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*Biochemistry*, **Just Accepted Manuscript** • DOI: 10.1021/acs.biochem.8b00600 • Publication Date (Web): 10 Jul 2018

# Extracellular Electron Transfer by the Gram-positive Bacterium *Enterococcus faecalis*

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**ABSTRACT:** Extracellular electron transfer (EET) in microbial cells is essential for certain biotechnological applications and contributes to the biogeochemical cycling of elements and syntrophic microbial metabolism in complex natural environments. The Gram-positive lactic acid bacterium *Enterococcus faecalis*, an opportunistic human pathogen, is shown to be able to transfer electrons generated in fermentation metabolism to electrodes directly and indirectly via mediators. By exploiting *E. faecalis* wild-type and mutant cells it is demonstrated that reduced demethylmenaquinone in the respiratory chain in the bacterial cytoplasmic membrane is crucial for the EET. Heme-proteins are not involved and cytochrome *bd* oxidase activity was found to attenuate EET. These results are significant for the mechanistic understanding of EET in bacteria and for design of microbial electrochemical systems. The basic findings infer that in dense microbial communities, such as in biofilm and in the large intestine, metabolism in *E. faecalis* and similar Gram-positive lactic acid bacteria might be electrically connected to other microbes. Such an intercellular electron transfer might confer syntrophic metabolism that promote growth and other activities of bacteria in the microbiota of humans and animals.

## INTRODUCTION

Electron transfer (ET) reactions are fundamental in living organisms. Many microorganisms are able to connect their metabolism to extracellular solid materials (electrodes and natural minerals) donating or accepting electrons, directly or via mediators, i.e., are capable of extracellular ET (EET). This ability is currently receiving intense attention within different disciplines such as bioelectrochemistry, biotechnology, and microbial ecology and cell physiology.[1-6] Microbe-electrode electron exchange interaction offers various promising practical applications if applied to bioremediation, microbial fuel cells (MFCs), and microbial electrosynthesis.[7-8] EET is the underlying concept in both current consuming and current producing bioelectrochemical systems. Cell-to-cell electrical connection also relies on EET. Electron exchange between microbial cells to support syntrophic metabolism and growth might be very common in natural environments such as biofilms and sediments, but is largely unexplored and has been convincingly demonstrated only in a few cases.[9-12] Transcellular electron transfer in microbial communities, e.g., in the human microbiota, potentially has a significant impact on health and disease.

From the fundamental point of view, knowledge about mechanisms behind transcellular ET is necessary for a detailed understanding of the biogeochemical cycles occurring on our planet. Recent studies stress that in order to estimate the ecological niche for electroactive microbial species[4] and define microbial electroactivity itself[5] one needs research on EET mechanisms including a description of electron flow during bacterial metabolism. To date, cell molecular aspects of EET have predominantly been studied in depth within a narrow set of electrogenic Gram-negative bacteria that rely on cell surface exposed cytochromes for the oxidation or reduction of minerals extracellularly.[1,10,13,14] Very little is known about electroactivity in Gram-positive bacteria. Compared to Gram-negative bacteria their cell envelope is without an outer membrane and the peptidoglycan layer is thicker (20–35 nm)[15] and contains substantial amounts of teichoic acids, which are covalently bound anionic polymers. Gram positive bacteria are thought to be poor in current production but can donate electrons to an external conductive acceptor[16] and are frequent members of the microbial community in MFCs.[17] Thus, there is a demand for knowledge about the cell molecular details behind EET in Gram-positive bacteria[2,6,18,19]

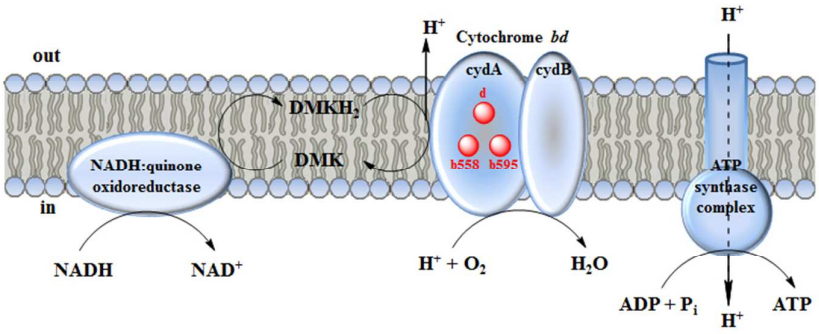


**Figure 1.** Schematic representation of the aerobic respiratory chain of *E. faecalis* as assembled in the cytoplasmic membrane when the bacterium is supplied with heme. The respiratory chain is comprised of NADH:quinone oxidoreductase, the demethylmenaquinone (DMK) pool and the quinone oxidase cytochrome *bd*. The proton gradient across the membrane generated by the activity of the respiratory chain is used by ATP synthase for ATP synthesis. The three heme prosthetic groups in the CydA subunit of cytochrome *bd* are indicated. In and out indicate the cytoplasmic and periplasmic sides of the bacterial membrane, respectively.

In this work we make use of the Gram-positive lactic acid bacterium *Entercoccus faecalis*, an opportunistic human pathogen found in the intestine of mammals.[20] *E. faecalis* cells have essentially a fermentative metabolism and cannot synthesize heme. However, if the cell is provided with heme from the environment two canonical heme proteins, catalase[21] and cytochrome *bd*,[22,23] are assembled. The cytochrome is the terminal oxidase in a minimal respiratory chain, where it oxidizes reduced demethylmenaquinone (DMK) to reduce molecular oxygen to water (Figure 1). Cytochrome *bd* consists of two protein subunits, CydA and CydB, and three heme prosthetic groups - heme *b*558, heme *b*595, and heme *d*.[24,25] Reduction of DMK is performed by various membrane associated dehydrogenases including a type-II NADH:quinone oxidoreductase. Thus when supplied with heme, glucose and molecular oxygen, *E. faecalis* cells respire by the oxidation of NADH generated in glycolysis and by pyruvate dehydrogenase activity. This yields four moles of NADH per mole of glucose potentially providing the respiratory chain with eight electrons that in principle can be transferred to an electrode. By using the laboratory wild-type *E. faecalis* strain OG1RF and mutants thereof we have investigated EET by this bacterium and identified cellular components crucial for this ET.

## EXPERIMENTAL DETAILS

**Bacterial strains and growth of bacteria.** The *E. faecalis* parental strain OG1RF[26] and its mutant derivatives EMB1 (*katA*), EMB4 (*katA cydC*)[27] and WY84 (*menB*)[28] were maintained on Todd-Hewitt agar plates. Cells were grown at +37°C and 200 rpm in tryptic

soy broth, a heme-free medium, supplemented with 1% (w/v) D-glucose. Hemin, when used, was added to the growth medium from a 0.2 or 2 mM stock solution in dimethylsulfoxide. Cells for electrochemical experiments were washed in 50 mM phosphate buffer, pH 7.40, and re-suspended in the phosphate buffer to a cell density of 1 g $mL^{-1}$ (wet weight) and used immediately.

**Immunoblot analysis of subcellular fractions.** Cells in samples of 45 ml from exponentially growing cultures were collected by centrifugation at 5,000 x g for 10 min at +4°C and the pellet was washed in 10 ml of 50 mM sodium phosphate buffer, pH 7.40. The cells were finally suspended in 1.3 ml of 50 mM potassium phosphate buffer, pH 8.00, containing 50 μg $ml^{-1}$ chloramphenicol and incubated at +25°C for 16 h to allow proteolytic degradation of apo-hemoproteins in the cells. The cell suspension was then added to 2 ml screw cap tubes containing 1.75 g of zirconia-silica glass beads (diameter = 0.1 mm) and the cells were lysed using a FastPrep-24 (3 cycles of 20 s at 6.5 M/S). Samples were kept on an ice bath between cycles and were after cell lysis placed on ice for 10 min to let the glass beads sediment. The supernatant was transferred to 2 ml tubes prior to centrifugation at 5 000 x g for 5 min. The final supernatant was saved as cell lysate.

Antiserum against *E. faecalis* CydA was generated by immunization of rabbits with a synthetic peptide (CEFQFGMNWSDYSRFVGD), corresponding to a hydrophilic loop in the protein, conjugated by the N-terminal cysteine residue to the carrier protein KLH. Cell lysates were subjected to SDS-polyacrylamide gel electrophoresis in precast 10% Bis-Tris Midi NuPage gels (Invitrogen, Carlsbad, CA, USA) using MOPS running buffer. Immunoblot was performed as described before[29] using primary antisera diluted 2,000 fold.

**Isolation and analysis of membranes.** The particulate fraction of lysed cells, i.e., membrane fraction, was isolated as described before[27] except that membranes were pelleted by centrifugation at 100,000 x g (35,000 rpm in Beckman Ti50.2 rotor) for 90 min and finally suspended in 20 mM sodium MOPS buffer, pH 7.40. Heme was determined by the pyridine hemochromogen method.[30] Redox (reduced minus oxidized) difference light absorption spectra were recorded at room temperature with an upgraded (OLIS Instruments) Aminco DW-2 spectrophotometer (slit 1 nm). NADH oxidase activity at +30° C in aerated 50 mM potassium phosphate buffer, pH 7.4, was determined by dual-wavelength (340 versus 400 nm) spectrophotometry using an initial NADH concentration of 0.16 mM.

**Electrode preparation.** Os RP, $[Os(2,2'\text{-bipyridine})_2\text{-poly(N-vinylimidazole)}_{10}Cl]^{2+/+}$, with a formal potential, E°', equal to +420 mV vs. SHE was synthesized as described in.[31] Graphite electrodes (Alfa Aesar GmbH& Co KG, Germany, diameter = 3.05 mm, surface

area = 0.0731 $cm^2$) were polished on a fine wet abrasive paper sheet (Norton P1200Tufbak T489, USA) and rinsed with a strong stream of water. After that 5 μL of Os RP solution (10 mg $mL^{-1}$ in water) was spread over the electrode surface, which was allowed to dry at room temperature for 20 min and then modified with 1 μL of *E. faecalis* cell suspension and dried again at room temperature for 5 min. To avoid washing off the cells from the electrode surface it was covered with a dialysis membrane (Spectrum Laboratories Inc., USA, molecular mass cutoff: 6-8 kDa) presoaked in buffer.

**Electrochemical measurements.** All electrochemical measurements were carried out in 50 mM phosphate buffer, pH 7.40, containing 0.1 M potassium chloride. As an alternative to Os RP mediation 0.5 mM potassium ferricyanide or menadione sodium bisulfite was added to the buffer.

The amperometric measurements were done using a three electrode flow through electrochemical wall jet cell including a working graphite, a platinum counter and a reference Ag|AgCl (0.1 M KCl) (Beta Sensor AB, Södra Sandby, Sweden) electrode controlled by a potentiostat (Zäta Elektronik, Höör, Sweden). All potentials in this work are given versus SHE. The applied potential of the working electrode was set to +588 mV (a value more positive than the E°' value of the Os RP to ensure a potential independent current response). The flow rate of the electrolyte solution was maintained at 0.5 mL $min^{-1}$ by a peristaltic pump. The distance between the nozzle and the working electrode surface was about 1 mm allowing passage of the pumped liquid through the wall-jet cell. The current responses were recorded with a strip chart recorder (Kipp&Zonen, Delft, The Netherlands), a representative flow chart is presented in Supporting Information (Figure S1). The buffer solution was purged with argon prior to the experiments to remove air bubbles that could plug the capillaries of the set-up.

Cyclic voltammetry (CV) was performed using a potentiostat (AUTOLAB PGSTAT 30, Eco Chemie, Utrecht, The Netherlands) equipped with GPES 4.9 software and with a three electrode electrochemical cell including a platinum foil as a counter electrode, a Ag|AgCl (sat. KCl) reference electrode (Sensortechnik Meinsberg, Germany) and a working electrode with immobilized bacterial cells. The scan rate was 0.5 mV $s^{-1}$.

Electrochemical impedance spectroscopy (EIS) measurements were performed in the same electrochemical cell at an applied potential, $E_{app}$, of +588 mV and in the presence of 10 mM D-glucose in the buffer solution. The swept frequency range was from 5 kHz to 0.1 Hz with a voltage amplitude perturbation of 5 mV. The experimental impedance data were analyzed using ZSimpWin software from Princeton Applied Research (Oak Ridge, TN, USA).

Presented data were obtained with at least three measurements for each experiment using different electrodes prepared independently.

**Miscellaneous methods.** Catalase activity of cell lysates was determined as described before.[29] Protein concentrations were determined using the BCA protein assay (Pierce Chem Co.). For preparation of all aqueous solutions Milli-Q (Millipore, Bedford, MA, USA) deionized water was used.

## RESULTS AND DISCUSSION

### Direct and mediated ET from *E. faecalis* cells to electrodes

Heme-proteins, especially cytochromes, are important components for EET in many Gram-negative bacteria and a few Gram-positive bacteria.[1,13,14,19,32] Assuming involvement of cytochromes, we recently demonstrated[33] that *E. faecalis* cells supplied with hemin are able to undergo mediated ET. ET to gold electrodes was not observed under those experimental conditions unless an osmium (Os) redox polymer (RP) ([Os(2,2′-bipyridine)$_2$-poly(N-vinylimidazole)$_{10}$Cl]$^{2+/+}$) with a redox potential, E°', of +420 mV vs. standard hydrogen electrode (SHE) was provided as electron mediator. Os RPs are widely applied in bioelectrochemical applications due to the combination of efficient electron transfer ability and nontoxic matrix supportive properties.[16,34,35] In this work, to in detail investigate features of EET by *E. faecalis*, the bacterium was cultivated also in the absence of hemin. Strain OG1RF heme-free cells directly immobilized on a graphite electrode demonstrated a small but clearly detectable biocatalytic current in response to D-glucose (Figure S2a). Generally the efficiency of ET between microbial cells and electrodes can be enhanced by the presence of redox mediator compounds. Applying the same type of Os RP as before and using CV we found that heme-free bacterial cells showed a well-defined anodic biocatalytic current response to D-glucose (Figure S2d). Electron flow to the graphite electrode was enhanced also when the cells were supplied with the freely diffusible redox compound menadione (Figure S2b) or ferricyanide (Figure S2c).

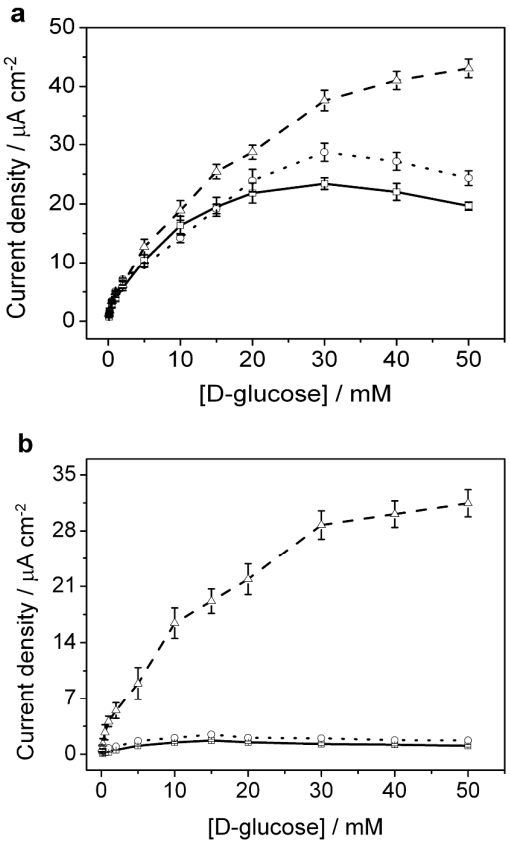


**Figure 2.** Current density responses of heme-free *E. faecalis* a) wild-type strain OG1RF and b) DMK-deficient WY84 cells on a graphite electrode mediated by different types of compounds: Os RP coated electrode (solid lines), 0.5 mM potassium ferricyanide (dotted lines), and 0.5 mM menadione sodium bisulfite (dashed lines). The amperometric experimental conditions were: carrier solution 100 mM KCl in 50 mM phosphate buffer, pH 7.40; flow rate 0.5 mL $min^{-1}$; $E_{app}$ +588 mV vs. SHE.

To evaluate the ET efficiency quantitatively, chronoamperometry under flow injection conditions at a stationary applied potential, $E_{app}$, of +588 mV was performed with injections of D-glucose in the range 0.1 to 50 mM (Figure 2a). The OG1RF cells showed a well established ET via the Os RP matrix with a current density up to 23.4±0.9 μA $cm^{-2}$ (in response to 30 mM D-glucose). The current response upon injection of glucose into the electrochemical wall jet cell was fast and the current stable over time as shown in Figure S1. The decrease in current at very high substrate concentrations (> 30 mM glucose) is attributed to local pH changes inside the cell or/and product inhibition as observed for other bacteria.[36,37] Current densities of 28.7±1.5 and 43.1±1.6 μA $cm^{-2}$ were obtained in the presence of ferricyanide and menadione, respectively (Figure 2a).

**Heme proteins are not required for EET**

The effect of the heme protein content in *E. faecalis* cells on EET was examined using chronoamperometry under flow injection conditions (Figure 3a). The catalase polypeptide, KatA, and the cytochrome *bd* polypeptides, CydA and CydB, are synthesized also in heme-free *E. faecalis* cells. KatA polypeptides without heme incorporated are in contrast to the holoprotein susceptible to proteolytic degradation in the cell.[29] We found that the CydA polypeptide similarily is unstable in heme-free cells and made use of this property to determine the extent of hemylation of CydA in *E. faecalis* cells depending on the availability of hemin in the growth medium. Cells were grown in the presence of different concentrations of hemin, harvested, incubated at +25° C for degradation of the apo-proteins and finally cell extracts were probed with KatA and CydA antisera in immunoblots (Figure S3). Based on the result, hemin at the concentrations 0.2 and 2.0 μM in the growth medium were selected to obtain heme-limited cells and heme-sufficient cells, respectively. After growth at 0.2 μM hemin the catalase and cytochrome *bd* contents were both reduced to about 30% compared to heme-sufficient cells, as determined by catalase activity (20 U $mg^{-1}$ protein in heme-sufficient cells), by the amount of hemylated CydA polypeptide and by visible light spectroscopy of cytochrome in isolated membranes. OG1RF cells with different heme protein contents immobilized on Os RP coated electrodes showed all current generation in response to D-glucose. Unexpectedly, in the perspective of the general importance of heme proteins for EET in electroactive bacteria, the current response was lower for *E. faecalis* cells supplied with hemin and those grown at the highest concentration of hemin showed the lowest current response (Figure 3a).

To analyze the ET processes occurring in the *E. faecalis* cells-Os RP-graphite electrode system EIS[38] was carried out in the presence of 10 mM D-glucose. Nyquist plots of the obtained impedance spectra fitted best with the equivalent circuit shown in Figure 3b. The proposed circuit model has been used for characterization of surfaces with attached Gram-positive bacterial cells[39,40] as well as for conducting polymer-coated electrodes.[41] A satisfactory approximation expresses how accurate the fitting is with regard to the experimental results and was about $10^{-4}$ for all the obtained EIS data, indicating a good fitting. Charge transfer resistance, $R_{ct}$, is an indirect measure and one way to interpret the kinetics of the reaction occurring at the electrode surface. The $R_{ct}$ values obtained from the EIS data were significantly lower for heme-free cells compared to those containing heme

(Table 1). This indicated enhanced ET for the heme-free *E. faecalis* cells, in full agreement with the amperometric data.

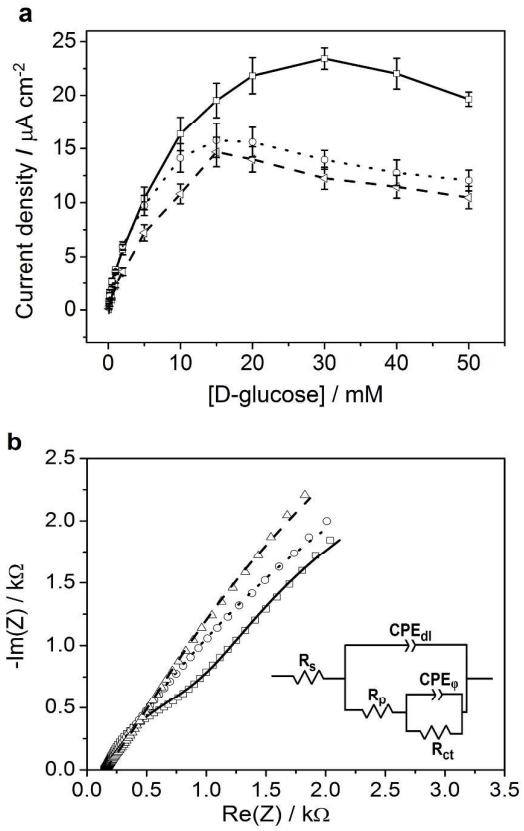


**Figure 3.** *E. faecalis* OG1RF cells cultivated in the absence (solid line) and in the presence of 0.2 μM hemin (dotted line) or 2 μM hemin (dashed line) and immobilized on an Os RP coated graphite electrode. a) Current density responses to various D-glucose concentrations. The experimental conditions were as in Figure 2. b) Nyquist plots of data obtained in the presence of 10 mM D-glucose. Squares, circles, and triangles show the experimental data, whereas the lines (solid, dotted, and dashed) represent equivalent circuit fitting. The inset shows the equivalent circuit used for our modeling, including electrolyte resistance ($R_s$), polarization resistance ($R_p$), charge transfer resistance ($R_{ct}$) and non-ideal double layer and pseudocapacitance, represented by two constant phase elements ($CPE_{dl}$ and $CPE_{\varphi}$, respectively).

## Cytochrome *bd* activity negatively affects EET

Catalase and cytochrome *bd* are the only heme proteins in *E. faecalis*.[23] To determine how these two proteins affect the ET efficiency from cells to the electrode, the electrochemical activity of strain EMB1 lacking catalase and EMB4 devoid of both catalase and cytochrome *bd* were investigated. The catalase-depleted strain behaved essentially as the wild-type (Figure S4a). In contrast, the strain lacking cytochrome *bd* showed no dependence on hemin-supplementation (at ≤ 15 mM D-glucose concentrations) (Figure S4b). The $R_{ct}$ values

for EMB1 and EMB4 cells were estimated from impedance spectra measured under the same conditions as for strain OG1RF (Table 1). The catalase-depleted strain showed the same behavior as the wild-type, i.e., the $R_{ct}$ increased with increased heme content in the cell. The cytochrome *bd*-deficient strain displayed no effect of heme on the kinetics of the cell-electrode communication. These results verified that ET from the cells to the electrode does not require catalase or cytochrome *bd*. Furthermore, it clearly showed that lack of cytochrome *bd* oxidase activity promotes EET. The latter effect, as well as the negative effect of heme on ET from OGR1F and EMB1 cells to the electrode, indicated that the capacity for EET depends on the level of reduced quinone in the cytoplasmic membrane, i.e., in the case of cytochrome *bd* deficiency (caused by the lack of heme or *cyd* gene inactivation) the menaquinone pool is in a more reduced state compared to when cytochrome *bd* oxidase activity is present.

**Table 1**. Summary of charge transfer resistance ($R_{ct}$) values for *E. faecalis* strains grown at different concentrations of hemin and immobilized on an Os RP modified graphite electrode in the presence of 10 mM D-glucose. Figure 3 shows the experimental details for strain OG1RF.

| **Strain** | **Relevant phenotype** | **$R_{ct}$, kΩ [hemin] added to the growth medium** | | |
|---|---|---|---|---|
| | | **0 μM** | **0.2 μM** | **2 μM** |
| OG1RF | Wild-type | 5.9 ± 0.4 | 10.8 ± 1.0 | 14.2 ± 0.9 |
| EMB1 | Lacks catalase | 11.2 ± 0.7 | 13.9 ± 0.7 | 17.3 ± 1.2 |
| EMB4 | Lacks catalase and cytochrome *bd* | 11.0 ± 0.9 | 11.4 ± 1.0 | 11.4 ± 0.7 |
| WY84 | Lacks DMK | 14.6 ± 0.9 | 18.7 ± 1.2 | 24.9 ± 1.6 |

**Quinone is essential for EET**

To determine the importance of the respiratory chain quinone for EET we analysed the electrochemical behavior of strain WY84 which is deleted for the *menB* gene (encodes 1,4-dihydroxy-2-naphthoyl-CoA synthase) and therefore blocked in the synthesis of DMK.[42] The mutant grown in the presence of hemin showed low respiratory activity (less than 5% compared to the parental strain OG1RF) and contained a normal amount of cytochrome *bd*, as determined by NADH oxidase activity and redox difference spectroscopy of isolated membranes, respectively. The WY84 cells immobilized on Os RP modified electrodes showed poor current generation capacity in response to glucose (Figures 2b and S5) and

high $R_{ct}$ (Table 1). The residual low electrochemical activity of the WY84 cells might be explained by the presence of a small amount of quinone in the mutant cells resulting from endogenous components in the complex growth medium or that the *menB* mutation does not completely block synthesis of quinone. Despite the low current response, the WY84 cells responded to heme supplementation like the wild-type OG1RF (Figure S5). These results indicated the crucial role of DMK/$DMKH_2$ for electrochemical communication between *E. faecalis* cells and the electrode.

When supplied with the water-soluble DMK analogue menadione strain WY84 showed enhanced electrochemical activity and with no inhibitory effect at high glucose concentrations (Figure 2b). The wild-type, OG1RF, behaved similarly in the presence of menadione (Figure 2a). Ferricyanide, however, did not promote ET from the WY84 cells to the electrode, as was the case for the OG1RF cells (Figure 2). This suggests that reduced DMK in the cell is the direct reductant of ferricyanide and thereby a key component also for biofilm formation promoted by EET.[43] Additionally, and in contrast to the wild-type, when WY84 cells were directly immobilized on a graphite electrode they did not show detectable electrochemical activity (Figure S2a). Thus, DMK-deficient *E. faecalis* cells produced only a low current and supplementation with menadione restored the ET properties. Furthermore, in cells containing DMK complete absence (*cyd* mutant) or depletion (heme deficiency) of the terminal respiratory enzyme cytochrome *bd*, promoted EET apparently due to hyperreduction of the quinone pool. Based on these findings we conclude that electrons generated by glucose fermentation are transferred from the cells to the electrode by, or at least via, reduced DMK.

A role of excreted quinones for EET has been observed for other bacteria.[44] EET to an electrode without added soluble mediator was reported[45] for *Lactococcus lactis*, which is a Gram-positive bacterium closely related to *E. faecalis*. In this case the cells were under the measurements provided with nutrients for growth and the current increased due to synthesis of 2-amino-3-dicarboxy-1,4-naphthoquinone in the cells incubated in contact with the electrode. In our electrochemical experiments *E. faecalis* was only supplied with glucose in phosphate buffer and we did not observe an increased current with time. Since growth of *E. faecalis* requires several amino acids and vitamins it is unlikely that some soluble diffusible compound synthesized from glucose during our measurements mediates ET from reduced DMK in the cytoplasmic membrane to the electrode surface. The redox active

naphthoquinone headgroup of DMK is reduced by NADH:quinone dehydrogenase on the cytoplasmic side of the membrane and flipped across the membrane lipid bilayer (Figure 1). *E. faecalis* is physically a robust Gram-positive bacterium with a thick peptidoglycan layer,[32] which presumably prevents direct contact between the cytoplamic membrane and an electrode surface. The positively charged Os RP network on the electrode probably intercalates with the abundant negatively charged teichoic acids in the cell envelope to reach positions close enough to the membrane to allow efficient ET from reduced DMK to the electrode. Peptidoglycan and teichoic acids in combination with bound reversibly reducible metal ions have been proposed to mediate ET across the cell wall in Gram-positive bacteria.[29]

## CONCLUSIONS

The findings presented in this work are highly relevant for biotechnology (e.g., construction of MFCs and biosensors), medicine (e.g., understanding the physiology of the gut microbiota) as wells as biogeochemistry (e.g., understanding global cycling of elements). We demonstrate that *E. faecalis* cells can transfer electrons to a graphite electrode directly and more efficiently via an Os RP network or via freely difffusable monomeric redox mediators. The mechanistic description of Os RP-mediated electron shuttling between microbes and electrodes remains unclear.[35] However, this is the first study reporting evidence-based identification of the quinone pool in the respiratory chain as a key component for EET in a Gram-positive bacterium. Cytochromes are in many cases crucial components for EET in bacteria.[8] We here show that heme proteins are not required for EET in *E. faecalis* and that cytochrome *bd* activity attenuates EET. Further studies are required to establish whether the mechanism for EET in Gram-positive bacteria in general is similar to that in the lactic acid bacterium *E. faecalis*.

In terms of biotechnological applications Gram-positive bacteria are “weak” current generators but frequently are participants of microbial consortia in MFCs. New findings at the cellular and molecular levels are fundamental to achieve a better understanding of electroactive microbial communities where syntrophic cooperation is crucial for electricity generation.[46-49] Knowledge about the mechanism(s) behind EET are expected to lead to improved design of bioelectrochemical systems since both cell-electrode and cell-cell interactions rely on electrical properties of individual microbes and are essential aspects to consider when developing self-sustained mirobial systems.

An important aspect of our results reported in this study is that EET from a bacterium to conductive surfaces presumably reflects the ability of ET to other cells. There are findings suggesting fermentative metabolism in *Enterococcus* sp. coupled to ET to methanogens.[50] In the perspective of medicine, *E. faecalis* cells in the large intestine of a mammal (which is an oxygen- and nutrient-limited dense bacterial multispecies environment) metabolise carbohydrates but are starved for heme and molecular oxygen. These cells therefore would be deficient in cytochrome *bd* oxidase activity leading to hyperreduction of the DMK pool. EET to another cell, that is not dependent on exogenous heme and with an anaerobic metabolism, would relieve the hyperreduction of the DMK pool and could promote metabolism in both cells. Such electric synthrophy would be beneficial for the collective survival and growth of gut microbiota and contribute to the balance between health and disease. Hyperreduction of the quinone pool in *E. faecalis* cells is a source of reactive oxygen species that can promote generation of sporadic adenomatous polyps in the colon and colorectal cancer.[51] With knowledge about molecular mechanisms and extent of trans-celluar ET in microbial communities we can better judge the importance of this process and act rationally. Finally it can be noted that cytochrome *bd* is unique to bacteria and important to many pathogens.[25,52] Wiring respiratory ET from a bacterium such as *E. faecalis* to an electrode could be used, for example, as a sensor in screens for antimicrobial compounds acting by inhibition of cytochrome *bd.*[53]

## SUPPORTING INFORMATION

Representative recording of amperometric measurement under flow injection conditions (Figure S1), CVs showing electrochemical communication between heme-free *E. faecalis* wild-type cells and a graphite electrode in absence or presence of various redox mediators (Figure S2), heme-dependent assembly of catalase and cytochrome *bd* in *E. faecalis* (Figure S3), negative affect of cytochrome *bd* activity on extracellular electron transfer by *E. faecalis* (Figure S4), and electrochemical activity of DMK-deficient *E. faecalis* cells cultivated in the absence or presence of heme (Figure S5).

## ACKNOWLEDGEMENTS

The authors thank Mark Huycke, University of Oklahoma, for generously providing strain WY84, Astrid Teubenbacher for technical assistance, Dr. Peter Ó Conghaile, NUI Galway, for Os RP synthesis, and the following agencies for financial support; the European Commission (”Bioenergy” FP7-PEOPLE-2013-ITN-607793) to DL, GP and LG, and the Swedish Research Council (2014-5908) to LG and (2015-02547) to LH.

**Author contributions**

LH initiated the study. GP, LG and LH planned experiments. GP performed all electrochemical experiments and assembled data. LH made biochemical analyses, DL provided Os RP. GP, LG and LH interpreted results and wrote the manuscript.

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**Table of Contents Graphic.**