamycin resistance cassette, it is expected that the differences in the Micredox® assay are due to the gene deletion. Some effect might also be due to kanamycin resistance expression, or to changes in expression of other genes linked to the insertion; however the significant Micredox® results were consistent with the known functions of the replaced genes.

Electron transfer in the Micredox® assay is not necessarily analogous to electron transfer in a mediator-less MFC. In the Micredox® assay, potassium hexacyanoferrate (III) comes into contact with the cell wall. It also passes into the periplasm through porins in the outer membrane and can interact with proteins embedded in both the outer and

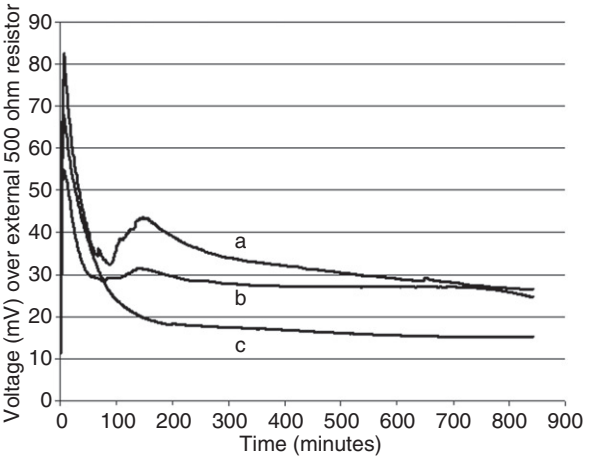

Figure 4. Comparison of *E. coli* strains in a mediated Microbial Fuel Cell fed glucose with methylene blue as the mediator. a: *arcA* mutant; b: wild-type; c: *nuoA* mutant. Data are the average of three independent reps for *nuoA* and *arcA* and six independent reps for the wild-type.

inner membranes [48]. In a mediator-less MFC, the anode can only directly interact with secreted compounds or with compounds on the outside surface of the cell. Mediated MFCs, containing a soluble mediator, are likely to be more closely analogous to the Micredox® assay than mediator-less MFCs.

Our results suggest that in the mediator-less MFCs, with *E. coli* as the bio-component, a significant part of the current, and possibly all of the current, is coming from compounds secreted from the cells. This is clearest in the MFCs that have a platinum catalyst on the anode. In the MFCs without a catalyst, we also detected no current specifically from free cells in the anolyte (data not shown). Some current was detected from filterable compounds in the anolyte. It is also possible that some current is coming directly from cells attached to the anode but we could not distinguish that. Previous work has also indicated that *E. coli* secretes electro-active substances in MFCs which do not have platinum catalysts [44,45].

To see if the Micredox® assay results were applicable to other bio-electric systems, two of the strains that had the most significant Micredox® assay result were tested in mediated and mediator-less MFCs. The *arcA* mutant strain consistently outperformed the wild type strain in the Micredox® assay and marginally outperformed the wild type strain both in mediator-less MFCs with a Pt catalyst on the anode and in mediator-less MFCs without a Pt catalyst on the anode. The *arcA* gene encodes a cytosolic response regulator which, along with the membrane-bound sensor ArcB, forms a two component regulatory system which is activated during the transition from aerobic to micro-aerobic growth in response to change in the redox state of the cell and remains activated during anaerobic growth. ArcA has a pervasive role as a global regulator and loss of ArcA has multiple physiological effects [49]. Compared to the wild type, *arc*A mutant strains have changes in expression of TCA and electron transfer chain genes, a higher redox potential and greatly increased flux through the TCA cycle in both anaerobic and aerobic environments [50–52]. It is likely that this higher reducing potential explains the superior performance of the *arcA* mutant in the Micredox® assay.

The marginally higher rate of electron transfer within the mediator-less MFCs by the *arcA* mutant is unlikely to be due to increased electron transfer through the respiratory chain as direct electron transfer from the cells was not detected in the MFCs and the current depended on anodic oxidation of extracellular substances. In experiments with the Pt anode mediator-less MFCs, while all strains were at the same cell concentration, the small increase in current may be due to higher rates of metabolism in the *arcA* strain. In the carbon fibre anode, mediator-less MFCs, cell concentrations changed over the course of the experiment and this could explain the higher current in the *arcA* strain as it tended to grow to a slightly higher concentration than the other strains.

The *nuoA* gene encodes a protein that forms part of the NADH dehydrogenase I complex; it links NADH oxidation to the respiratory chain and functions in both aerobic and anaerobic respiration. Expression of *nuoA* is regulated by ArcA, plays a minor role in aerobic respiration and is stimulated during early exponential growth [50,53]. In the mediator-less MFCs, the *nuoA* mutant was not deficient in electron transfer compared to the wild type, indicating that the rate of respiration, as measured in the Micredox® assay, did not limit the MFC current.

We conclude that the Micredox® assay is not suitable for predicting the performance of *E. coli* mutant strains in mediator-less MFCs but appears to be an appropriate method to screen mutants for altered electron transfer in mediated MFCs. This observation likely reflects significant differences between the mechanism by which electrons are passed from *E. coli* to solid, external electron acceptors and the mechanism by which *E. coli* transfers electrons to soluble mediators, such as potassium hexacyanoferrate (III) and methylene blue, that can penetrate into the cell periplasm and make contact with components of the electron transport chain.

This is the first report that we are aware of where an *E. coli* strain with a specific gene deletion has been shown to have different electron transfer capabilities in the Micredox® assay and in microbial fuel cells. The Micredox® assay has proven to be a rapid and repeatable method for screening libraries for genes involved in electron transfer, at least in mediated systems. The Micredox® results likely have limited applicability to non-mediated electron transfer systems. Based on these results, we are proceeding to screen all 3700 Keio collection mutants using the Micredox® assay and so far over 500 strains have been tested. It is anticipated that this work will reveal greater understanding of the mechanisms involved in electron transfer in *E. coli*. It is also expected to identify potential targets for genetically engineering the Micredox® assay to make it more sensitive and specific. This research could also have applications in other bio-electrical systems such as mediated MFCs.

Acknowledgements

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References

[1] T. Baba, T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K.A. Datsenko, M. Tomita, B.L. Wanner, and H. Mori, Mol. Systems Biol. **2**, 8 (2006).

[2] N. Pasco, K. Baronian, C. Jeffries, J. Webber, and J. Hay, Biosens. Bioelectron. **20**, 524 (2004).
[3] N. Pasco, J. Hay, and J. Webber, Biomarkers **6**, 83 (2001).
[4] V.G. Debabov, Microbiol. **77**, 123 (2008).
[5] C.E. Reimers, L.M. Tender, S. Fertig, and W. Wang, Environ. Sci. Technol. **35**, 192 (2001).
[6] L.M. Tender, S.A. Gray, E. Groveman, D.A. Lowy, P. Kauffman, J. Melhado, R.C. Tyce, D. Flynn, R. Petrecca, and J. Dobarro, J. Power Sources **179**, 571 (2008).
[7] P. Clauwaert, P. Aelterman, T.H. Pham, L. De Schamphelaire, M. Carballa, K. Rabaey, and W. Verstraete, Appl. Microbiol. Biot. **79**, 901 (2008).
[8] B.H. Kim, I.S. Chang, G.C. Gil, H.S. Park, and H.J. Kim, Biotechnol. Lett. **25**, 541 (2003).
[9] B.E. Logan and J.M. Regan, Trends Microbiol. **14**, 512 (2006).
[10] K. Rabaey, N. Boon, S.D. Siciliano, M. Verhaege, and W. Verstraete, Appl. Environ. Microb. **70**, 5373 (2004).
[11] J.R. Kim, B. Min, and B.E. Logan, Appl. Microbiol. Biot. **68**, 23 (2005).
[12] D.E. Holmes, D.R. Bond, R.A. O'Neil, C.E. Reimers, L.R. Tender, and D.R. Lovley, Microbial Ecol. **48**, 178 (2004).
[13] D.R. Bond, D.E. Holmes, L.M. Tender, and D.R. Lovley, Science **295**, 483 (2002).
[14] S. Ishii, Y. Hotta, and K. Watanabe, Biosci. Biotech. Bioch. **72**, 286 (2008).
[15] B.H. Kim, I.S. Chang, and G.M. Gadd, Appl. Microbiol. Biot. **76**, 485 (2007).
[16] D.R. Bond and D.R. Lovley, Appl. Environ. Microb. **71**, 2186 (2005).
[17] K. Rabaey and W. Verstraete, Trends Biotechnol. **23**, 291 (2005).
[18] C.J. Sund, S. McMasters, S.R. Crittenden, L.E. Harrell, and J.J. Sumner, Appl. Microbiol. Biot. **76**, 561 (2007).
[19] D.R. Bond and D.R. Lovley, Appl. Environ. Microb. **69**, 1548 (2003).
[20] Y.A. Gorby, S. Yanina, J.S. McLean, K.M. Rosso, D. Moyles, A. Dohnalkova, T.J. Beveridge, I.S. Chang, B.H. Kim, K.S. Kim, D.E. Culley, S.B. Reed, M.F. Romine, D.A. Saffarini, E.A. Hill, L. Shi, D.A. Elias, D.W. Kennedy, G. Pinchuk, K. Watanabe, S. Ishii, B. Logan, K.H. Nealson, and J.K. Fredrickson, Proc. Natl Acad. Sci. U.S.A. **103**, 11358 (2006).
[21] G. Reguera, K.P. Nevin, J.S. Nicoll, S.F. Covalla, T.L. Woodard, and D.R. Lovley, Appl. Environ. Microb. **72**, 7345 (2006).
[22] S.K. Chaudhuri and D.R. Lovley, Nat. Biotechnol. **21**, 1229 (2003).
[23] C.E. Turick, F. Caccavo, and L.S. Tisa, Fems Microbiol. Lett. **220**, 99 (2003).
[24] S. Srikanth, E. Marsili, M.C. Flickinger, and D.R. Bond, Biotechnol. Bioeng. **99**, 1065 (2008).
[25] T.H. Pham, N. Boon, P. Aelterman, P. Clauwaert, L. De Schamphelaire, L. Vanhaecke, K. De Maeyer, M. Hofte, W. Verstraete, and K. Rabaey, Appl. Microbiol. Biot. **77**, 1119 (2008).
[26] D.K. Newman and R. Kolter, Nature **405**, 94 (2000).
[27] J.B. McKinlay and J.G. Zeikus, Appl. Environ. Microb. **70**, 3467 (2004).
[28] T. Mehta, M.V. Coppi, S.E. Childers, and D.R. Lovley, Appl. Environ. Microb. **71**, 8634 (2005).
[29] D.R. Lovley, D.E. Holmes, and K.P. Nevin, Adv. Microb. Physiol. **49**, 219 (2004).
[30] Z.W. Du, H.R. Li, and T.Y. Gu, Biotechnol. Adv. **25**, 464 (2007).
[31] D.E. Holmes, D.R. Bond, R.A. O'Neill, C.E. Reimers, L.R. Tender, and D.R. Lovley, Microb. Ecol. **48**, 178 (2004).
[32] M. Vargas, K. Kashefi, E.L. Blunt-Harris, and D.R. Lovley, Nature **395**, 65 (1998).
[33] J.E. Champine, B. Underhill, J.M. Johnston, W.W. Lilly, and S. Goodwin, Anaerobe **6**, 187 (2000).
[34] G.J. Newton, S. Mori, R. Nakamura, K. Hashimoto, and K. Watanabe, Appl. Environ. Microb. **75**, 7674 (2009).
[35] D.E. Holmes, T. Mester, R.A. O'Neil, L.A. Perpetua, M.J. Larrahondo, R. Glaven, M.L. Sharma, J.E. Ward, K.P. Nevin, and D.R. Lovley, Microbiol.-Sgm **154**, 1422 (2008).
[36] B.C. Kim and D.R. Lovley, Fems Microbiol. Lett. **286**, 39 (2008).
[37] D.E. Holmes, S.K. Chaudhuri, K.P. Nevin, T. Mehta, B.A. Methe, A. Liu, J.E. Ward, T.L. Woodard, J. Webster, and D.R. Lovley, Environ. Microbiol. **8**, 1805 (2006).

[38] H. Richter, K.P. Nevin, H.F. Jia, D.A. Lowy, D.R. Lovley, and L.M. Tender, Energ. Environ. Sci. **2**, 506 (2009).
[39] M.E. Hernandez and D.K. Newman, Cell. Mol. Life Sci. **58**, 1562 (2001).
[40] D.H. Park and J.G. Zeikus, J. Bacteriol. **181**, 2403 (1999).
[41] M. Allen, Bacteriol. Rev. **30**, 80 (1966).
[42] D.H. Park and J.G. Zeikus, Appl. Environ. Microb. **66**, 1292 (2000).
[43] I.A. Ieropoulos, J. Greenman, C. Melhuish, and J. Hart, Enzyme Microb. Tech. **37**, 238 (2005).
[44] T. Zhang, C. Cui, S. Chen, X. Ai, H. Yang, P. Shen, and Z. Peng, Chem. Communications **31**, 3306 (2007).
[45] T. Zhang, C. Cui, S. Chen, H. Yang, and P. Shen, Electrochem. Commun. **10**, 293 (2008).
[46] T. Zhang, Y.L. Zeng, S.L. Chen, X.P. Ai, and H.X. Yang, Electrochem. Commun. **9**, 349 (2007).
[47] N. Pasco, J. Hay, A. Scott, and J. Webber, Aust. J. Chem. **58**, 288 (2005).
[48] P. Ertl, U. Unterladstaetter, K. Bayer, and S.R. Mikkelsen, Anal. Chem. **72**, 4949 (2000).
[49] G. Sawers, Curr. Opin. Microbiol. **2**, 181 (1999).
[50] S. Shalel-Levanon, K.Y. San, and G.N. Bennett, Metab. Eng. **7**, 364 (2005).
[51] S. Shalel-Levanon, K.Y. San, and G.N. Bennett, Biotechnol. Bioeng. **92**, 147 (2005).
[52] A. Perrenoud and U. Sauer, J. Bacteriol. **187**, 3171 (2005).
[53] B. Wackwitz, J. Bongaerts, S.D. Goodman, and G. Unden, Mol. Gen. Genet. **262**, 876 (1999).