



Tactic Response of *Shewanella oneidensis* MR-1 toward Insoluble Electron Acceptors

Joseph Oram,^a Lars J. C. Jeuken^a

^aSchool of Biomedical Sciences and the Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, United Kingdom

ABSTRACT Exoelectrogenic bacteria are defined by their ability to respire on extracellular and insoluble electron acceptors and have applications in bioremediation and microbial electrochemical systems (MESs), while playing important roles in biogeochemical cycling. *Shewanella oneidensis* MR-1, which has become a model organism for the study of extracellular respiration, is known to display taxis toward insoluble electron acceptors, including electrodes. Multiple mechanisms have been proposed for MR-1's tactic behavior, and, here, we report on the role of electrochemical potential by video microscopy cell tracking experiments in three-electrode electrochemical cells. MR-1 trajectories were determined using a particle tracking algorithm and validated with Shannon's entropy method. Tactic response by MR-1 in the electrochemical cell was observed to depend on the applied potential, as indicated by the average velocity and density of motile ($>4 \mu\text{m/s}$) MR-1 close to the electrode ($<50 \mu\text{m}$). Tactic behavior was observed at oxidative potentials, with a strong switch between the potentials -0.15 to -0.25 V versus the standard hydrogen electrode (SHE), which coincides with the reduction potential of flavins. The average velocity and density of motile MR-1 close to the electrode increased when riboflavin was added ($2 \mu\text{M}$), but were completely absent in a $\Delta\text{mtrC}/\Delta\text{omcA}$ mutant of MR-1. Besides flavin's function as an electron mediator to support anaerobic respiration on insoluble electron acceptors, we propose that riboflavin is excreted by MR-1 to sense redox gradients in its environment, aiding taxis toward insoluble electron acceptors, including electrodes in MESs.

IMPORTANCE Previous hypotheses of tactic behavior of exoelectrogenic bacteria are based on techniques that do not accurately control the electrochemical potential, such as chemical-in-plug assays or microscopy tracking experiments in two-electrode cells. Here, we have revisited previous experiments and, for the first time, performed microscopy cell-tracking experiments in three-electrode electrochemical cells, with defined electrode potentials. Based on these experiments, taxis toward electrodes is observed to switch at about -0.2 V versus standard hydrogen electrode (SHE), coinciding with the reduction potential of flavins.

KEYWORDS *Shewanella oneidensis*, extracellular electron transfer, flavin, insoluble electron acceptors, microbe-mineral interactions, motility, respiration, taxis, tracking microscopy

Exoelectrogens, such as *Shewanella oneidensis* MR-1, are a group of microbes able to transfer respiratory electrons to extracellular and insoluble electron acceptors. They have been employed in the development of microbial electrochemical systems (MESs) and bioremediation and are implicated in biogeochemical cycling (1–6). The role of exoelectrogens in biogeochemistry, MESs, and bioremediation is underpinned by their ability for extracellular electron transfer (EET). Besides EET efficiency, performance is dependent on the exoelectrogens' ability to migrate toward and colonize insoluble electron acceptors. There has been a steady flow of reports focused on MR-1's tactic

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Address correspondence to Lars J. C. Jeuken, L.J.C.Jeuken@leeds.ac.uk.

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response to soluble electron acceptors, which can form concentration gradients (7–11), but, notably, contrasting models have been proposed for taxis of MR-1 toward insoluble electron acceptors (9, 10, 12–14).

Here, we decided to revisit the function of electrochemical potentials in the tactic behavior of MR-1 toward electrode surfaces. An experimental platform was developed that can specifically inspect taxis of MR-1 around electrodes with defined electrode potentials (in a three-electrode system), such that the redox state of extracellular redox compounds such as flavins can be controlled. The set-up was designed with the aim to analyze both individual- and population-level behaviors of MR-1 and $\Delta mtrC/\Delta omcA$ MR-1 ($\Delta mtrC/\Delta omcA$ MR-1 is a mutant strain deficient in outer membrane cytochromes that are required for EET).

Tracking analysis. Within the first 20 min after loading and sealing an electrochemical cell with MR-1, a high proportion (>70%) of bacteria displayed high motility throughout the cell with no relationship toward the electrode. This is expected as after sealing the cell, MR-1 will consume oxygen still dissolved in the medium. Between 20 and 60 min the motility gradually decreases as oxygen is consumed.

Microscope videos (at 10 FPS) were recorded of the electrochemical cell while applying various potentials to the gold working electrode. Motility of bacteria was analyzed using a tracking algorithm described in the supplemental material (Text S1). Similar to previous observations (12–14), only a subpopulation of the bacteria was observed to be motile. Here, only motile bacteria are considered, defined as bacteria with velocities $>4 \mu\text{m/s}$, and it is observed that $50\% \pm 10\%$ of the bacteria are motile. Two key parameters are presented here: average velocity of all motile bacteria within an area (for bacteria with velocities $>4 \mu\text{m/s}$) and motile population density, which is the density of bacteria with mean velocities $>4 \mu\text{m/s}$ in a specified area.

Taxis is dependent on electrode potential. Tracking analysis shows that when oxidative potentials are applied (0.3 V versus SHE), motile MR-1 bacteria accumulate close to the electrode (Fig. 1). These effects are stronger if an additional $2 \mu\text{M}$ riboflavin is added to the capillary cell (see Fig. S1). Importantly, $\Delta mtrC/\Delta omcA$ MR-1 does not respond to the applied potential, indicating accumulation and motility are dependent on MR-1's ability for EET (Fig. S1).

Switching to a relatively reducing potential of -0.3 V results in a sudden drop in average velocity and motile population density close to the electrode (3 to $23 \mu\text{m}$, red lines in Fig. 1A and B). Somewhat unexpectedly, at first glance, is the increase in motile population density further away from the electrode (83 to $103 \mu\text{m}$, black lines in Fig. 1B). On further analysis, however, this is due to MR-1 migrating away from the electrode upon applying a reducing potential. In doing so, motile MR-1 bacteria pass through areas further away from the electrode, explaining the increase in motile population density further away from the electrode. The latter is also apparent when analyzing the directional bias of motile bacteria (Fig. 1C), which is quantified by treating the tracked trajectories as vectors with a magnitude and a directional component. Here the changes in net swimming direction can be clearly seen and coincide well with the changes in applied potential.

To validate the tracking analyses, videos were also analyzed with a method based on Shannon's entropy, which does not require locating bacteria and their trajectories, both of which can be biased depending on the tracking algorithm used. Shannon's entropy is the entropy for each pixel in the video based on intensity fluctuations over a specified duration. Shannon entropy values for pixels in areas of high bacterial motility will thus be higher than areas of low or no motility. Shannon's entropy has previously been used in the analysis of time series in numerous fields, including bacterial population motility by Nisenbaum et al. (15). Qualitatively identical results were obtained using the two unrelated techniques (Fig. S2). Agreement between results obtained from tracking analysis and Shannon's entropy confirms the validity of the analytical methods.

Tactic behavior switches at about -0.2 V . By applying a series of potentials, while measuring the bacterial response through quantifiable parameters (average velocity,

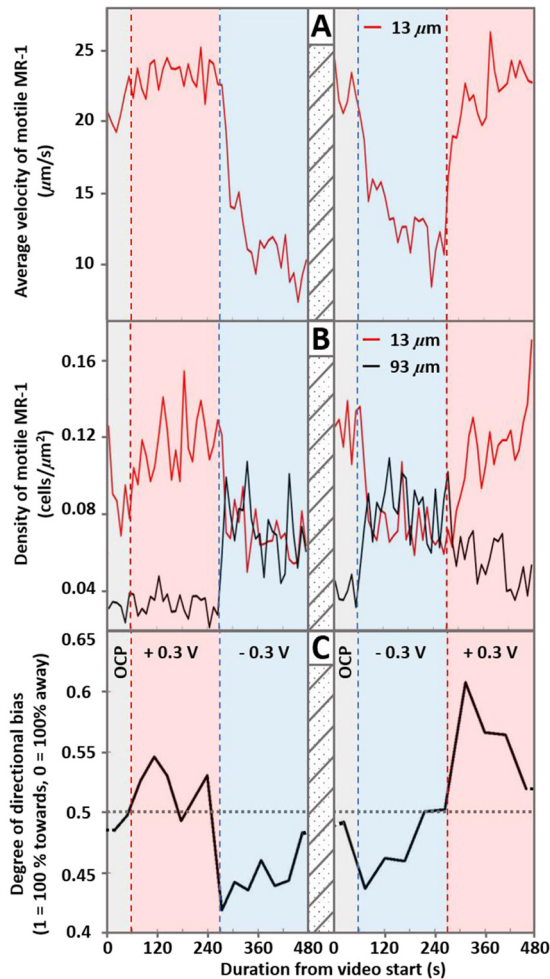


FIG 1 Time traces of (A) average velocity, (B) motile population density, and (C) directional bias of MR-1 in electrochemical cells during the application of different potentials. Changes in applied potential are indicated by the blue (switch to -0.3 V SHE) and red (switch to $+0.3$ V SHE) dashed lines and shaded areas. (A) Average velocity of motile MR-1 close to the electrode surface (between ~ 3 and 23 μm , mid-distance = 13 μm). (B) MR-1 motile population density calculated for the available area close to the electrode (between ~ 3 and 23 μm , mid-distance = 13 μm , red line) and the area further away from the electrode (between 83 and 103 μm , mid-distance = 93 μm , black line). (C) The average direction of all motile bacterial movement relative to the electrode in the form of a fraction, where 1 indicates a complete and direct migration toward the electrode and 0 indicates migration away from the electrode. Each data point was calculated as the weighted mean from 211 to 773 complete traces. The first video (left) was taken after ~ 60 min from loading and sealing the cell with MR-1 suspension. The second video (right) was taken 30 min after the first. All samples consist of the bacteria (MR-1) grown semianaerobically in LB and diluted 5-fold with 20 mM MOPS, 30 mM Na_2SO_4 , pH 7.4, supplemented with 50 mM lactate and 2 μM riboflavin. During recording of the first video (left), the potential remained at open cell potential (OCP, -0.18 V) for the first 60 s, after which potentials of $+0.3$ V and -0.3 V were applied for 210 s each. During recording of the second video (right), the potential remained at OCP (-0.18 V) for the first 60 s, after which potentials of -0.3 V and $+0.3$ V were applied for 210 s each.

motile population density, and directional bias), the potential can be identified at which the change in bacterial behaviors occurs. Throughout all the video microscopy experiments carried out using MR-1, with or without added riboflavin, the shift in motile behavior is observed between -0.25 and -0.15 V. For MR-1 with added riboflavin, the window where the change occurred is narrower and between -0.25 V and -0.205 V, which coincides with the reduction potential of riboflavin (Fig. 2).

Here, tactic behavior was mostly absent in $\Delta\text{mtrC}/\Delta\text{omcA}$ MR-1. Although in this work only riboflavin was added, it is well known that MtrC reduces other flavins besides riboflavin, in particular flavin mononucleotide (FMN) (16, 17). MR-1 is known to excrete flavins, reaching concentrations between 100 and 200 nM in “closed” electrochemical

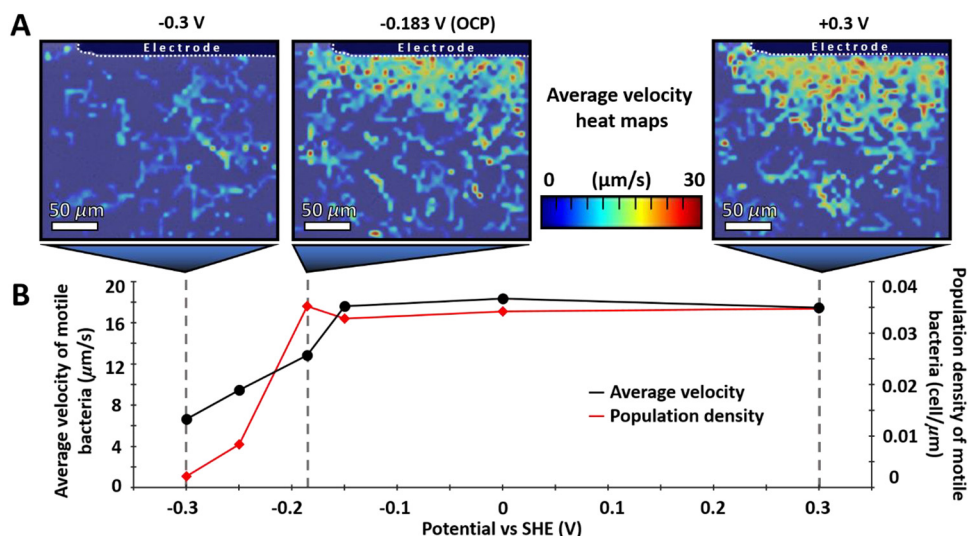


FIG 2 (A) Average velocity of MR-1 (presented as heatmaps) for three potentials (-0.3 , -0.183 , and $+0.3$ V versus SHE) representing the data used to calculate three of the data points shown in panel B. (B) Overlaid plots of the motile population density (red) and average velocity (black) of MR-1 within $20\ \mu\text{m}$ of the electrode surface as a function of the electrochemical potential. Each applied potential was held for at least 3 min, and only data collected in the third minute were used. The data used to produce panel B were collected over two consecutive videos at the location/experiment. The first video was taken after 60 min from loading and sealing the cell with MR-1 suspension. The cell was loaded with bacteria (MR-1) grown semianaerobically in LB and diluted 5-fold with 20 mM MOPS, 30 mM Na_2SO_4 , pH 7.4, supplemented with 50 mM lactate and $2\ \mu\text{M}$ riboflavin.

systems (17). Besides secreted flavins, flavins are found in yeast extract (18) and vitamin mixes used to prepare growth media. We estimate that in our experiments (using a one-in-five dilution of LB), even without any additional riboflavin, flavin concentrations could reach up 160 to 240 nM, explaining why tactic behavior is also observed in the absence of added riboflavin (Fig. S2).

Flavins, including riboflavin (vitamin B_2), are a vital metabolite for MR-1, and control experiments in the complete absence of (ribo)flavin are not feasible. In spite of this, we propose that MR-1 utilizes riboflavin, and possibly other flavins, as a means to migrate toward insoluble electron acceptors (e.g., metal oxides) and initiating colonization of the surface. This function would be in addition to the multiple roles of flavins in EET respiration. First, flavins act as an electron shuttle for mediated electron transfer, enabling respiration to continue in situations where the bacterium is in a planktonic state with limited soluble electron acceptors and no insoluble electron acceptors in its immediate vicinity (17). Second, flavins act as cofactors for MtrC and OmcA, enabling enhanced direct electron transfer (16). The concentrations required for efficient energy taxis could be much lower than for substantial mediated electron transfer. We thus propose that self-secreted flavins are not only redox mediators, but also redox sensors, and could be an important factor for MR-1's survival in electron acceptor-limited niches.

The MATLAB codes used for the tracking and Shannon entropy analysis are openly available from the University of Leeds Data Repository (<https://doi.org/10.5518/409>).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/mBio.02490-18>.

TEXT S1, DOCX file, 1.3 MB.

FIG S1, TIF file, 0.4 MB.

FIG S2, TIF file, 0.9 MB.

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REFERENCES

- Hartshorne RS, Reardon CL, Ross D, Nuester J, Clarke TA, Gates AJ, Mills PC, Fredrickson JK, Zachara JM, Shi L, Beliaev AS, Marshall MJ, Tien M, Brantley S, Butt JN, Richardson DJ. 2009. Characterization of an electron conduit between bacteria and the extracellular environment. *Proc Natl Acad Sci U S A* 106:22169–22174. <https://doi.org/10.1073/pnas.0900086106>.
- Shi L, Rosso KM, Clarke TA, Richardson DJ, Zachara JM, Fredrickson JK. 2012. Molecular underpinnings of Fe(III) oxide reduction by *Shewanella oneidensis* MR-1. *Front Microbiol* 3:50. <https://doi.org/10.3389/fmicb.2012.00050>.
- Pant D, Van Bogaert G, Diels L, Vanbroekhoven K. 2010. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. *Bioresour Technol* 101:1533–1543. <https://doi.org/10.1016/j.biortech.2009.10.017>.
- Logan BE, Rabaey K. 2012. Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies. *Science* 337:686–690. <https://doi.org/10.1126/science.1217412>.
- Beliaev AS, Klingeman DM, Klappenbach JA, Wu L, Romine MF, Tiedje JA, Nealson KH, Fredrickson JK, Zhou J. 2005. Global transcriptome analysis of *Shewanella oneidensis* MR-1 exposed to different terminal electron acceptors. *J Bacteriol* 187:7138–7145. <https://doi.org/10.1128/JB.187.20.7138-7145.2005>.
- Rotaru AE, Calabrese F, Stryhanyuk H, Musat F, Shrestha PM, Weber HS, Snoeyenbos-West OLO, Hall POJ, Richnow HH, Musat N, Thamdrup B. 2018. Conductive particles enable syntrophic acetate oxidation between *Geobacter* and *Methanosarcina* from coastal sediments. *mBio* 9:e00226-18. <https://doi.org/10.1128/mBio.00226-18>.
- Baraquet C, Théraulaz L, Iobbi-Nivol C, Méjean V, Jourlin-Castelli C. 2009. Unexpected chemoreceptors mediate energy taxis towards electron acceptors in *Shewanella oneidensis*. *Mol Microbiol* 73:278–290. <https://doi.org/10.1111/j.1365-2958.2009.06770.x>.
- Nealson KH, Moser DP, Saffarini DA. 1995. Anaerobic electron-acceptor chemotaxis in *Shewanella putrefaciens*. *Appl Environ Microbiol* 61:1551–1554.
- Li R, Tiedje JM, Chiu C, Worden RM. 2012. Soluble electron shuttles can mediate energy taxis toward insoluble electron acceptors. *Environ Sci Technol* 46:2813–2820. <https://doi.org/10.1021/es204302w>.
- Bencharit S, Ward MJ. 2005. Chemotactic responses to metals and anaerobic electron acceptors in *Shewanella oneidensis* MR-1. *J Bacteriol* 187:5049–5053. <https://doi.org/10.1128/JB.187.14.5049-5053.2005>.
- Li R, Auchtung JM, Tiedje JM, Worden RM. 2011. *Shewanella oneidensis* MR-1 chemotaxis in a diffusion gradient chamber. *Environ Sci Technol* 45:1014–1020. <https://doi.org/10.1021/es102425p>.
- Harris HW, El-Naggar MY, Nealson KH. 2012. *Shewanella oneidensis* MR-1 chemotaxis proteins and electron-transport chain components essential for congregation near insoluble electron acceptors. *Biochem Soc Trans* 40:1167–1177. <https://doi.org/10.1042/BST20120232>.
- Harris HW, El-Naggar MY, Bretschger O, Ward MJ, Romine MF, Obraztsova AY, Nealson KH. 2010. Electrokinetics is a microbial behavior that requires extracellular electron transport. *Proc Natl Acad Sci U S A* 107:326–331. <https://doi.org/10.1073/pnas.0907468107>.
- Kim BJ, Chu I, Jusuf S, Kuo T, TerAvest MA, Angenent LT, Wu M. 2016. Oxygen tension and riboflavin gradients cooperatively regulate the migration of *Shewanella oneidensis* MR-1 revealed by a hydrogel-based microfluidic device. *Front Microbiol* 7:1438. <https://doi.org/10.3389/fmicb.2016.01438>.
- Nisenbaum M, Maldonado E, Martinez Arca J, Gonzalez JF, Passoni LI, Murialdo SE. 2016. Video processing analysis for the determination and evaluation of the chemotactic response in bacterial populations. *J Microbiol Methods* 127:146–153. <https://doi.org/10.1016/j.mimet.2016.06.006>.
- Xu S, Jangir Y, El-Naggar MY. 2016. Disentangling the roles of free and cytochrome-bound flavins in extracellular electron transport from *Shewanella oneidensis* MR-1. *Electrochim Acta* 198:49–55. <https://doi.org/10.1016/j.electacta.2016.03.074>.
- Marsili E, Baron DB, Shikhare ID, Coursolle D, Gralnick JA, Bond DR. 2008. *Shewanella* secretes flavins that mediate extracellular electron transfer. *Proc Natl Acad Sci U S A* 105:3968–3973. <https://doi.org/10.1073/pnas.0710525105>.
- Lee JS, Little BJ. 2015. Technical note: electrochemical and chemical complications resulting from yeast extract addition to stimulate microbial growth. *Corrosion* 71:1434–1440. <https://doi.org/10.5006/1833>.