Screening, Identification, Performance Enhancement, and Electron Transfer Mechanism of Highly Active Electrogenic Microorganisms in Microbial Fuel Cells (Postprint)

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Abstract

Microbial Fuel Cells (MFCs) are a type of bioreactor that utilize exoelectrogenic microorganisms as catalysts to directly convert chemical energy from organic (or inorganic) matter into electrical energy, holding broad application prospects in bioenergy production and wastewater treatment. Our research group initiated and operated MFCs using sludge from the Tianjin TEDA Wastewater Treatment Plant as inoculum. Multiple isolates were obtained from the biofilm enriched on the anode. Among them, isolates P2-A-1 and P2-A-5 exhibited high electrochemical activity and were identified as Tolumonas osonensis and Kocuria rhizophila, respectively, representing the first report of electricity-producing capability for these microbial species/genera. In addition to optimizing electricity-producing conditions, cell permeabilization treatment represents an effective approach for enhancing MFCs' power output. Chemical agent treatment increases bacterial adhesion efficiency to electrodes, reduces MFC internal resistance, enhances cell permeability and cell membrane fluidity, and increases the content of the key electron carrier CoQ10. Through construction of membrane-encapsulated MFCs, cyclic voltammetry scanning of anolyte, and GC-MS component analysis, the extracellular electron transfer mechanisms of the strains were elucidated. Using the model exoelectrogenic microorganism Pseudomonas aeruginosa as the target, global transcription machinery engineering was employed to introduce a series of endogenous and exogenous global transcription factors into Pseudomonas aeruginosa. Leveraging metabolic engineering strategies, intracellular electron generation and extracellular electron transfer were enhanced to explore novel strategies and methods for constructing high electricity-producing activity engineered strains. Among these, the introduction of the exogenous global transcription factor IrrE could significantly improve the electricity-producing performance and environmental stress tolerance

of Pseudomonas aeruginosa. Further analysis revealed that the introduction of this transcription factor exerted significant effects on the expression levels of genes related to central metabolic pathways, biofilm formation, mediator synthesis, quorum sensing systems, and general stress response. This demonstrates that IrrE plays a global regulatory role in the host organism. The achievements of this project hold significant importance for enriching the diversity of exoelectrogenic microorganisms and enhancing the power output of microbial fuel cells.

Full Text

Preamble

Research Title: Screening, Identification, and Performance Enhancement of Highly Active Electrogenic Microorganisms in Microbial Fuel Cells, and Investigation of Their Extracellular Electron Transfer Mechanisms

Luo Jianmei is a professor and master' s supervisor at the College of Bioengineering, Tianjin University of Science and Technology, where she also serves as Director of the Pharmaceutical Engineering Department. She is a member of the Chinese Microbiology Society and affiliated with the Key Laboratory of Industrial Fermentation Microbiology (Ministry of Education) and the Tianjin Key Laboratory of Industrial Microbiology. Over the past five years, she has led more than ten research projects funded by the National Natural Science Foundation of China, International Science Foundation, Tianjin Science and Technology Commission, Tianjin Education Commission, Key Laboratory open funds, and industry collaborations. She has published over 50 scientific papers, contributed to one provincial/ministerial-level textbook, and holds 15 authorized or pending national invention patents. Her research focuses on biotechnology, encompassing: (1) screening and improvement of microbial strains with important industrial, pharmaceutical, and environmental applications; (2) breeding and physiological mechanism studies of stress-tolerant microorganisms, as well as construction and application of stress-resistant strains; and (3) microbial cell catalytic reactions and process control.

Microbial Fuel Cells (MFCs) are bioreactors that utilize electrogenic microorganisms as catalysts to directly convert chemical energy from organic (or inorganic) compounds into electrical energy, offering promising applications for bioenergy production and wastewater treatment. Our research team initiated and operated MFCs using sludge from the Tianjin TEDA Wastewater Treatment Plant as inoculum. Multiple isolates were obtained from the enriched biofilm on the anode, among which strains P2-A-1 and P2-A-5 exhibited high electrochemical activity. These were identified as *Tolumonas osonensis* and *Kocuria rhizophila*, respectively, representing the first report of electrogenic capability for these species.

In addition to optimizing electrogenic conditions, cell permeabilization proved to be an effective strategy for enhancing MFC power output. Chemical treatment increased bacterial adhesion efficiency to electrodes, reduced MFC internal resistance, enhanced cell membrane permeability and fluidity, and elevated the content of the key electron carrier CoQ10. The extracellular electron transfer mechanisms of these strains were elucidated through construction of membrane-wrapped MFCs, cyclic voltammetry scanning of anolyte, and GC-MS compositional analysis.

Using the model electrogenic microorganism *Pseudomonas aeruginosa* as the target, we employed global transcription machinery engineering to introduce a series of endogenous and exogenous global transcription factors. Adopting a metabolic engineering approach, we strengthened intracellular electron generation and extracellular electron transfer, exploring novel strategies for constructing high-performance electrogenic engineered strains. Notably, introduction of the exogenous global transcription factor IrrE significantly enhanced the electrogenic performance and environmental stress tolerance of *P. aeruginosa*. Further analysis revealed that this transcription factor markedly influenced expression levels of genes related to central metabolic pathways, biofilm formation, mediator synthesis, quorum sensing systems, and general stress responses, demonstrating its global regulatory role in the host organism. These findings are significant for enriching the diversity of electrogenic microorganisms and improving MFC power output.

Recent Publications (Past 5 Years)

1. Luo J.M.*, Li M., Zhou M.H.*, Hu Y.S. Characterization of a novel strain phylogenetically related to *Kocuria rhizophila* and its chemical modification to improve performance of microbial fuel cells[J]. *Biosensors and Bioelectronics*, 2015, 69: 113-120. (Top Journal)

2. Luo J.M.*, Yang J., He H.H., Jin T., Zhou L., Wang M., Zhou M.H.* A new electrochemically active bacterium phylogenetically related to *Tolumonas osonensis* and power performance in MFCs[J]. *Bioresource Technology*, 2013, 139: 141-148. (Top Journal)

3. Luo J.M.*, Li J.S., Liu D., Liu F., Wang Y.T., Song X.R., Wang M. Genome shuffling of* Streptomyces gilvosporeus* for improving natamycin production[J]. *Journal of Agricultural and Food Chemistry*, 2012, 60(23): 6026-6036. (Top Journal)

4. Luo J.M., Liang Q.K., Shen Y.B., Chen X., Yin Z.N., Wang M.*. *Biotransformation of bavachinin by three fungal cell cultures[J].* Journal of Bioscience and Bioengineering*, 2014, 117(2): 191-196.

5. Luo J.M., Ning J., Wang Y.X., Cheng Y.X., Zheng Y., Shen Y.B., Wang M.*. *The effect of ethanol on cell properties and steroid 1-en-dehydrogenation biotransformation of* Arthrobacter simplex*[J].* Biotechnology and Applied Biochemistry*, 2014, 61(5): 555-564.

6. Luo J.M., Chi M.L., Wang H.Y., He H.H., Zhou M.H.* Electrochemical surface modification of carbon mesh anode to improve the performance of air-cathode microbial fuel cells[J]. *Bioprocess and Biosystems Engineering*, 2013, 36: 1889-1896.

7. Yang J, Li M, Zhu S.Y., Luo J.M.*, Zhou M.H., Wang M. Optimization of electrogenic performance of* Tolumonas osonensis* strain in microbial fuel cells[J]. *Journal of Chemical Engineering of Chinese Universities*, 2015, 29(2): 352-358.

8. Li M, Liang X, Luo J.M.*, Zhou M.H.*. Isolation and optimization of electrogenic performance of an electrogenic bacterium *Kocuria rhizophila*[J]. *Acta Scientiae Circumstantiae*, DOI: 10.13671/j.hjkxxb.2014.1038.

Note: Figure translations are in progress. See original paper for figures.