

Enhancing the Performance of Microbial Fuel Cells via Metabolic Engineering of *Escherichia coli* for Phenazine Production

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One of the central roles in the design, development, and application advances in mediator-based microbial electrochemical systems, such as microbial fuel cells (MFCs), is the establishment of efficient and successful communication between conductive electrode surfaces and microorganisms via modes of extracellular electron transfer (EET). Most microbial-based systems require the use of artificial electroactive mediators in order to facilitate and/or enhance electron transfer. Our previous work established an exogenous phenazine-based library as a mediator system to enable EET from the model microorganism *Escherichia coli* as a promising biotechnological host. However, the addition of exogenous mediators to a microbial electrochemical system has certain limiting downsides, specifically with regard to mediator toxicity to cells and increased operational expenses. Herein, we demonstrate the metabolic and genetic engineering of *E. coli* to self-generate phenazine metabolites endogenously by introducing the phenazine biosynthetic pathway from *P. aeruginosa* into *E. coli*. This biosynthetic pathway contains a phenazine cluster of seven genes, namely, *phzABCDEFG* (*phzA-G*), responsible for the synthetic conversion of phenazine-1-carboxylic acid (PCA) from chorismic acid, and two additional phenazine accessory genes *phzM* and *phzS* to catalyze the transformation of PCA to pyocyanin (PYO). We present the characterization of the engineered *E. coli* cells that were collected via electrochemical measurements, RNA sequencing, and microscopy imaging. Finally, the engineered *E. coli* cells were used for the design of a microbial fuel cell with enhanced performances, demonstrating a maximum power density increase from $127 \pm 5 \text{ mW m}^{-2}$ with nonengineered *E. coli* cells to $806 \pm 7 \text{ mW m}^{-2}$ with genetically engineered, phenazine-producing *E. coli*. Our results indicate that the introduction of a heterologous electron shuttle into *E. coli* is not only an efficient, but also a promising strategy toward establishing efficacious electron mediation in living bioelectrochemical systems and enhancing the overall MFC performance related to the MFC current generation and power output.