Real-time electrochemical monitoring of gene expression in transformed bacteria using a microbial chip

K Nagamine, T Kaya, T Yasukawa, H Shiku, and T Matsue

Graduate School of Environmental Studies, Tohoku University, Sendai, Aramaki Aoba 07 980-8579, Japan.

A number of microbial genomes are providing the opportunity to comprehensively and efficiently survey the transcriptional profile of bacteria under different conditions and well-defined genotypes. Whereas the currently used DNA microarray employs the lysate solution prepared from a large number of cells, it is difficult to monitor the real-time gene expression in the whole cells. The on-line analysis of gene expression in the intact cell should be appropriate to monitor the dynamic processes during the gene expression in the cells.

Previously, we fabricated a microbial chip with collagen gel spots entrapping living bacterial cells and applied for real-time detection of the β-galactosidase expression induced by isopropyl-β-D-thiogalactopyranoside (IPTG) in a small number of the wild-type *Escherichia coli* K12[1]. Figure 1(A), (B) shows the on-line culture system and the design of the microbial chip. A Pt microelectrode was patterned on the glass substrate near the square-shaped micropore entrapping the *E. coli* cells. In the present work, the β-galactosidase is used as a reporter, to assess the dynamic response during a transformation process on the microbial chip. *E. coli* JM109 was transformed by a cloning vector pBR322 containing the gene encoding for a β-galactosidase.

The plasmid pBR322-lacZ (NIPPON GENE) was introduced into *E. coli* JM109 cells using calcium chloride method. The transformed bacteria was mixed with Collagen type I solution, a 10-times concentrated form of PBS(-) in 2:8:1 proportion. The microbial chip was prepared by gelling the mixed solution prepared above in the micropores (geometry of 300 μm squares and 50 μm depth), and the *E. coli* JM109 cells were embedded in the collagen gel microstructure. The number of embedded *E. coli* JM109 in the collagen gel microstructure was 10^3 cells. The activity of β-galactosidase was determined by using 2.0 mg/ml *p*-aminophenyl β-D-galactopyranoside as substrate. The product of the enzymatic reaction, *p*-aminophenol, was oxidized at Pt microelectrode.

Figure1(C) shows the current responses of (●) transformed and (○) wild-type *E. coli* JM109 in the collagen gel microstructure detected at the Pt microelectrode on the glass substrate. In wild-type, no current response was detected. This is because wild-type *E. coli* JM109 cannnot grow in the nutrient medium with ampicillin lacking ampicillin resistance. In the transformed *E. coli* JM109, on the other hand, the current response increased gradually around 8 hr and a peak current was detected around 16 hr after on-chip incubation. This current response clearly reflects the expression of β-galactosidase induced by IPTG. Therefore, we achieved amperometric detection of gene expression in transformed *E. coli*. Now we attempt to apply this sensing device to DNA cloning.

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Reference:

[1] T. Kaya, K. Nagamine, N. Matsui, T. Yasukawa, H. Shiku and T. Matsue, *Chem. Comm.*, 2, **2004,** 248.

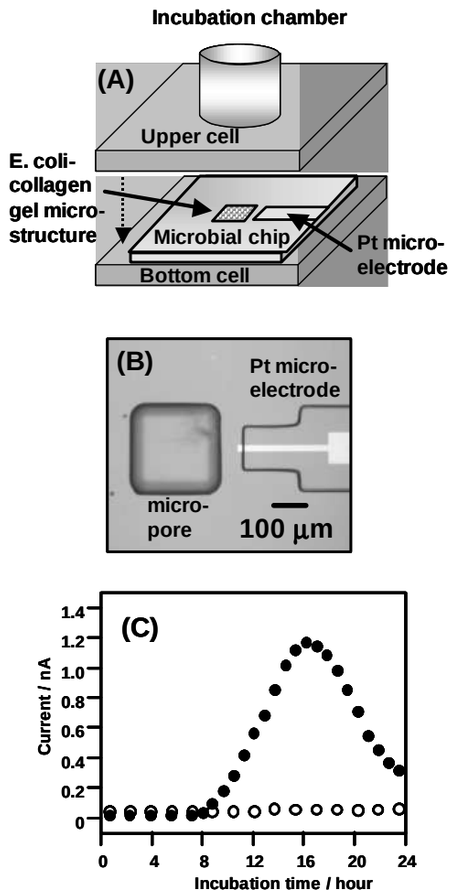

Figure 1 (A)Schematic drawing of the on-chip culture system. (B) An optical microscopic image of micropore and microelectrode on the microbial chip. (C) The current responses of (●) transformed and (○) wild-type *E. coli* JM109 in the collagen gel microstructure detected at the Pt microelectrode on the glass substrate.