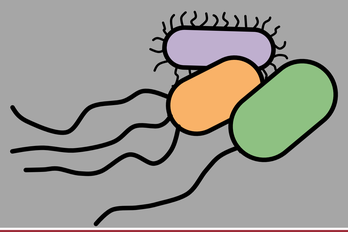

Microscopic Investigations of Physiology and Respiration in Electrode Oxidizing Microorganisms

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INTRODUCTION:

Using microscopic techniques coupled to electrochemical conditions probing for electron uptake, we hope to investigate the energetic conditions that support respiration and NADH production in electrode-oxidizing bacteria as this physiology is poorly understood. The *Alphaproteobacteria Thioclava electrotropha* and *Gamaproteobacteria Idomarina loihiensis* were isolated from a marine sediment using electrodes poised at electron donating potentials[1]. These microbes also grow heterotrophically, making them ideal model systems for studying this novel physiology. To investigate the metabolic consequences of electron uptake, we are testing microscopic techniques that assess the cellular electrochemical gradient (Nernstian voltage indicator, ThT) and NADH concentration (NADH-binding protein Peredox). To establish the validity of fluorescent markers for biosynthetic capacity and respiration, *T. electrotropha* and *I. loihiensis* were studied under heterotrophic growth conditions where the concentration of acetate and/or the presence of oxygen was used to modulate their energetic state.

Can changes in the electrochemical gradient and NADH pool of electrode oxidizing microbes be observed?

APPROACH:

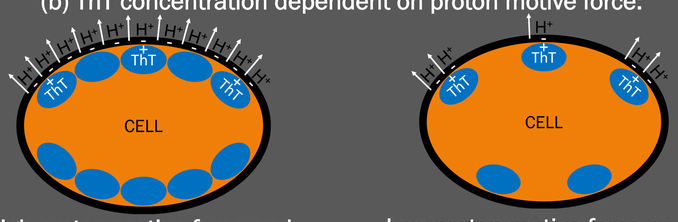
The physiology of the electron uptake in microbes was studied using two different fluorescent markers: the Peredox-mCherry NADH-binding protein and Thioflavin T (ThT). Peredox binds to reduced NADH in the cell, while ThT associates with the membrane across an electrochemical gradient.

(a) Peredox-mCherry fluorescence dependent on NADH-NAD$^+$ pool:

High NADH/NAD$^+$ and high Peredox:mCherry fluorescence

Low NADH/NAD$^+$ and low Peredox:mCherry fluorescence

(b) ThT concentration dependent on proton motive force:



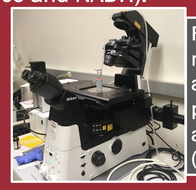
High proton motive force and high ThT fluorescence (BLUE)

Low proton motive force and low ThT fluorescence (blue)

Figure 1. Hypothetical models of fluorescent markers: (a) Peredox-mCherry protein fluorescence is dependent on the amount of NADH present in the cell. The protein binds to NADH as its configuration changes and Peredox protein becomes fluorescent. When NAD$^+$ is attached, no purple fluorescence is observed. mCherry can be used to normalize fluorescence to protein abundance[2]. Ratios of Peredox:mCherry fluorescence provides a means of assessing NADH concentration. (b) ThT (a Nernstian voltage indicator) is positively charged and is retained in cells with a negative electrical membrane potential, due to the proton motive force. Cells with a more negative membrane potential will retain more ThT[3].

METHODS:

A Nikon Eclipse Ti2 microscope was used with a plastic and glass reactor to capture images of the cells over 2 hours. All microbes were grown in minimal media (SWB) and different concentrations of acetate (0 mM, 1 mM, 10 mM). Acetate was used as an electron donor and carbon source to grow the cells[1] and can be adjusted to modify cellular respiration (proton motive force and NADH).



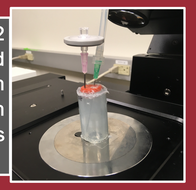
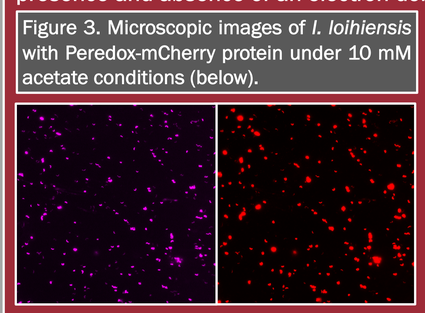


Figure 2. Nikon Eclipse Ti2 microscope (left) used to capture and analyze images. Reactor (right) with plastic tube and glass cover slip with air hose and filter to pump in gas (oxygen or nitrogen).

RESULT 1: Peredox-mCherry protein in *I. loihiensis* shows fluorescent difference in plus/minus acetate conditions

After the successful insertion of the Peredox protein into *I. loihiensis,* different concentrations of acetate (0 mM, 1 mM, 10 mM) were used to grow the cells. The different Peredox/m-Cherry fluorescence intensities were compared, suggesting a potential correlation between fluorescence and the presence and absence of an electron donor:

Figure 3. Microscopic images of *I. loihiensis* with Peredox-mCherry protein under 10 mM acetate conditions (below).



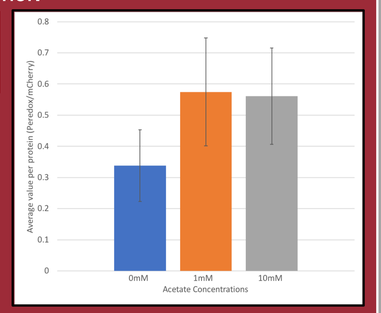


Figure 4. Comparative difference between average of Peredox:mCherry intensities under different acetate conditions. Presence of acetate as an electron donor yielded higher Peredox:mCherry intensities, while absence resulted in lower intensities.

RESULT 2: ThT fluorescent concentration in *T. electrotropha* changes due to acetate concentrations

T. electrotropha was grown in different acetate concentrations (0 mM, 1 mM, 10 mM) with 100 µM ThT added to visualize differences in ThT intensity. 1 mM and 10 mM cells were exposed to oxygen, while 0 mM was exposed to nitrogen. ThT intensities were compared, suggesting membrane potential differences between presence and absence of acetate:

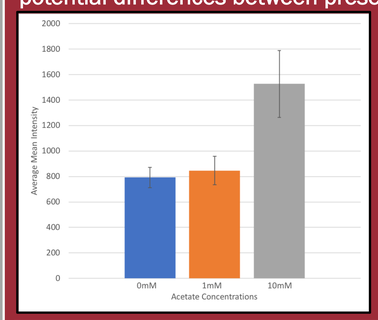


Figure 5. Microscopic images of *T. electrotropha* with 100 µM ThT dye under 0 mM and 10 mM acetate conditions (below).

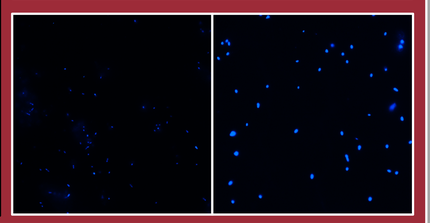


Figure 6. Noticeable differences in ThT concentration between 10 mM and 0 mM conditions were observed, suggesting the 10 mM condition to have more proton motive force compared to 0 mM or 1 mM conditions.

CONCLUSIONS:

Comparisons of the NADH-NAD$^+$ redox pool (with the Peredox-mCherry protein and its fluorescent ratio) and electrochemical gradient due to the proton motive force (ThT dye concentration) were made between *Idomarina loihiensis* and *Thioclava electrotropha*, respectively. Both were affected by the addition of acetate, changing intensities between the presence and absence of the electron donor. The addition of acetate resulted in a higher intensity of both fluorescent markers (ThT and Peredox-mCherry) in the cells. The absence of acetate resulted in considerably lower intensities. More in-depth analysis of ThT (electrochemical gradient) and Peredox-mCherry (NADH pool) levels in *I. loihiensis* and *T. electrotropha* will need to be performed to confirm these preliminary results, and lead to investigation of electron uptake under electrochemical conditions.

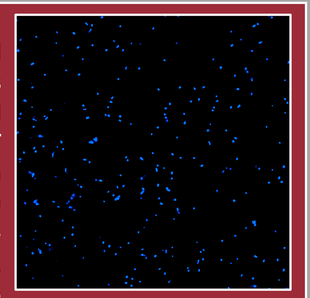


Figure 7. Microscopic image of *T. electrotropha* under electrochemical conditions with a electrode potential of -400mV (for electron uptake). 10 µM ThT used for imaging.

FUTURE WORK:

- Insertion of the Peredox-mCherry protein into *T. electrotropha* for comparison of the NADH pool fluorescence.
- Further investigations into the Peredox-mCherry protein intensity relation to ThT concentration intensity in *T. electrotropha* for publishable data.
- Investigations into ThT intensity differences in *I. loihiensis.*
- Using *T. electrotropha* on electrodes, comparing Peredox protein and ThT dye intensities to assess the effects of electron uptake with production of an electrochemical gradient and/or NADH.
- Incorporating *T. electrotropha* and *I. loihiensis* into the same environment and investigating any interspecies electron transfer.

ACKNOWLEDGEMENTS:

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