Electronic Supplementary Information

Electrochemical Detection of Pathogenic Bacteria by Using Glucose Dehydrogenase Fused Zinc Finger Protein

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Materials and Methods

Colorimetric measures of Zif-268 GDH activity

Biotinylated target dsDNA or non-target dsDNA was prepared at 300 nM in TBS buffer (10 mM Tris-HCl, 100 mM NaCl, and 90 μM $ZnCl_2$, pH 7.0), and 100 μL of this dsDNA solution was added to streptavidin-coated wells of a 96-well plate (Thermo Scientific, MA, USA). After incubation at room temperature for 30 min, each well was washed three times with TBS-T buffer (10 mM Tris-HCl, 100 mM NaCl, and 0.05% Tween20, pH 7.0). Then, purified Zif268-GDH was prepared at 100 nM in TBS buffer that contained 4% skimmed milk and 200 μl of this Zif268-GDH solution was added to each well. After incubation for 30 min, each well was washed three times with TBS-T buffer and twice with TBS buffer. Finally, 200 μL of assay buffer (10 mM Tris-HCl, 100 mM NaCl, 90 μM $ZnCl_2$, 0.06 mM DCIP, 0.6 mM PMS, and 100 mM glucose, pH 7.0) was added to each well. DCIP absorbance at 600 nm was monitored with a plate reader (PerkinElmer, Kanagawa, Japan).

Primer pairs for pathogen detection

For *E. coli* O157 detection, we used a primer pair that targeted the fimbrial protein gene. The specificity of this primer pair among all genomes was assessed using a BLAST search. These primers were purchased from Operon and their sequences are shown in Table 1.

Bacterial culture and genomic DNA extraction.

E. coli O157 isolates from 24 cattle and eight human stool samples were cultured and genomic DNA was extracted. Eleven serovars of *S. enterica* subspecies *enterica* (Hadar, Infantis, Thompson, Oranienburg, Litchfield, Montevideo, Braenderup, Tennessee, Virchow, Agona, and Enteritidis) were cultured and genomic DNA was extracted. DNA extraction was done using FastPure DNA Kits (Takara, Shiga, Japan).

Detecting synthetic oligonucleotides and PCR products using the Zif268-GDH fusion protein.

Different concentrations of biotinylated dsDNA were immobilized on NeutrAvidin beads agarose resin (Thermo Fisher Scientific Inc., MA). The sequences of the synthetic oligonucleotides are shown in Table 1. After incubation for 15 min, bead samples were centrifuged and washed 3 times with binding buffer (90 μM $ZnCl_2$, 100 mM NaCl, and 10 mM Tris-HCl, pH7.0). Then, purified Zif268-GDH was prepared at 100 nM in binding buffer that contained 4% skim milk and added to the bead samples. After incubation for 30 min, each sample was washed 3 times with binding buffer that contained 0.05 % (v/v) Tween20 and twice with binding buffer. Finally, 15 μL of a bead sample and 15 μL of binding buffer that contained 6 mM 1-methoxyphenazine methosulphate (m-PMS) were added on a DEP Chip (BioDevice Technology, Ishikawa, Japan). After adding a glucose solution (f.c. 100 mM), the response current was measured at a potential of 0.1 V (vs. Ag/AgCl). The applied potential was previously determined by CV measurements, and we confirmed this potential was able to effectively oxidize m-PMS.[1] We defined the delta current as the difference in current between without and with 100 mM glucose.

To detect PCR products, PCR solution (1 μM forward Primer, 1 μM reverse Primer, 1.25 U of

TaKaRa EX Taq HS DNA polymerase, and 200 μM of each dNTP) that contained 10–10^5 copies of *E. coli* O157 genomic DNA was prepared. The target region that included the binding sequence of Zif268-GDH was amplified by PCR: (98 ℃/10 s→48 ℃/30 s→72 ℃/30 s) × 35 cycles. A 40 μL volume of PCR products was added directly to 15 μL of NeutrAvidin beads agarose resin.

Reference

1 K. Ikebukuro, Y. Kohiki and K. Sode, *Biosens. Bioelectron.*, 2002, **17**, 1075–1080.