A Synthetic Biology Approach to: Microbial Fuel Cell Development

By Marcus Hayer, Patrick VerSteeg, Nichole Hurd, Patrick Martin, Sooyoung Shin, Drew Menke, Amanda Foster, Meghan Ray, Helen Cardwell, Erica Shannon, and Ben Heimann

The Departments of Chemical and Biological Engineering & Biological Sciences
Missouri University of Science and Technology

Prelude

Optimization of electron transfer 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 extracellularly. E. coli was the chassis for this experiment due to its well documented use and the fact that its genome already contains many of the key proteins in our preferred pathway. The proteins, such as extracellular pilin, MacA, and many other cytochromes, and certain electron transfer proteins were isolated from Geobacter sulfurreducens and introduced into E. coli to form the most optimal pathway for generating electrical 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- and extracellular 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.

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, Pce, and cytochromes complexes therefore the selected genes for this synthetic biology approach in order to induce electrical stimulation in E. coli were: OmcB, OmcE, OmcS, and MacA. Some other related genes which were also probable candidates for insertion into E. coli were: OmEC, MtrA, MtrB, MucC, and CymA, but E. coli also already contains a few of these genes in its own genome.

Materials & Methods

Media: NB fumarate (NBAF) using 100X NB mix via D. Lovely
• Primers acquired via DNA Technologies
• PCR: Master Mix, H2O, 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 H2O)
• 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 Shewenella oneidensis or Geobacter sulfurreducens with this E. coli or with each other to obtain desired electrical output.

Materials & Methods

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 Shewenella oneidensis or Geobacter sulfurreducens with this E. coli or with each other to obtain desired electrical output.

Acknowledgments

Much appreciation and gratitude is due for Dr. David Westenberg and Dr. Katie Shannion for their guidance, patience, and devotion to iGEM and its students. A special thanks to Dr. Derek Lovely, Susan Childers, and their research group at The University of Massachusetts. The Materials Research Center along with Becky Treu and Ron Haas aided our research group in its intellectual quest. Thanks also goes out to those who sponsored our research and travels; Missouri S&T Chemical and Biological Engineering, Missouri S&T Biological Sciences, Missouri S&T Chemical and Biological Engineering, Missouri S&T Chemical and Biological Engineering, Missouri S&T Chemical and Biological Engineering, Missouri S&T Environmental Research Center, and the Missouri S&T cDNA laboratory.

References

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