A Synthetic Biology Approach to: Microbial Fuel Cell Development

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Prelude

Optimization of electron shuffle to external surfaces such as anodes was a primary goal. Geobacter *sulfurreducens* happened to be our model bacteria due to its ability in nature to efficiently export electrons extracelluarly. E. coli was the chassis for this experiment due to its well documentation and the fact that its genome already containing some key proteins in our preferred pathway. The proteins, such as extracellular pilin, MacA, and many other cytochromes, which E. coli does not have were isolated from Geobacter *sulfurreducens* and introduced into E. coli to formulate the most optimal pathway for generating electromotive force in a microbial fuel cell apparatus.

Some problems were faced concerning plasmid engineering and the simple fact that Geobacter is anaerobic and E. coli is aerobic. The current work includes production and optimization of a microbial fuel cell into which our modified bacteria will be placed.

Background

Dr. Derek Lovely of The University of Massachusetts has researched Geobacter and other bacteria, which may prove favorable for electrical production. His and along with other research has shown validity to electrical production via the breakdown and processing of carbon sources. Some research has dissected certain pathways, which are believed to involve nanowires and certain intra-, extra-, and trans-membrane proteins [1]. In nature, Geobacter *sulfurreducens* reduces local metals and materials in order to harvest energy thus creating electron potentials [2]. Specific genes have shown as a promising pathway for electrical stimulation [3]. Our work is a novel approach to integrating a well known bacterium, Escherichia *coli*, into an electron producing entity.

Fig. 1 – Microbial Fuel Cell constructed by members of iGEM and ChemE Car.

Selected Genes

The pathway elucidated by Figure 2 shows genes OmcB, OmcE, and OmcS along with MacA and some related cytochromes. E. coli has native MacA, PcpA, and cytochromes complexes therefore the selected genes for this synthetic biology approach in order to induce electrical stimulation in E. coli were: OmcB, OmcT, OmcS, and OmcE. Some other related genes which were also probable candidates for insertion into E. coli were OmcA, MtrA, MtrB, MtrC, and CymA, but E. coli also already contained a few of these genes in its own genome.

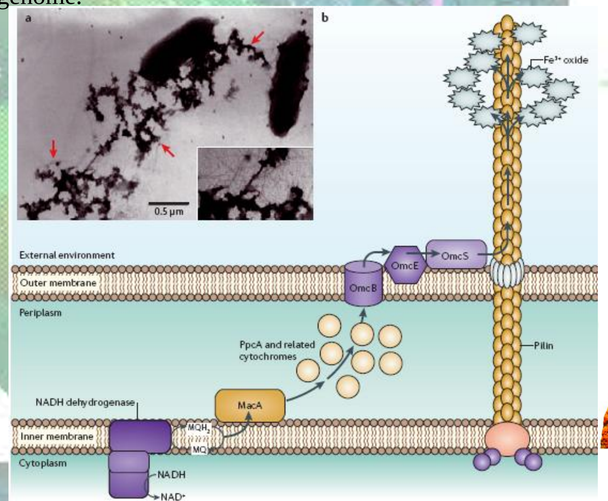

Figure 5 | **A mechanism for extracellular electron transfer by** *Geobacter sulfurreducens*. a | A transmission electron micrograph showing the association of Fe^{3+} oxide (indicated by arrows) with pilin expressed by *Geobacter sulfurreducens*. The inset shows pili that are intertwined with Fe^{3+} oxides. b | Potential route for electron transfer to Fe^{3+} oxides by *Geobacter sulfurreducens*. This model is based on a previous model[58] and subsequent findings[41,69]. MacA, PpcA, OmcB, OmcE and OmcS are c-type cytochromes which genetic studies have indicated are required for optimal Fe^{3+} reduction. The proposed electron flow between the cytochromes is based on their reported location within the bacterial cell. It is important to note that the genome of *G. sulfurreducens* contains genes that encode approximately 100 c-type cytochromes[64], some of which might also participate in this electron transfer process. MQH_2, menaquinol; MQ, menaquinone. Panel (a) is reproduced with permission from Nature REF. 41 © (2005) Macmillan Publishers Ltd.

Fig. 2 – Taken via Macmillan Publishers Ltd., Derek Lovely: Bugjuice: Harvesting energy from microorganisms. This shows the theoretically proposed pathway for electrons to the extracellular space of Geobacter *sulfurreducens*. Note OmcB, OmcE, OmcS.

Materials & Methods

- Media: NB fumarate (NBAF) using 100X NB mix via D. Lovely
- Primers acquired via DNA Technologies
- PCR: Master Mix, H_2O, Primers reacted in cDNA laboratory
- Ligation comprised of T vectors
- Transformation using competent E. coli cells made using plates with composition: 10 g Tryptone, 5 g Yeast Extract, 5 g NaCl, 15 g Agar, 1000 uL Kan, and 1000 uL X-gal (all per 1 L H_2O)
- Microbial Fuel Cell: polycarbonate casing, Nafion 117, carbon felt, butyl rubber, E-TEK ELAT GDE, media, copper wire

Conclusions

The fundamentals of experimental design are that one's first attempt may not always be successful, but will always give incite for future attempts and direct one on the desired path.

We had trouble with the anaerobic nature of Geobacter. Another problem was successfully transforming cells due to a lack of competency. The fuel cell came together rapidly, but one problem with Nafion stabilizing in 100% EtOH brought its formation to a halt. Nevertheless, four genes were sequenced and submitted to the registry: OmcB, OmcE, OmcS, and OmcT ranging from BBa_K269000 to BBa_K269003.

Future Work

Our research group desires to transform successful E. coli cells then test them in the microbial fuel cell. We might vary the amounts of glucose feed or media conditions. Other approaches may include coupling Shewnella *oneidensis* or Geobacter *sulfurreducens* with this E. coli or with each other to obtain desired electrical output.

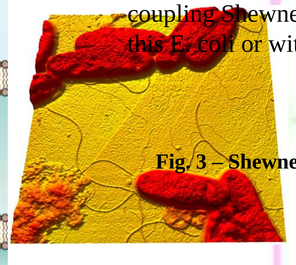

Fig. 3 – Shewnella o. reducing metal. Image via Asylum Research: M. El-Naggar, USC and Y. Gorby, J. Craig Venter Institute

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References

[1] Bug juice: harvesting electricity with microorganisms, Derek R. Lovely

[2] Possible Nonconductive Role of Geobacter *sulfurreducens* Pilius Nanowires in Biofilm Formation, Reguera et al.

[3] Microarray and genetic analysis of electron transfer to electrodes in Geobacter *sulfurreducens*, Holmes et al.