

CHEMICAL SCREENING FOR SINGLE BACTERIAL ACTIVITY USING BACTERIA IMMOBILIZATION INTO MICROPOROUS

Tomonori Kano, Tomomi Inaba, Kazuhiko Higashi, Norihisa Miki

Keio University, Japan

ABSTRACT

In this paper we demonstrate chemical screening using a microbial micro reactor immobilizing microbes by dielectrophoresis into micro holes, as shown in Fig.1. The flow-type microbial micro reactor facilitates collecting and evaluating reaction products while immobilization of microbes enables quantification of the number of microbes involved in the reaction and making their reactions conditions consistent. We applied this system to investigate the effects of chemicals on microbial activities of *Corynebacterium glutamicum*, which is widely used to produce beneficial chemicals in industry. The proposed system will be of great help to reduce the time and cost to find optimum biochemical microbial reactions.

KEYWORDS

Micro reactor, Chemical screening, Dielectrophoresis, Bacteria, Immobilization.

INTRODUCTION

A microbe enables multi-step biochemical reactions using containing multiple enzymes. Extremely wide varieties of bacteria have been discovered or genetically modified to develop the desirable fermentation process and are industrially used to produce various useful chemicals [1,2]. Precise control of the reaction conditions and the number of bacteria involved in the biochemical reaction is crucial to effectively screen them to efficiently produce useful chemicals by fermentation, such as organic acids and drugs [3,4].

In our prior work, we developed the microbial micro reactor which can handle and immobilize live bacteria into micro holes by positive DEP as shown in Fig. 1. The media including reactive agents can be flown into the chip continuously and therefore, the reaction products can be continuously collected, which facilitates evaluation of the bacterial reaction. Different from traditional evaluate method using bulk condition, this micro reactor we proposed can quantify the number of microbes involved in the reaction and make their reactions conditions consistent. These advantages enable us to evaluate the production capacity of a single bacterium. By using micro reactor, we screened microbes to find the most efficient species [5].

In this paper, in order to demonstrate the performance of the micro reactor, we used the developed micro reactor to evaluate biological activity by chemical substance. We used *Corynebacterium glutamicum* as sample bacteria, which can produce more lactic acid by the addition of pyruvic acid or sodium bicarbonate [6,7]. After bacteria immobilization, we introduced culture media including pyruvic acid or sodium bicarbonate. By measuring the amount of lactic acid produced by the immobilized microbes in the micro reactor, we experimentally revealed biological activity by chemical substance.

FABRICATION

The fabrication process is shown in Fig. 2. The microbial micro reactor consists of two electrode, a fluid channel, and micro holes. Micro holes were formed by patterning negative photoresist (ZPN 1150-90, Nippon Zeon) 4 μm in thickness. We prepared the chip containing holes 5 μm in diameter. A hot melt sealing foil (Meltonix 1170-60, SOLARONIX) was patterned by a cutting machine (Craft ROBO Pro CE5000-40-CRP, Graphtec co.) and formed a micro channel when sandwiched by ITO electrodes and heat-treated at 100 $^{\circ}\text{C}$. The channel height was determined by the foil thickness of 60 μm .

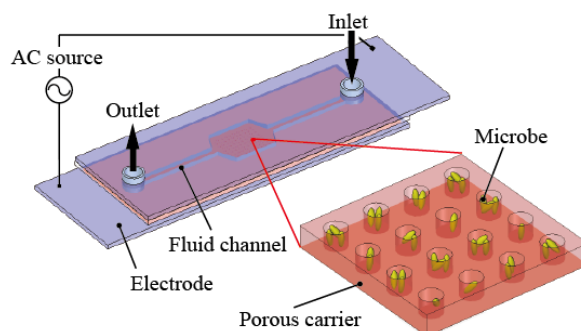


Figure 1: Schematic image of microbial micro reactor.

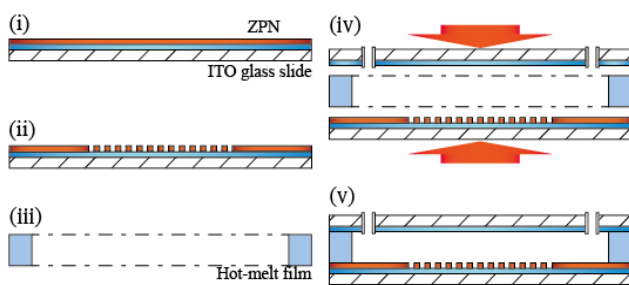


Figure 2: Fabrication process. (i) Negative photoresist on ITO glass slide. Micro holes were formed by patterning negative photoresist 4 μm in thickness. (ii) Photoresist patterning. We prepared the holes 5 μm in diameter. (iii) Fluid channel patterning. A hot melt sealing foil was patterned by a cutting machine (iv) Thermo compression bonding. A micro channel is formed when sandwiched by ITO electrodes and heat-treated at 100 °C. (v) Completion. The channel height was determined by the foil thickness of 60 μm.

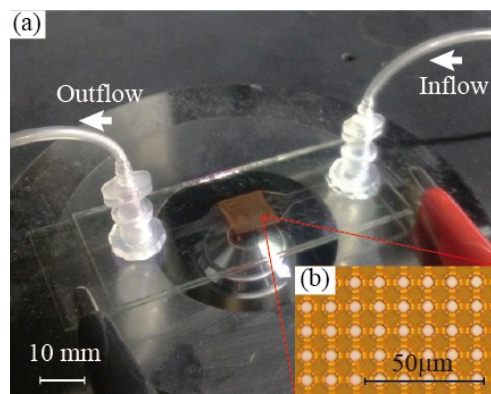


Figure 3: (a) Photo of the microbial micro reactor. (b) Photo of the 5 μm-diameter micro holes formed by photoresist.

EXPERIMENT

The micro reactor enables us to investigate the effects of chemicals on microbial activities. To demonstrate it, we evaluated change of the production rate of lactic acid depend on the presence and absence of pyruvic acid or sodium bicarbonate. We tested *Corynebacterium glutamicum*, which can produce lactic acid by the addition of pyruvic acid or sodium bicarbonate.

Live and dead bacteria were dyed with SYTO9 and PI, respectively. Figure 4 shows the bacteria evaluation process. First, microbial suspension (5×10^8 cells in 1 ml of DI water) was introduced into the fluid channel and an electric voltage of 10 Vpp at 10 MHz was applied, which was found to be most effective to selectively immobilize live bacteria [8]. Strong electric fields are generated in the micro holes and microbes are attracted towards the pores and immobilized by positive DEP. After bacteria immobilization, we rinsed non-immobilized microbes away by flowing DI water at a flow rate of 2.5 μl/min for 20 min. We investigated the live/dead bacteria selectivity by measuring the fluorescent intensity of SYTO9 and PI. Then, culture media including pyruvic acid or sodium bicarbonate was introduced at a flow rate of 2.5 μl per minute. The product was collected for 70 min and the amount of lactic acid was measured using UV/Vis.

RESULT AND DISCUSSION

Figure 5 show fluorescent images of immobilized microbes. The green fluorescence indicates live bacteria dyed with SYTO9 and the blue fluorescence indicates dead bacteria dyed with PI. The Fig.5(A) and (B) were taken at the same positions but filtered by different wavelengths. 7.3 live bacteria in average were immobilized into 5-μm-diameter holes with a live/dead selectivity of approximately 3.5. These results confirm that live bacteria can be preferentially collected using a positive DEP process.

The microbial micro reactor can investigate the effects of chemicals on microbial activities. Figure 6 and 7 show the the production rate of lactic acid of a shingle *C. glutamicum* as a function of the concentration of pyruvic acid and sodium bicarbonate, respectively. The lactic acid production capacities of a single bacterium were deduced by dividing the total amount of the produced lactic acid by the number of the immobilized bacteria in the chip. The production rate increase from 0.26 pg/min to 0.41 pg/min and 0.95 pg/min by addition of pyruvic acid and sodium bicarbonate. It increased 1.7 times by the addition of pyruvic acid and increased 3.8 times by sodium bicarbonate.

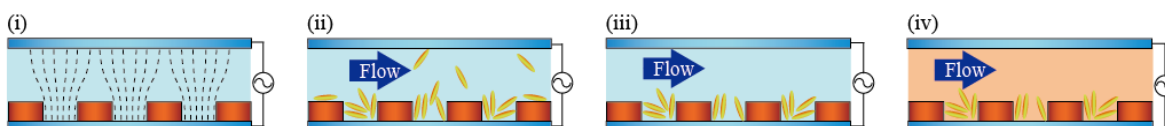


Figure 4: Schematic image of bacteria evaluation process. (i) An electric voltage of 10 Vpp at 10 MHz was applied. (ii) Microbial suspension was introduced into the fluid channel. Positive DEP immobilized microbes into the holes. (iii) Non-immobilized microbes were rinsed away by DI water. (iv) Culture media were introduced into fluid channel. Immobilized bacteria produced lactic acid.

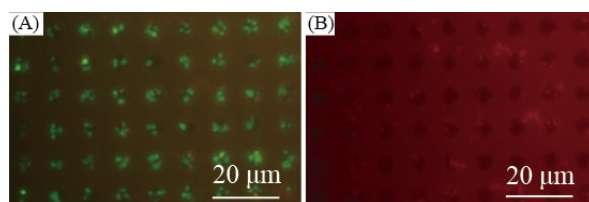


Figure 5: Immobilized bacteria in micro holes. (A) Live bacteria were dyed with SYTO9. (B) Dead bacteria were dyed with PI. The image (A) and (B) were taken at the same positions but filtered by different wavelengths.

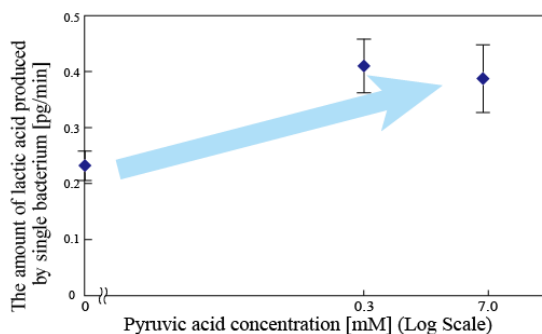


Figure 6: The production rate of lactic acid of *C. glutamicum* under various pyruvic acid concentrations.

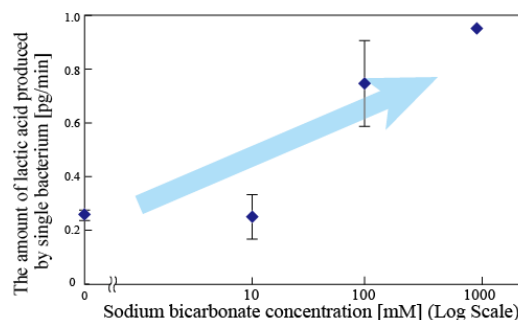


Figure 7: The production rate of lactic acid of *C. glutamicum* under various sodium bicarbonate concentrations.

CONCLUSION

We demonstrate chemical screening using a microbial micro reactor immobilizing microbes by dielectrophoresis into micro holes. The device can preferably collect live bacteria into micro hole made of non-conductive photoresist by positive DEP and quantify the total number of bacteria involved in the microbial reaction. Because of these advantages, the device enables us to evaluate activity of single bacterium. We applied this system to investigate the effects of chemicals on microbial activities of *C. glutamicum* and experimentally found that the production rate of lactic acid of *C. glutamicum* increased 1.7 times and 3.8 time by the addition of pyruvic acid and sodium bicarbonate.

We successfully investigated the effects of chemicals on microbial reactions, which can be readily applicable to chemical screening and microbial process development.

REFERENCES

- [1] BS. Jeon, BC. Kim, Y. Um, BI. Sang, *Production of hexanoic acid from D-galactitol by a newly isolated Clostridium sp. BS-1*, Appl Microbiol Biotechnol 88 (5):1161-1167 (2010).
- [2] M. Sasaki, H. Teramoto, M. Inui, H. Yukawa, *Identification of mannose uptake and catabolism genes in Corynebacterium glutamicum and genetic engineering for simultaneous utilization of mannose and glucose*, Appl Microbiol Biotechnol 89 (6):1905-16 (2011)
- [3] Z. Liang, Z. Han, S. Yang, X. Liang, P. Du, G. Liu, and Y. Yang, *A control strategy of partial nitritation in a fixed bed biofilm reactor*, Bioresource Technology, 12, 710-715 (2011).
- [4] F. K. Balagadde, L. You, C. L. Hansen, F. H. Arnold, and S. R. Quake, *Long-Term Monitoring of Bacteria Undergoing Programmed Population Control in a Microchemostat*, Science, 309, 137 (2005).
- [5] T. Kano, T. Inaba, G. Ye, and N. Miki, *A Continuous-Flow Microbial Microreactor Using Microbes Immobilized into A Microporous Carrier By Dielectrophoresis*, IEEE MEMS 2012.
- [6] M. Inui, S. Murakami, S. Okino, H. Kawaguchi, A. A. Vertes, and H. Yukawa, *Metabolic analysis of Corynebacterium glutamicum during lactate and succinate productions under oxygen deprivation conditions*, J Mol Microbiol Biotechnol, 7(4):182-96(2004)
- [7] S. Okino, M. Inui, H. Yukawa, *Production of organic acids by Corynebacterium glutamicum under oxygen deprivation*, Appl Microbiol Biotechnol 68, 475-480(2005)
- [8] T. Kano, G. Ye, T. Inaba and N. Miki, *Bacteria Immobilization in a Micro Porous Carrier by Dielectrophoresis*, microTAS 2011.

CONTACT

Tomonori Kano +81-45-566-1430 or kano@a7.keio.jp