Electrochemical Characterization of Bacteria from the Human Oral Microbiome

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Abstract

The field of microbial electrochemistry studies the ability of some bacteria to move electrons to terminal acceptors outside of their cell membranes such as an electrode or an insoluble metal. While there has been extensive research into the characterization of certain extracellular electron transport (EET) capable bacteria that live in soils such as *Shewanella oneidensis* and *Geobacter metallireducens,* little is known about the EET capabilities of bacteria in the human microbiome. In this study we looked at the EET capabilities of cultured bacteria from saliva samples, and more specifically into the EET capabilities of an oral pathogen, *Aggregatibacter actinomycetemcomitans (Aa).* We did this by culturing cells from saliva samples and isolates of *Aa* and performing cyclic voltammetry and chronoamperometry on the samples. We found that *Aa* is electroactive, that *Aa* performs EET through mediated transport, and that some cultured saliva bacteria are EET capable in vitro.

Background

Extracellular electron transport (EET) capable bacteria are a group of bacteria that can move electrons to a terminal electron acceptor located outside of the cell. Unlike most cells, which generally use oxygen or other soluble molecules as a terminal electron acceptor, EET capable bacteria can use metals like iron, manganese oxides, and electrodes. One such organism that is EET active in *Shewanella oneidensis* MR-1. *Shewanella* can perform EET in three ways (figure 1); they directly transfer electrons through the membrane to the oxides outside of the cell, they can use a shuttle-like particle that carry electrons to the electron acceptor, and they produce nanowire appendages to reach electron acceptors[1]. *Shewanella* was discovered in 1985[2] and since then, the field of bio-electrochemistry has grown to study the characteristics of EET capable bacteria.

Aggregatibacter actinomycetemcomitans (Aa) is a gram-negative bacteria that lives in the oral microbiome of some humans. It is an oral pathogen that is associated with aggressive periodontitis, commonly known as gum disease. *Aa* enables the acquisition of aggressive periodontitis because it is able to produce compounds that neutralize the damaging affects of oral defenses which can allow the growth of more harmful pathogens.[3] It has been shown that some strains of *Aa* are electroactive.[4]

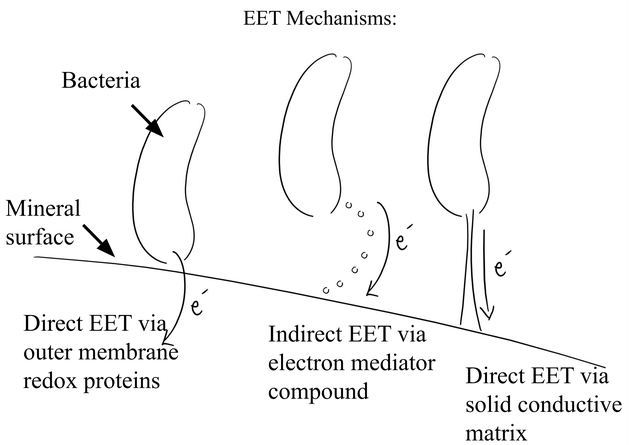

Figure 1: Illustration of the three modes of EET: Direct, Soluble, and Nanowire

Methods

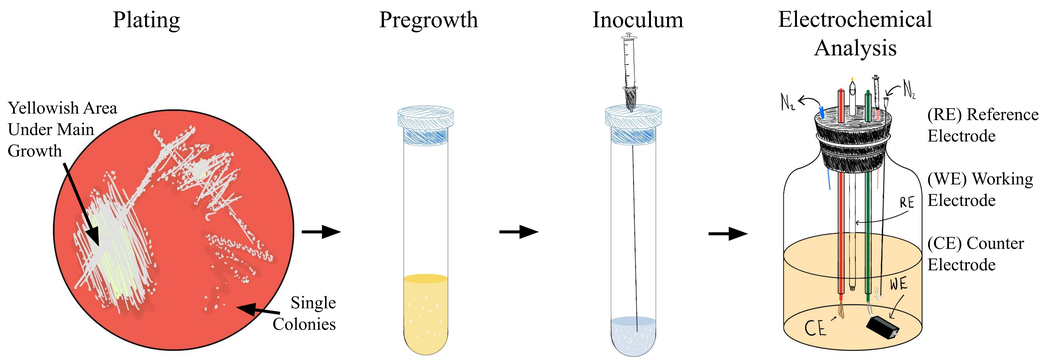

Figure 2: Illustration of experimental workflow. For *Aa,* we grew the bacteria on blood agar plates in anaerobic N2/CO2 filled and microaerobic conditions at 37 ˚C for 48 hours. We then transferred to a 25 mL of trypticase soy broth with yeast extract (TSBYE) and grew the bacteria for another 24 hours at 37 ˚C. Next, we harvested the cells by 10 minute centrifugation at 7800 rpm and washed the *Aa* three times in a media designed for electrochemical analysis (DM-Anode). Finally, the *Aa* were added to a reactor with either 25 mL of DM-Anode, or 23 mL of DM-Anode with 2mL of TSBYE for electrochemical analysis.

The saliva samples were either grown in a defined media in aerobic conditions or in an RPMI media with anaerobic conditions under N2/CO2 for 24 hours at 37 ˚C. We then harvested the cells via 10 minute centrifugation at 7800 rpm and washed the bacteria in DM-Wash solution three times. Finally, we inoculated the reactors with 25 mL of defined media under N_2 or RPMI under N2/CO2 for electrochemical analysis.

Aggregatibacter actinomycetemcomitans

Aa can perform extracellular electron transport

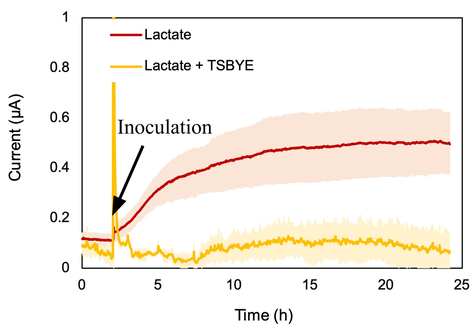

Figure 3: This Chronoamperometry was performed with the graphite felt working electrode poised at +200 mV against the reference electrode Ag/AgCl in 1M KCl. Reactors were kept anaerobic under N_2. The *Aa* cells were added two hours after the experiment began.

Aa transports electrons via indirect EET

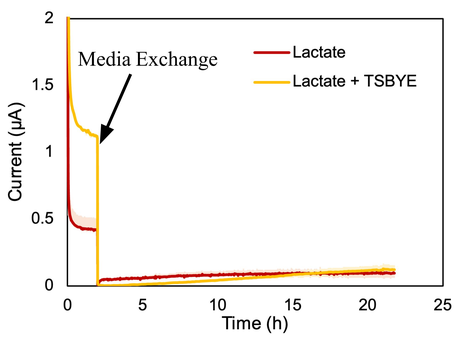

Figure 4: This Chronoamperometry was performed with the graphite felt working electrode poised at +200 mV against the reference electrode Ag/AgCl in 1M KCl. The reactors were kept anaerobic under N_2. We exchanged the media around two hours after the experiment began to see whether the cells were using direct EET or were using mediator compounds that were in solution.

Saliva

There Are Bacteria in Human Saliva Cultures That Are Electroactive

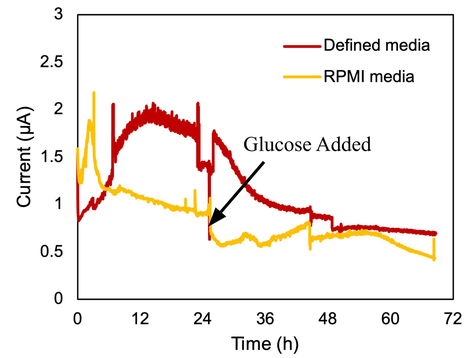

Figure 5: This Chronoamperometry was performed with the working electrode poised at +200 mV against the reference electrode's Ag/AgCl in 1M KCl. The reactors with the RPMI media were anaerobic under N2/CO2 while the reactors with the defined media were anaerobic under N_2. 40mM glucose was added to the reactors with RPMI at around 24 hours after the beginning of the experiment.

Conclusions

1. Chronoamperometry measurements showed that *Aa* can perform extracellular electron transport at +200 mV vs Ag/AgCl.
2. *Aa* transport electrons via indirect electron transfer.
3. There are bacteria in human saliva cultures that are electroactive in vitro.

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Acknowledgements

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