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Outline of Research Result

Mechanism of electricity generation based on the stress response of metal-reducing bacteria

Yoshihiro OJIMA

Department of Materials Engineering Science, Graduate School of Engineering Science, Osaka University

Research aims

Shewanella oneidensis is a gram-negative facultative anaerobe that can use a wide variety of terminal electron acceptors for anaerobic respiration.[1)] It plays an important role in many environmental and biotechnological processes, including a microbial fuel cell (MFC) system and in reduction of toxic metals, such as uranium and chromium. Our study aims at clarifying the mechanism of electricity generation of *S. oneidensis* in terms of stress response. *S. oneidensis* and other gram-negative bacteria (*Escherichia coli* etc.) have the *rpoS* gene, a global regulon gene, which regulates the transcription of structural genes responsible for adaptation to a variety of stresses, including oxidative and osmotic pressures, heat, starvation, acids. Additionally, *S. oneidensis* and *Escherichia coli* share many characteristics. For example, the plasmid used for gene recombination of *E. coli* can be used in the *S. oneidensis* cells.

In this study, I investigated the effects of deleting the stress response gene *rpoS* on electricity generation by *S. oneidensis* cells. Genetic engineering techniques using *E. coli* cells were applied to construct the *rpoS*-deficient *S. oneidensis* mutant.

Methods

In-frame deletion of *rpoS* from the *S. oneidensis* genome

rpoS (SO3442) was deleted in-frame from the *S. oneidensis* genome. Following multiple rounds of fusion PCR, the amplicon was cloned into the suicide plasmid pRE112, and then transformed into *E. coli* SM10 *λ*pir via the calcium chloride method. The resulting plasmid was transferred into recipient *S. oneidensis* MR-1 cells by conjugation, and the transformants were selected on the basis of chloramphenicol resistance. Integration of the mutagenesis construct into the chromosome was induced to generate the final deletion strain. The remaining plasmids were removed via sucrose-induced lethality. The deletion was verified by PCR amplification of *rpoS* and realtime-PCR analysis.

Microbial fuel cell

A two-chambered MFC was fabricated using two 300-ml Pyrex glass bottles that were manually modified. A Nafion® perfluorinated membrane was clamped between the adapters. The cathode and anode were graphite unrolled by 0.20 g of untreated carbon cloth. Copper wires were used to connect the circuit, and the connections were secured with alligator clips. The anode chamber was filled with 300 ml of lysogeny broth (LB) medium (10 g/l polypeptone, 5 g/l bacto-yeast extract, and 10 g/l NaCl). It also contained 2 g/l lactate as substrate. The initial pH of the medium was 7.0. The cathodic chamber was filled with 300 ml of 100 mmol/l phosphate buffer (pH 7.0) containing 320 mmol/l potassium ferricyanide. *S. oneidensis* cells were pre-cultured in LB medium and harvested by centrifugation. The concentrated *E. coli* cell suspension was added into the anodic chamber to obtain an initial optical density at 660 nm (OD_{660}) of ~1. The MFC was operated at room temperature (ca. 25°C) and the voltage was measured with a voltage logger by connecting to a 10-Ω resistor.

Results

In-frame deletion

To confirm the in-frame deletion of *rpoS*, genomic DNA was extracted from wild type *S. oneidensis* and the Δ*rpoS* mutant. The *rpoS* gene (981 bp) plus 250 bp upstream was amplified by PCR. The electrophoresis results are shown in Fig. 1. An approximately 1.2-kbp fragment was amplified from the wild type cells, corresponding to the combined size of *rpoS* plus surrounding regions. In contrast, a 250-bp

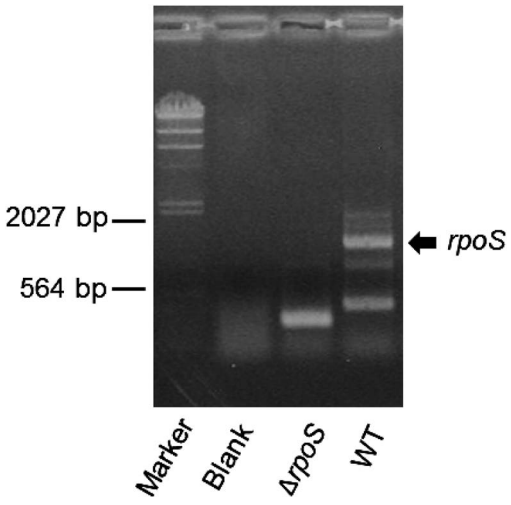

Fig. 1. Electrophoresis of *rpoS* fragment amplified from genomic DNA isolated from wild type (WT) and Δ*rpoS* strains.

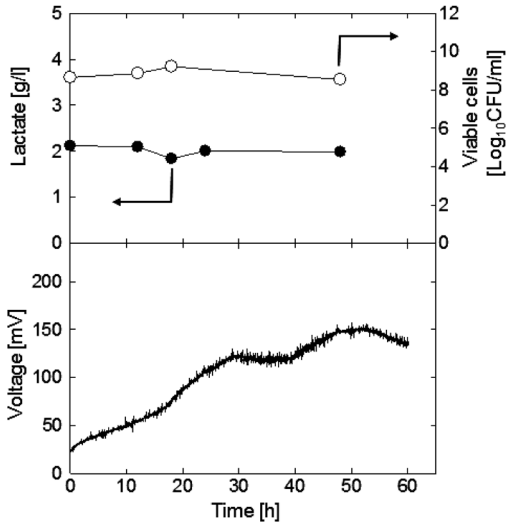

Fig. 2. Time profile of viable cells, lactate concentration, and voltage of the constructed MFC with wild type cells.

band was amplified from the putative Δ*rpoS* mutant cells, demonstrating that the *rpoS* region was successfully deleted from genomic DNA of *S. oneidensis*. Furthermore, real-time PCR analysis did not detect *rpoS* mRNA expression in the mutant cells. These results indicate the successful deletion of *rpoS* from the chromosome of *S. oneidensis*.

Microbial fuel cell

Time profile of viable cells, lactate concentration, and voltage of the constructed MFC with wild type cells are shown in Fig. 2. The voltage gradually increased and reached about 150 mV at 50 h, indicating that *S. oneidensis* wild type cells convert lactate to electric energy. Since viable cell concentration did not increase during the reaction period, it can be considered that the gradual increase in voltage was due to the increase of the efficiency of electron transfer from *S. oneidensis* cells to electrode. Lactate concentration slightly decreased from 2 g/l, but it was not completely consumed during the culture period, suggesting that the substrate supply does not limit the output of this system.

Next, Figure 2 shows the time profiles of the constructed MFC with Δ*rpoS* cells. The voltage rapidly increased and reached 90 mV at 5 h with no appreciable increase afterward. The value remained constant at around 90 mV. Meanwhile, time profiles of viable cells and lactate concentration

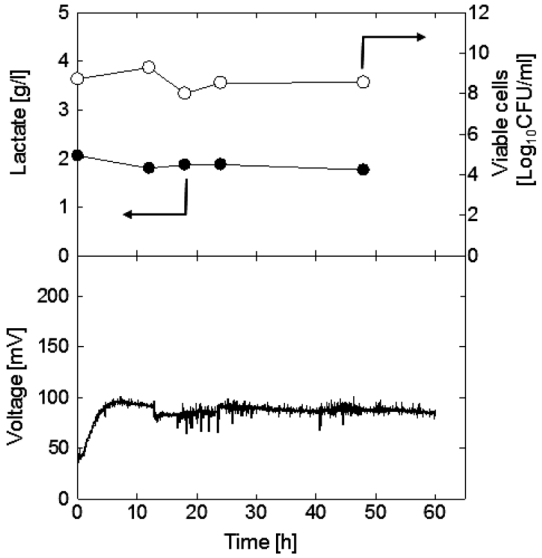

Fig. 3. Time profile of viable cells, lactate concentration, and voltage of the constructed MFC with Δ*rpoS* cells.

were not significantly different between wild type and Δ*rpoS* cells, suggesting that the deletion of *rpoS* negatively affects the electron transfer from cells to the electrode. Thus, it was clarified that the global stress response gene *rpoS* plays an important role in electricity generation of *S. oneidensis*.

Conclusion

The *rpoS* gene of *S. oneidensis* was successfully deleted in-frame to examine the effect of oxidative stress on electricity production. Evaluation of the constructed MFC system showed that the deletion of *rpoS* gene decreased the voltage of MFC without affecting the cell and lactate concentration. These results suggest that the *rpoS* gene is important for electricity generation by *S. oneidensis* cells.

References

1) Myers, C. R., and Nealson, K. H. (1988). Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. *Science* **240**: 1319–1321.
2) Ojima, Y., Kawata, T., Matsuo, N., Nishinoue, Y., and Taya, M. (2014) Recovery of electric energy from formate by using a recombinant strain of *Escherichia coli*. *Bioprocess and Biosystems Engineering* **37**: 2005–2008.