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Supplementary Materials

Detection of *Escherichia coli* by Combining an Affinity-Based Method with Atmospheric Pressure Ionization Mass Spectrometry

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Additional Experimental Details

1. Materials and reagents

Fused silica capillary (inner diameter: ~50 μm, outer diameter: ~360 μm) were purchased from Polymicro Technologies (Phoenix, AZ, USA). Ethanol was purchased from Echo (Miaoli, Taiwan). Luria-Bertani (LB) broth and tryptic soya broth (TSB) were purchased from Becton Dickinson (Franklin Lakes, NJ, USA). Yeast extract was purchased from Alpha Bioscience (Baltimore, MD, USA), whereas agarose was purchased from Amresco (Solon, OH, USA). Hydrofluoric acid (HF) was obtained from Merck (Darmstadt, Germany). Hydrogen peroxide was obtained from Showa (Japan). 3,3′,5,5′-tetramethylbenzidine (TMB), potassium chloride, sodium phosphate dibasic heptahydrate, potassium phosphate monobasic, and horseradish peroxidase (HRP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium acetate was purchased from Fluka (Seelze, Germany). *Staphylococcus aureus*, *Acinetobacter baumannii*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* were collected from the patients in the Tzu-Chi General Hospital (Hualien, Taiwan) and kindly provided by Prof. P.-J. Tsai (NCKU, Taiwan). *Escherichia coli* J96, *Escherichia coli* BOS117, *Escherichia coli* HB101, and *Escherichia coli* JM109, *Escherichia coli* UTI no. 37 were kind gifts from Dr. James Johnson (Minneapolis Veterans Affairs Medical Center and University of Minnesota, Department of Medicine). *Escherichia coli* O157:H7 and *Bacillus cereus* were purchased from the BioResource Collection and Research Center (Hsinchu, Taiwan).

2. Instrumentation

Either an amaZon SL mass spectrometer (Bruker Daltonics, Bremen, Germany) or a micrOTOF Q II mass spectrometer (Bruker Daltonics, Bremen, Germany) was used for C-API MS analysis. A CMOS camera (SG-210X) from Sage Vision (New Taipei City, Taiwan) was used for recording and taking images during MS analysis. Optical microscopic images were obtained from a Nikon Eclipse 80i microscope with a camera (DS-Ri1) (Tokyo, Japan). Gold coating on the surface of silica capillaries was conducted by using a 108-auto sputter coater from Ted Pella (Redding, CA, USA). Products obtained from the Maillard reaction were purified by high-performance liquid chromatography (HPLC) equipped with a LC-20AD pump and an SPD-M20A ultraviolet-visible detector from Shimadzu (Tokyo, Japan).

3. Preparation of tapered capillaries and gold-coated probes

A capillary was cut to ~4 cm using a ceramic glass cutter. The capillary was tapered by placing it vertically on a metal stand and clamping the upper and lower ends with two binder clips. The lower end of the capillary was hanged with a counterweight of 50 g. A butane torch was used to burn the capillary on the middle to have a narrow tip. The tapered end of the capillary was dipped to aqueous HF (24%, v/v) for 20 min followed by rinse with deionized water. The tapered capillary was thoroughly dried in the oven (60 °C) to remove water prior to the experiment. The diameter of the tapered tip of the

capillary was ~20 μm. The as-prepared tapered capillary (length: ~2 cm) was used as the C-API ionization emitter. In addition, we also modified the tapered capillary as the bacterial trapping probe. That is, the surface of the capillary was flushed with nitrogen gas (30 psi (= ~2.07 × 10^5 Pa) to remove impurities. The tapered capillary was then put inside the chamber of the sputter coater for gold coating. The gold coating of the capillary was conducted by setting the current at 40 mA for 30 s followed by switching on argon gas for 40 s.

4. Preparation of maltosylated-cysteine

β-Maltose derivatized by cysteine was conducted through the Maillard reaction. Cysteine (12 mg) and β-maltose (102 mg) with a mole ratio of 1/3 were dissolved in deionized water (1 mL). The mixture was sonicated for 10 min followed by lyophilization. The lyophilized mixture was subjected to an oil bath at 120 °C for 30 min. Yellowish maltosylated-cysteine (MALCY) product was formed. The product was re-suspended in deionized water (1 mL). MALCY was purified by HPLC and confirmed by mass spectrometry. C18 column (dimensions: 4.6 × 150 mm; diameter of stationary particles: 5 μm) was used in HPLC for separation. The composition of the mobile phase was methanol/ammonium bicarbonate (5 mM) (9:1, v/v) with a flow rate of 0.6 mL min^{-1}.

5. Quantitative analysis of glucose unit on the probe by phenol-sulfuric acid method

Glucose standard samples (5-2836 μg mL^{-1}, 0.2 mL) were prepared initially. Three gold-coated probes were put to each glucose standard sample. Phenol (53 M, 50 μL) was then added to glucose solution followed by sulfuric acid (18.4 M, 0.5 mL) in the ice bath, and the reaction stood for around 5 min. The color of the reaction solution slowly turned orange, and the reaction was cooled to room temperature for 10 min before examined by UV-Vis spectroscopy. Deionized water (0.2 mL) was added to the vial tube containing 3 MALCY-probe. Subsequently, phenol (53 M, 0.05 mL) was added to the solution followed by the addition of sulfuric acid (18.4 M, 0.5 mL) in an ice bath.

6. Peroxidation reaction of TMB catalyzed by HRP

Peroxidation reaction of TMB catalyzed by HRP was first conducted. HRP (10^{-5} M, 5 μL) was prepared in ammonium acetate (pH 4.5, 45 μL). Hydrogen peroxide (100 mM, 45 μL) was added to the HRP solution (50 μL) followed by the addition of TMB (1 mg mL^{-1}, 5 μL). The mixture changed its color from colorless to blue, indicating the occurrence of TMB oxidation occurred.[1] The resultant sample was 100-fold diluted with ethanol/deionized water (2:1, v/v) prior to C-API-MS analysis.

7. Preparation of artificial urine samples

Simulated urine samples were prepared by dissolving urea (121.3 mg), sodium chloride (50 mg), potassium chloride (30 mg), and sodium phosphate monobasic (32 mg) in deionized water (50 mL) [1]. The resultant solution was adjusted to pH 6.5. Creatinine (13.3 mg) and albumin from chicken egg (0.33 mg) were added to the artificial urine sample (50 mL) above to ensure similarity to human urine. The as-prepared artificial urine was 100-fold by PBS buffer (pH 7.5, 10 mM) prior to spiked with bacteria.

8. Additional Scheme

Scheme 1. Peroxidase reaction by using TMB as the substrate in the presence H_2O_2 [2].

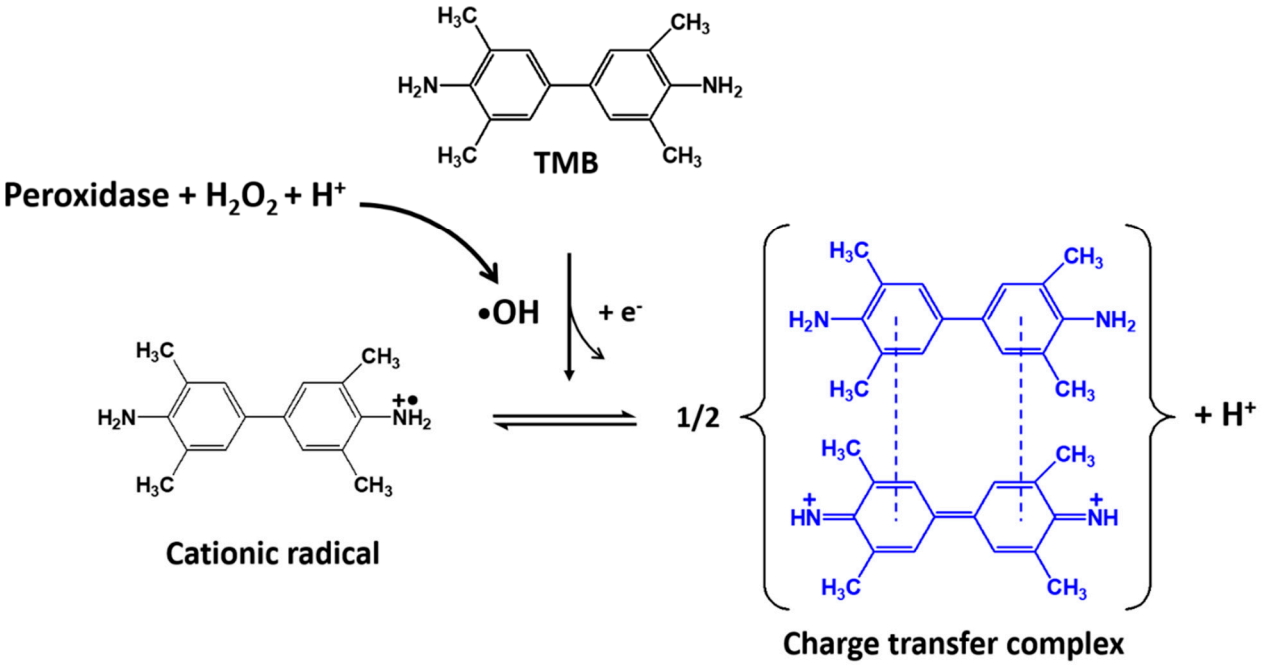

Table S1. Comparison of the current method with the existing methods for detection of *E. coli*.

Method	Sample type	Limit of detection	Analysis time (Approx.)	References
Real time-based PCR	Ground beef	5×10^2 cfu ml^{-1}	5 h 10 min	[3]
ELISA based PCR	Milk	10^2 cfu ml^{-1}	5 h	[4]
Paper-based ELISA	Buffer	10^4 cfu ml^{-1}	3 h	[5]
Fluorescence-based bacteriophage assay	Broth	10^2-10^3 cfu ml^{-1}	10 h	[6]
Colorimetric	Buffer	10^5 cfu ml^{-1}	N/A	[7]
Electrochemical immunoassay	Buffer	2×10^2 cfu ml^{-1}	3 h	[8]
Surface modified gold-nanorods	Broth	10^7 cfu ml^{-1}	8 h	[9]
MALDI-TOF-MS	Whole blood	8×10^3 cfu ml^{-1}	4 h	[10]
Glycan-based affinity method combined with MS	Buffer/orange juice	~10^2 cfu mL^{-1}	~2 h	This work

9. Additional Results and Discussion

Figure S1A shows the resultant LC chromatogram of the MALCY product. The fraction at 3.8-4.2 min was collected. **Figures S1B** and **S1C** show the electrospray ionization (ESI) mass spectra of the reaction product obtained before and after purification, respectively. Apparently, the ion peak at *m*/*z* 446 derived from protonated MALCY dominated the mass spectrum after purification (**Figure S1C**).

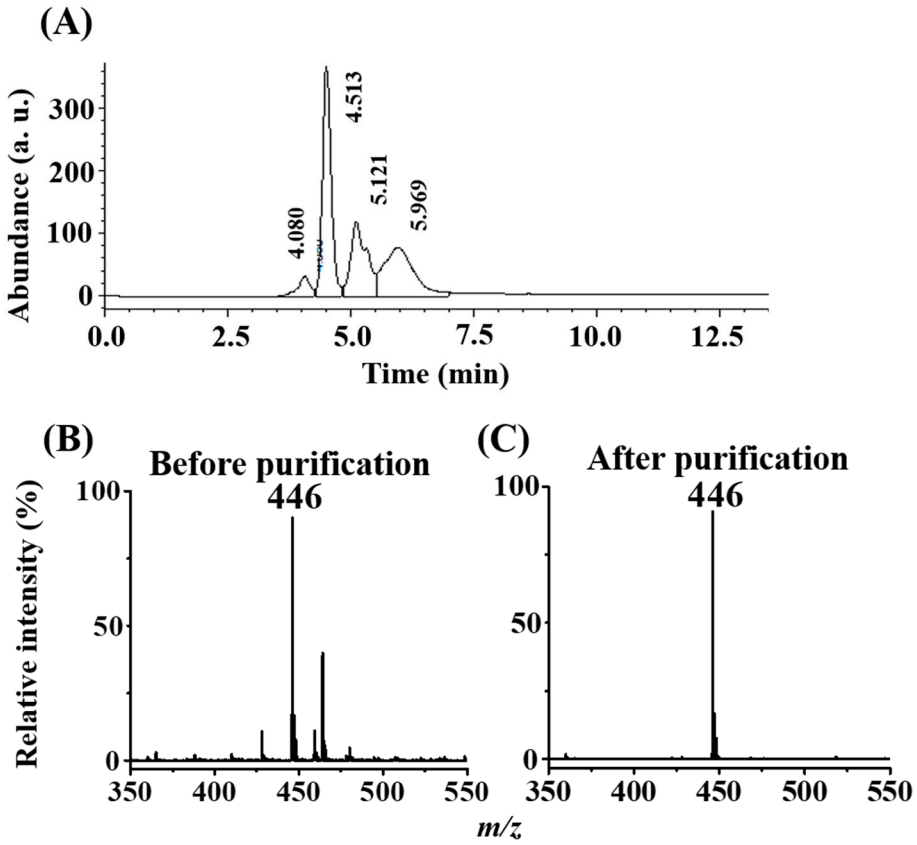

Figure S1. (A) LC chromatogram of the product derived from the Maillard reaction obtained by reacting cysteine and maltose with a mole ratio of 1/3 at 120 °C, monitored the wavelength at 210 nm by a photodiode array detector. ESI mass spectra of the reaction product obtained (B) before and (C) after purification by LC.

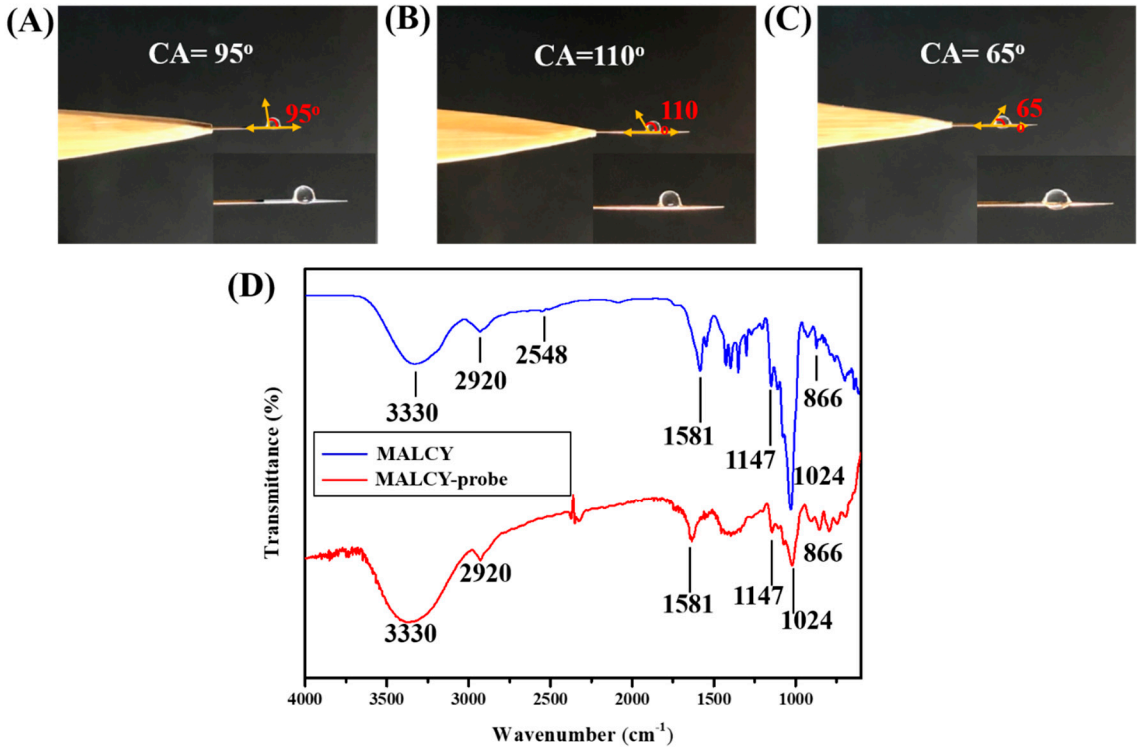

Figure S2. Photograph of the contact angles (CA) examined on the surfaces of (A) the unmodified capillary probe, (B) the gold-coated probe, and (C) the MALCY immobilized probe by depositing with a droplet of water (~4 μL). The inset photographs show the zoom-in pictures of the droplets on the as-prepared capillaries. (D) Infrared absorption spectra of the MALCY powder (blue line) and the MALCY-probe (red line). The characteristic bands derived from S-H at 2548 cm^{-1} no longer appeared in the spectrum of the MALCY-probe (red line). The band at 866 cm^{-1} were contributed by C-S stretching vibration mode. In addition, the characteristic band at 1581 cm^{-1} corresponded to asymmetric stretching C=O. The sharp peak at 1024 cm^{-1} was assigned to C–O single bond stretching of the C–O–C group. The band at ~3330 cm^{-1} corresponded to the stretching mode of OH group, and the band at 2920 cm^{-1} was assigned to stretching vibrations of C-H group in the glucose unit. The absorption band at 1147 cm^{-1} was assigned to weak C–O stretching mode from glycosidic bond.

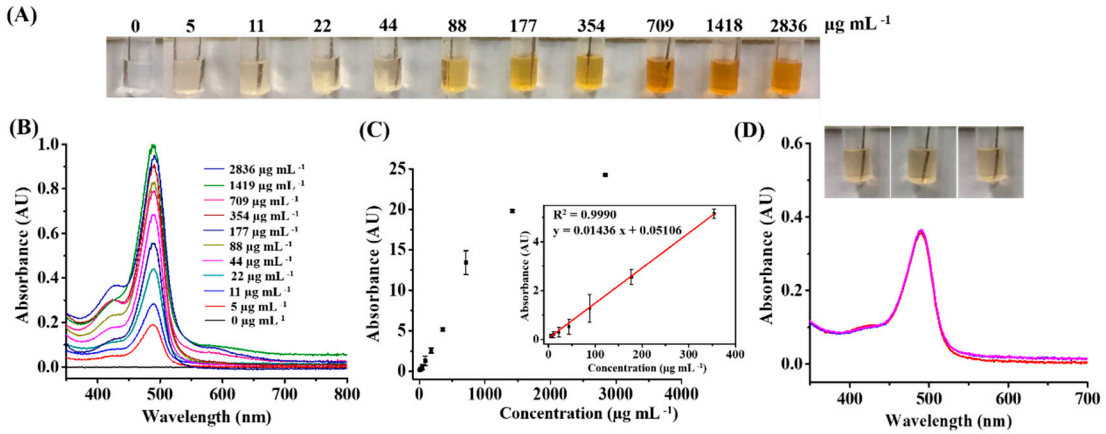

Figure S3. (A) Photograph of the samples containing glucose (5-2836 μg mL^{-1}, 0.2 mL) in the presence of 3 gold-coated probes obtained after reaction with sulfuric acid (18.4 M, 0.5 mL) followed by reaction with phenol (5.3 M, 5 μL) in an ice bath for 5 min and cooling down at room temperature for 10 min. (B) The corresponding UV-Vis absorption spectra of the samples shown in Panel A. (C) Calibration curve obtained by plotting the absorbance at 485 nm versus the concentration of glucose (5-2836 μg mL^{-1}) according to the results obtained in Panel B and Figure S5. The inset shows the linear dynamic range for the concentration range of glucose from 5 to 354 μg mL^{-1}. (D) UV-Vis absorption spectra the aqueous solution (0.2 mL) inserting with 3 MALCY-probes and reaction with sulfuric acid (18.4 M, 0.5 mL) and phenol (5.3 M, 5 μL) for 5 min in an ice bath and cooling down at room temperature for 10 min. Three replicates were shown in Panel D. The inset shows the photograph of the resultant sample from three replicates.

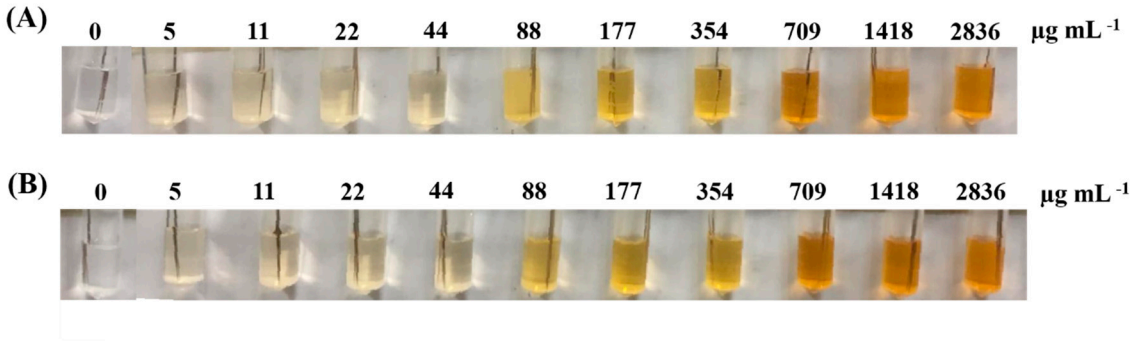

Figure S4. (A, B) Two other replicates of the samples as shown in Figure S3A.

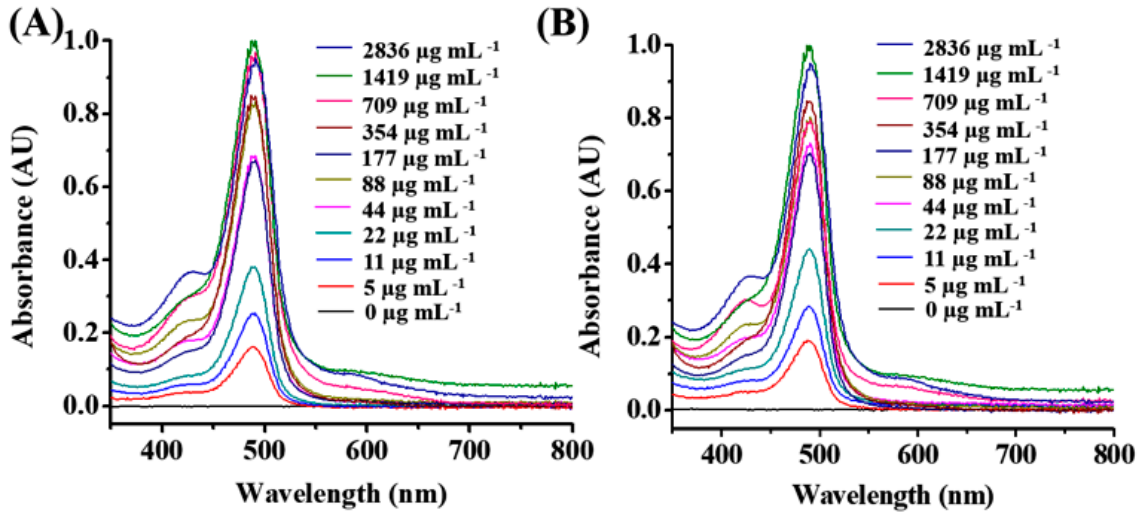

Figure S5. Corresponding UV-Vis absorption spectra of the samples shown in (A) Figure S4A and (B) Figure S4B.

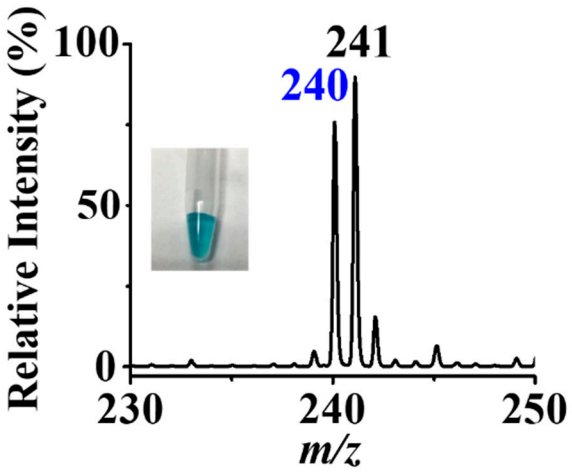

Figure S6. C-API mass spectrum of the sample obtained by using HRP to catalyze the peroxidase reaction of the sample containing TMB in the presence of H_2O_2. The resultant reaction sample was 100-fold diluted with ethanol/deionized water (2:1, v/v) prior to C-API-MS analysis. The inset image shows the photograph of the resultant sample obtained after reaction.

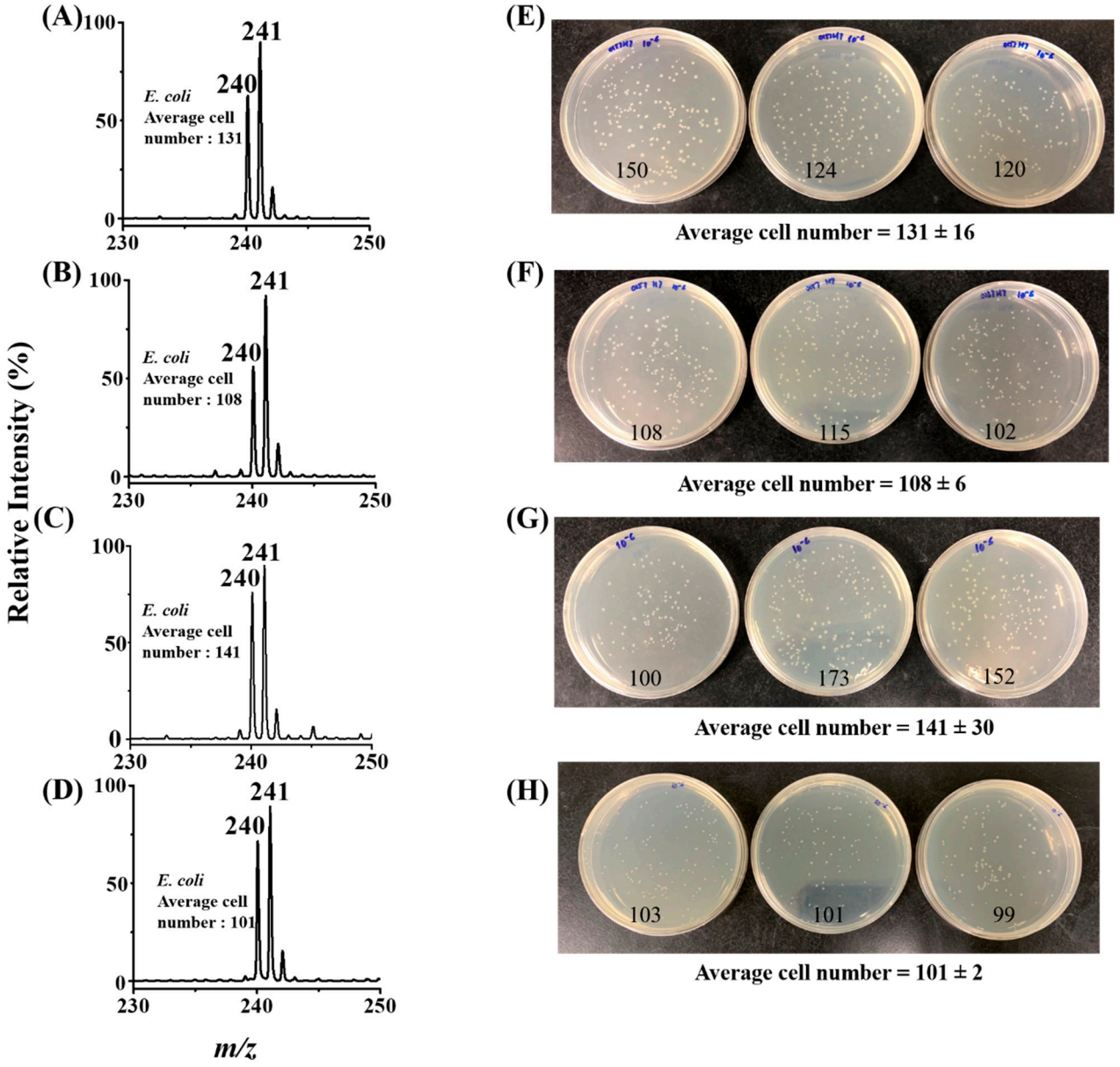

Figure S7. (A, B, C, and D) Four replicated experimental results for the sample in Figure 3B. (E, F, G and H) Photographs of the plate counting results derived from samples used to obtain Panels A, B, C and D. The bacterial samples (0.1 mL) were individually cultured on LB agar plate for overnight. Three replicates of overnight culture of each sample were performed for each sample.

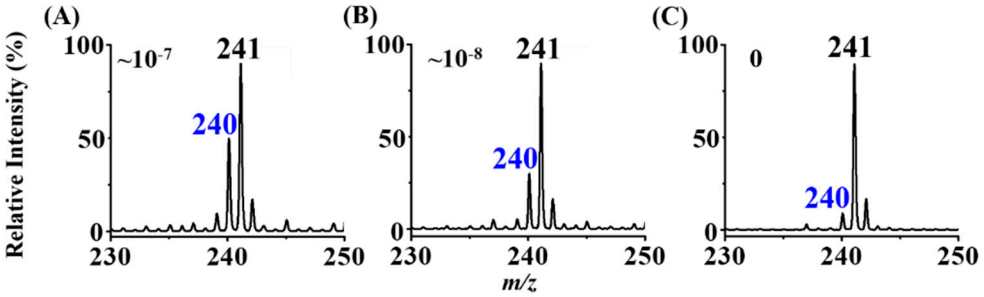

Figure S8. C-API mass spectra of the samples obtained by using the MALCY-probes to enrich target species from the samples containing *E. coli* O157:H7 with the concentrations of (A) OD_{600} of ~10^{-7}, (B) ~10^{-8}, and (C) 0, followed by releasing the bacteria from the probe for subsequent endogenous peroxidase reactions. The resultant samples were 100-fold diluted with ethanol/deionized water (2:1, v/v) prior to C-API-MS analysis. One droplet (5 μL) of the resultant samples were used for C-API-MS analysis.

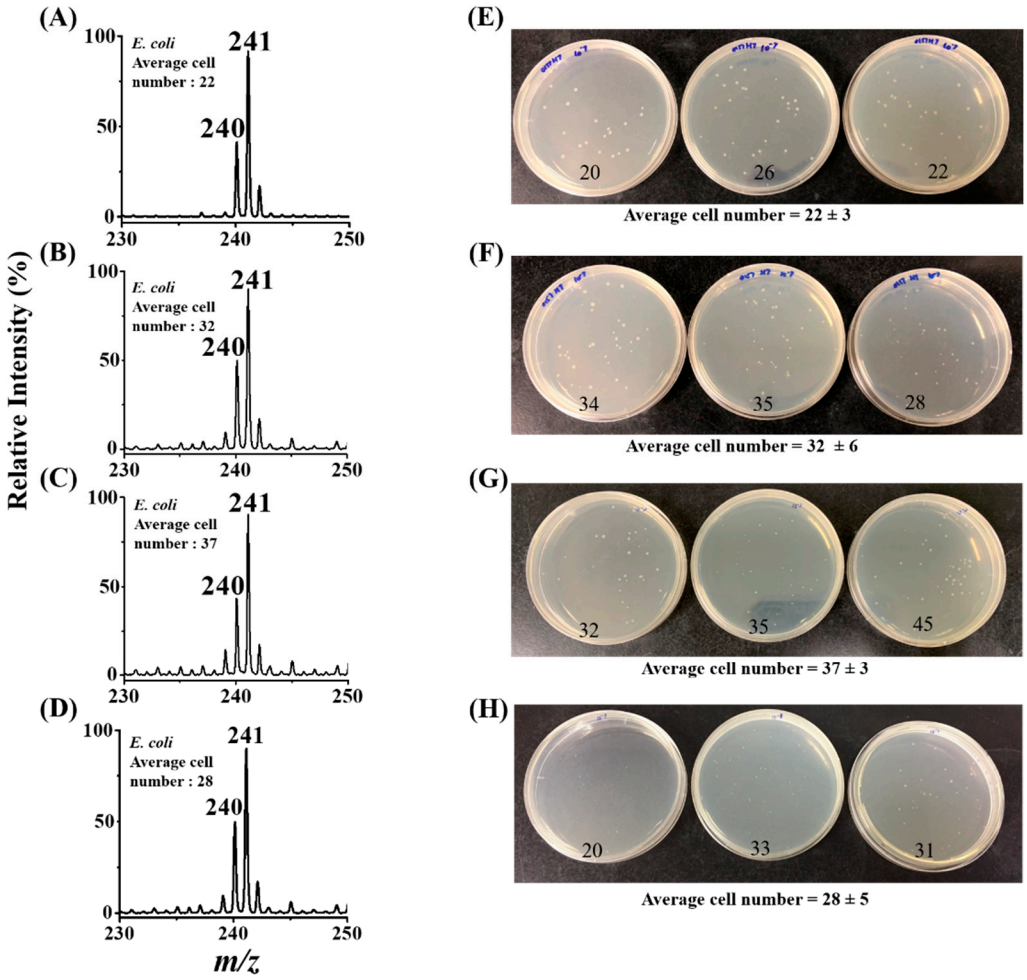

Figure S9. (A, B, C, and D) Four replicated experimental results of the sample in Figure S8A. (E, F, G, and H) Photographs of the plate counting results derived from samples used to obtain Panels A, B, C and D. The bacterial samples (0.1 mL) were individually cultured on LB agar plate for overnight. Three replicates of overnight culture of each

sample were performed for each sam

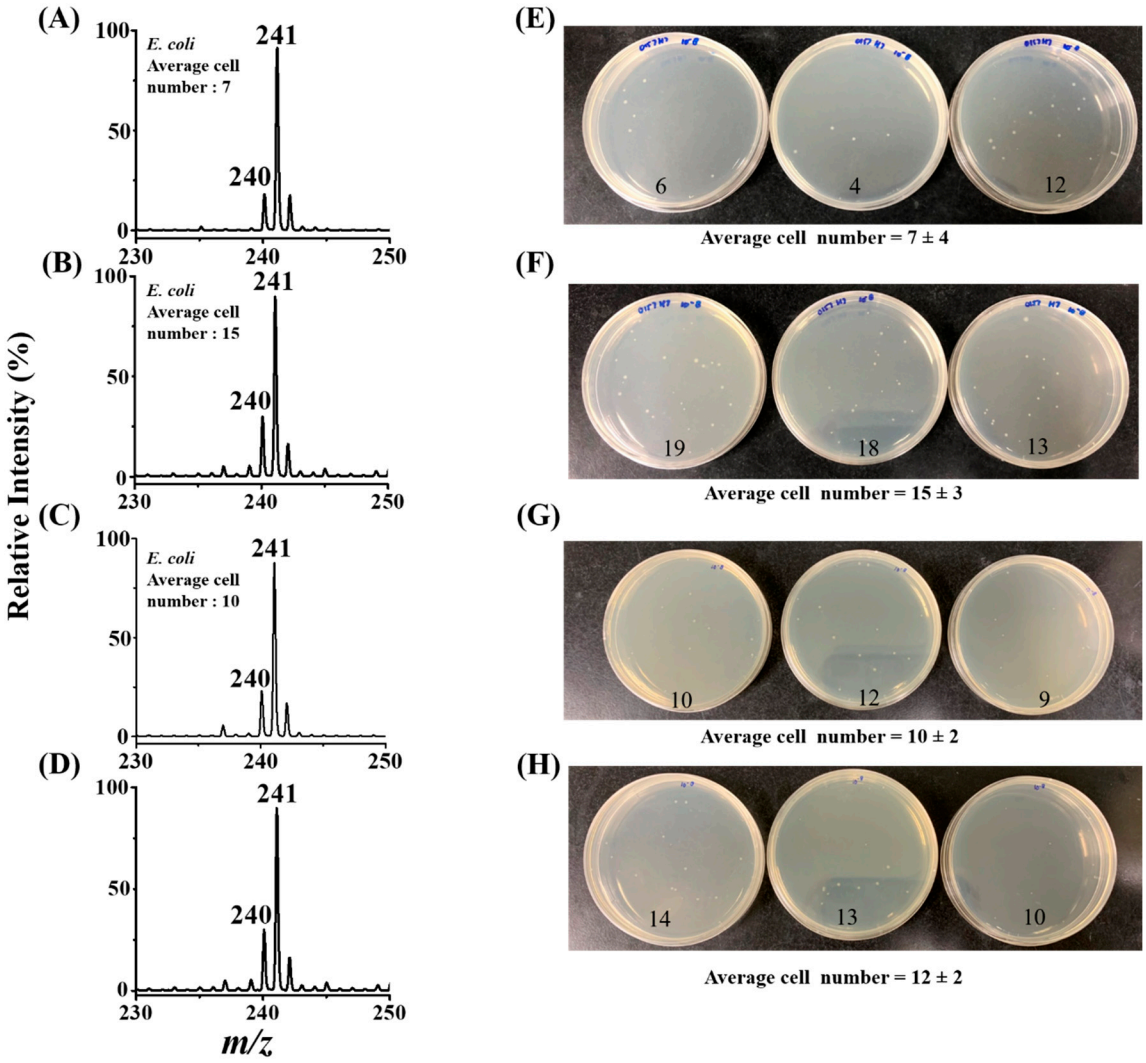

Figure S10. (A, B, C, and D) Four replicated experimental results of the sample in **Figure S8B**. (E, F, G and H) Photographs of the plate counting results obtained from overnight culture of the four samples used for obtaining Panels A, B, C, and D. The bacterial samples (0.1 mL) were individually cultured on LB agar plate for overnight. Three replicates of overnight culture of each sample were performed for each sample.

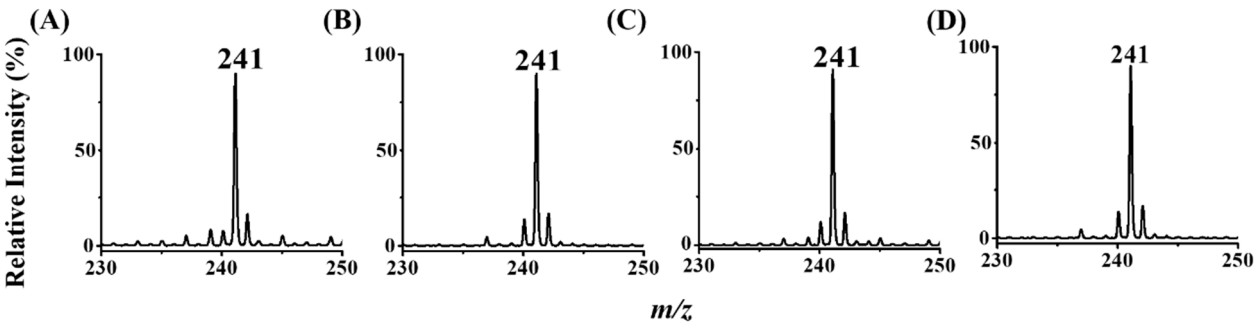

Figure S11. (A, B, C, and D) The resultant mass spectra of the blank samples from four replicates.

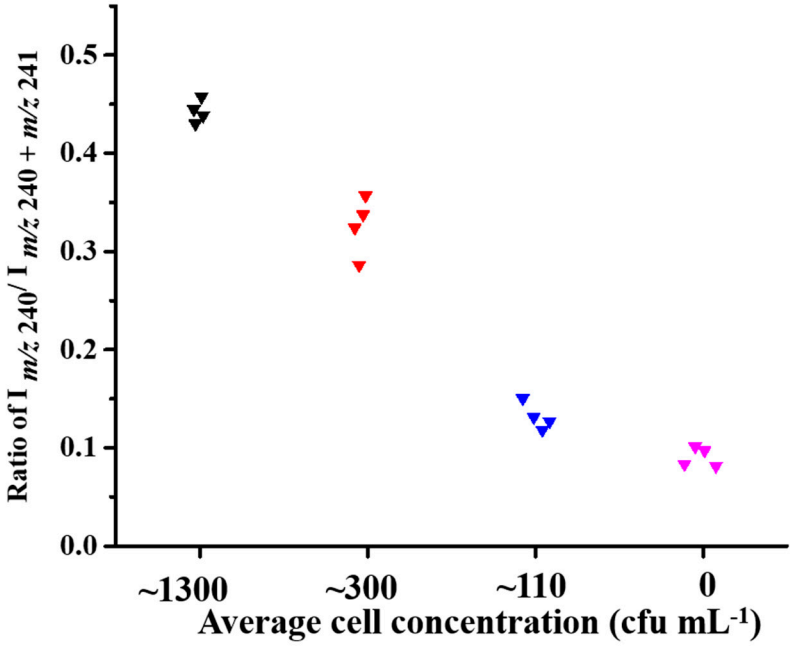

Figure S12. Examination of the variations of the ratio of the intensity of the ion peak at *m*/*z* 240 to the sum of the ion intensity at *m*/*z* 240 and *m*/*z* 241 versus the bacterial concentration. The results were obtained from 4 replicates shown in Figures S7 (black triangles), S9 (red triangles), and S10 (blue triangles), and S11 (purple triangles).

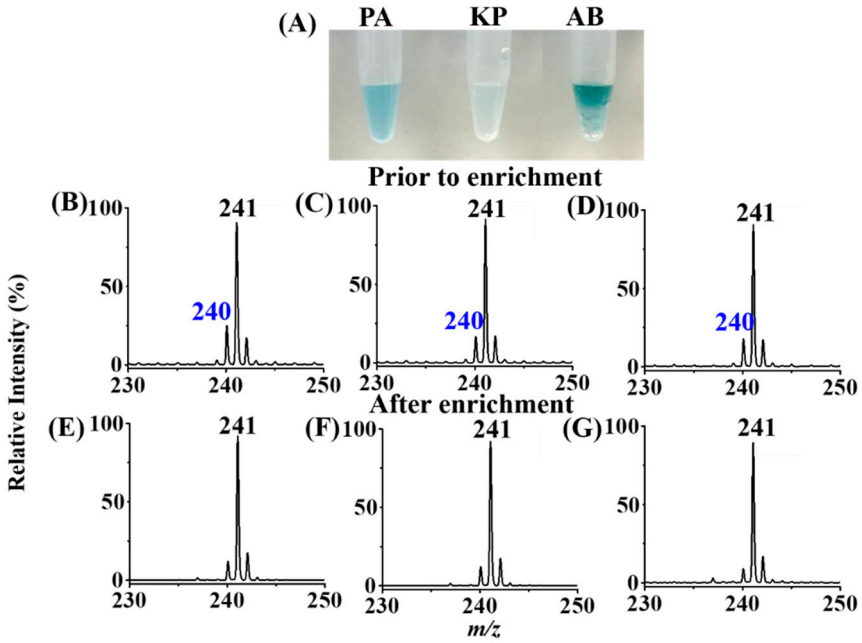

Figure S13. (A) Photograph of the samples (0.2 mL) containing *P. aeruginosa* (PA), *K. pneumonia* (KP), and *A. baumannii* (AB) at the concentrations of OD_{600} of ~1 obtained after conducting endogenous peroxidase reaction. C-API mass spectra of the bacterial samples containing (B) *P. aeruginosa*, (C) *K. pneumonia*, and (D) *A. baumannii* with the concentration of OD_{600} of ~10^{-5} obtained after bacterial peroxidase reactions prior to enrichment. C-API mass spectra of the samples obtained by using the MALCY-probes to enrich target species from the samples (3 mL) containing (E) *P. aeruginosa*, (F) *K.*

pneumonia, and (G) *A. baumannii* with the concentrations of OD_{600} of ~10^{-5} followed by releasing the bacteria from the probe and conduction of endogenous peroxidase reactions. The resultant samples were 100-fold diluted with ethanol/deionized water (2:1, v/v) prior to C-API-MS analysis. One droplet (5 μL) of the resultant samples were used for MS analysis.

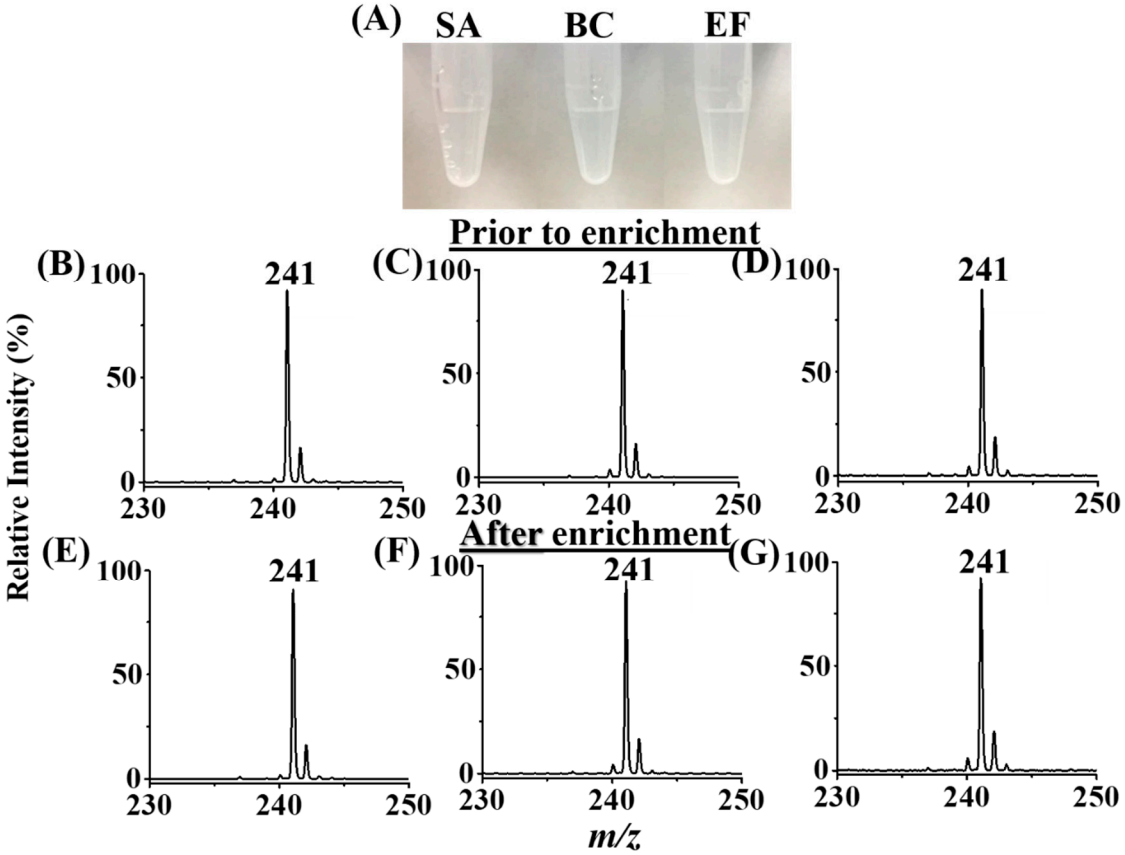

Figure S14. (A) Photograph of the bacterial samples (0.2 mL) containing *S. aureus* (SA), *B. cereus* (BC), and *E. faecalis* (EF) with the concentration of OD_{600} of ~1 prepared in ammonium acetate (10 mM, pH 4.5) with the addition of H_2O_2 (100 mM, 2 μL) and TMB (1 mg mL^{-1}, 2 μL). C-API mass spectra of the bacterial samples (0.2 mL) with the concentration of OD_{600} of ~10^{-5} containing (B) *S. aureus*, (C) *B. cereus*, and (D) *E. faecalis* prepared in ammonium acetate (10 mM) at pH 4.5 with the addition of H_2O_2 (100 mM, 2 μL) and TMB (1 mg mL^{-1}, 2 μL). C-API mass spectra of the bacterial samples (3 mL) containing (E) *S. aureus*, (F) *B. cereus*, and (G) *E. faecalis* with the concentration of OD_{600} of ~10^{-5} prepared in the phosphate buffered saline (PBS) buffer (10 mM, pH 7.5) obtained after enriched by MALCY-probe followed by releasing the bacteria from the probe to the ammonium acetate buffer (pH 4.5, 8 μL) followed by the addition of H_2O_2 (100 mM, 1 μL) and TMB (1 mg mL^{-1}, 1 μL). The samples were incubated for 15 min at room temperature. A droplet of sample (5 μL) was used for MS-analysis. All the resultant samples were 100-fold diluted with ethanol/deionized water (2:1, v/v) prior to the C-API-MS analysis.

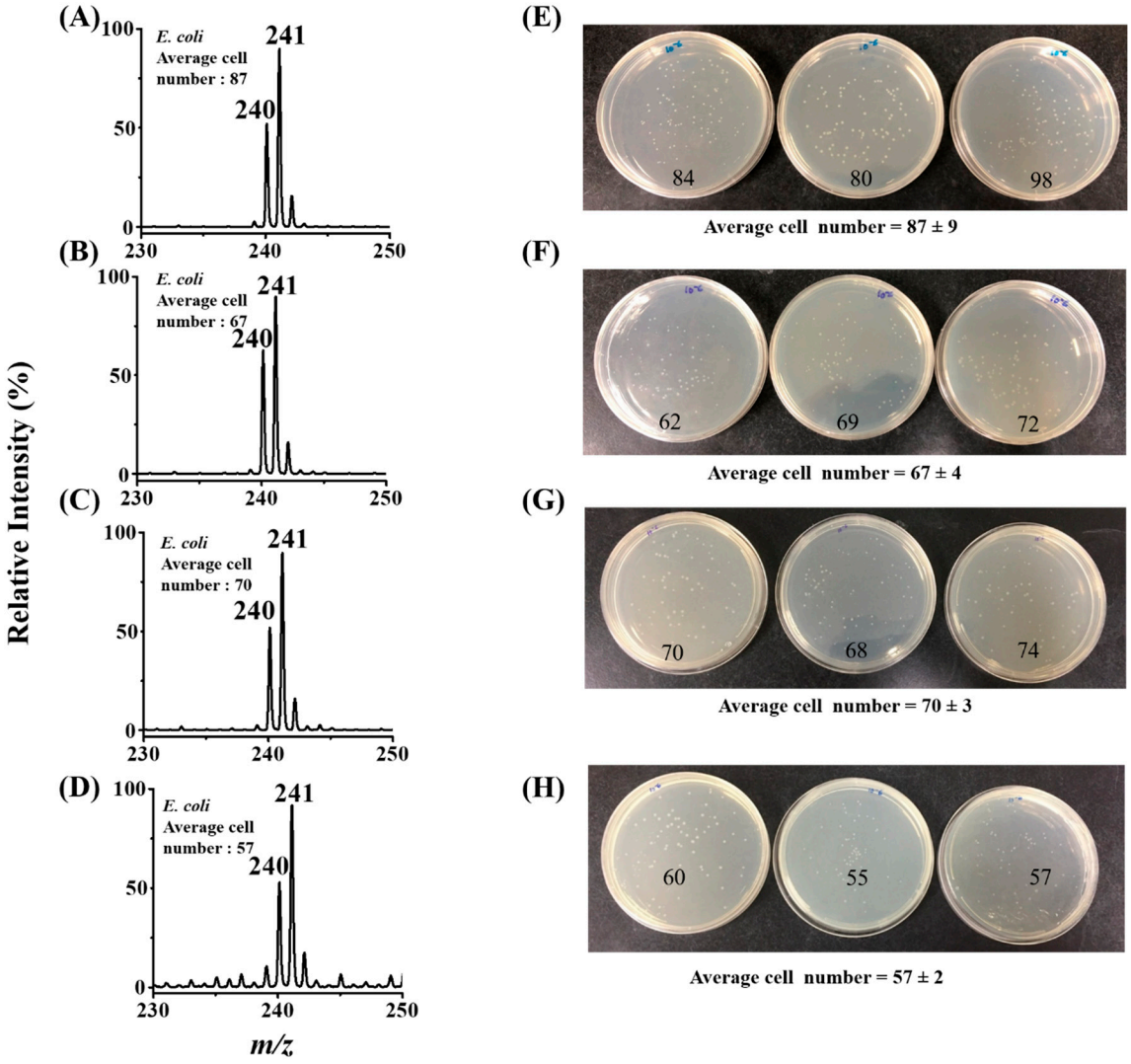

Figure S15. (A, B, C, and D) C-API mass spectra of the samples obtained by using the MALCY-probe to trap target species from the orange juice samples (3 mL) spiked with *E. coli* O157:H7 at the concentration OD_{600} of ~10^{-6} followed by releasing the trapped bacteria in ammonium acetate (pH 4.5, 8 μL) under shaking for 15 min and subsequent bacterial peroxidase reaction with the addition H_2O_2 (100 mM, 1 μL) and TMB (1 mg mL^{-1}, 1 μL) for another 15 min prior to MS analysis. (E, F, G, and H) Photographs obtained from three replicates of the plate counting results obtained from overnight-culture of the samples used in Panels A, B, C, and D. The bacterial samples (0.1 mL) were individually cultured on LB agar plate for overnight.

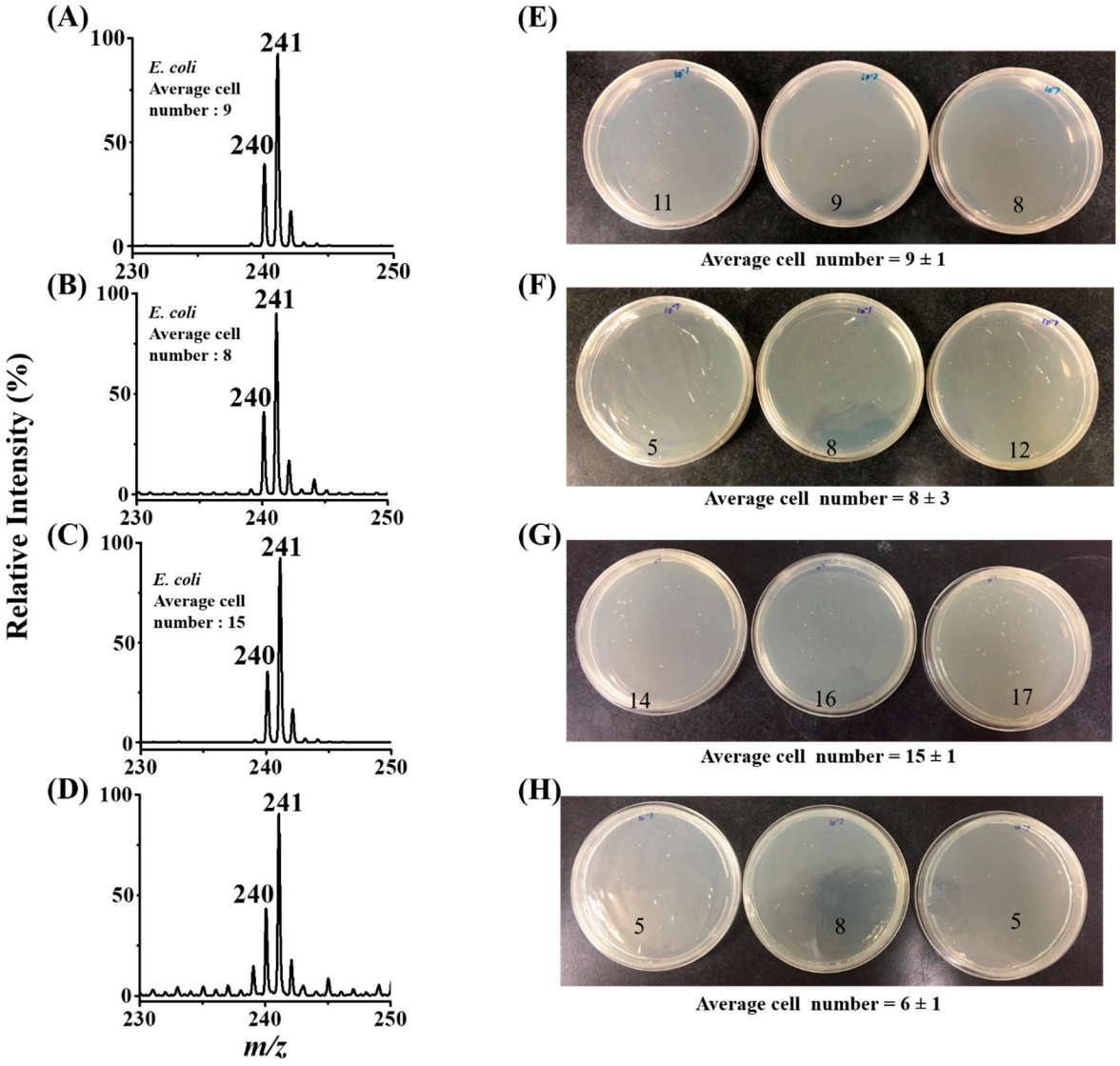

Figure S16. (A, B, C and D) C-API mass spectra of the samples obtained by using the MALCY-probe to trap target species from the orange juice samples (3 mL) spiked with *E. coli* O157:H7 at the concentration OD_{600} of ~10^{-7} followed by releasing the trapped bacteria in ammonium acetate (pH 4.5, 8 µL) under shaking for 15 min and subsequent bacterial peroxidase reaction with the addition H_2O_2 (100 mM, 1 µL) and TMB (1 mg mL^{-1}, 1 µL) for another 15 min prior to MS analysis. (E, F, G and H) Photographs obtained from three replicates of the plate counting results obtained from overnight-culture of the samples used in Panels A, B, C, and D. The bacterial samples (0.1 mL) were individually cultured on LB agar plate for overnight.

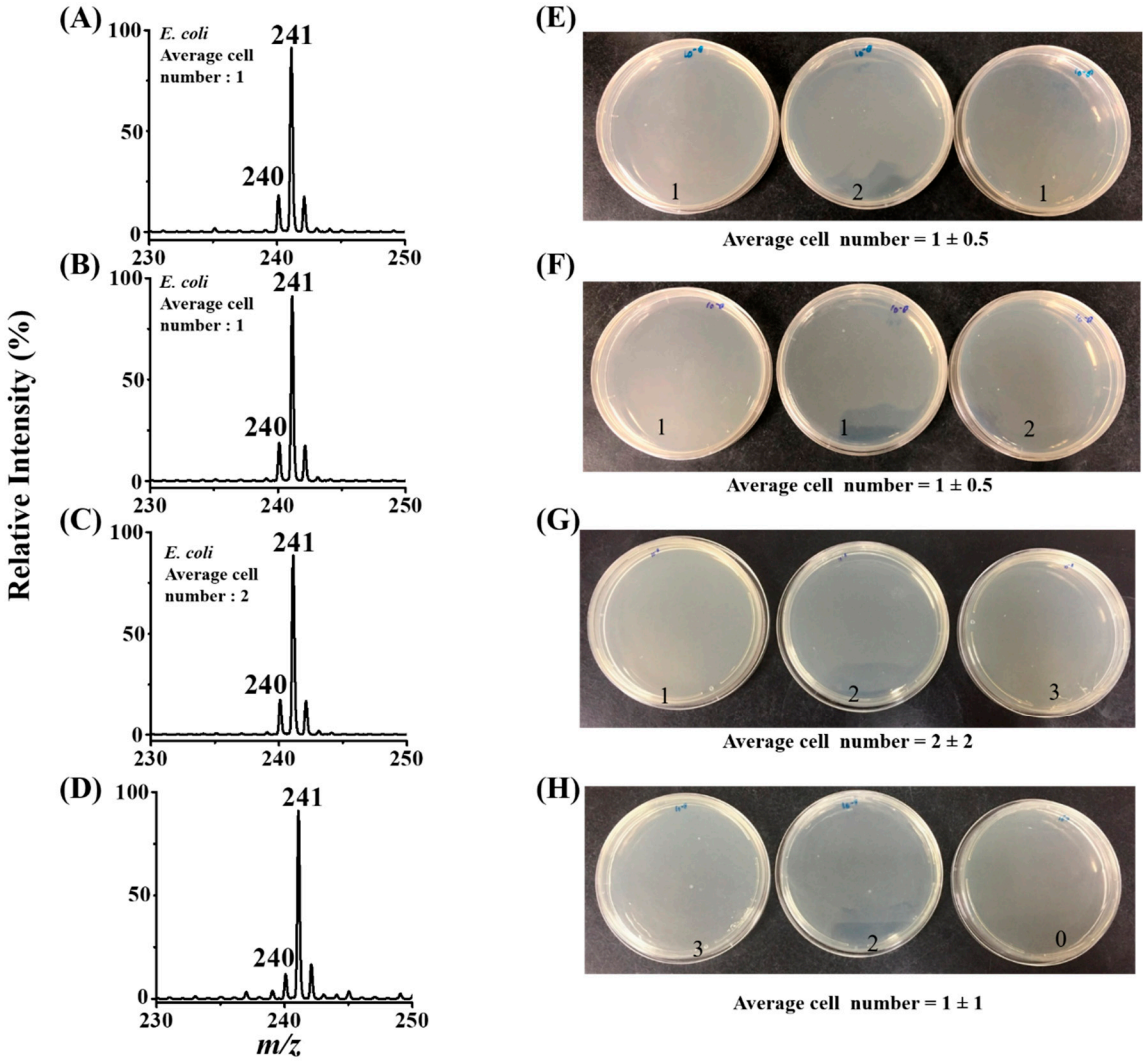

Figure S17. (A, B, C, and D) C-API mass spectra of the samples obtained by using the MALCY-probe to trap target species from the orange juice samples (3 mL) spiked with *E. coli* O157:H7 at the concentration OD_{600} of ~10^{-8} followed by releasing the trapped bacteria in the ammonium acetate buffer (pH 4.5, 8 μL) under shaking for 15 min and subsequent bacterial peroxidase reaction with the addition H_2O_2 (100 mM, 1 μL) and TMB (1 mg mL^{-1}, 1 μL) for another 15 min prior to MS analysis. (E, F, G and H) Photographs obtained from three replicates of the plate counting results obtained from overnight-culture of the samples used in Panels A, B, C, and D. The bacterial samples (0.1 mL) were individually cultured on LB agar plate for overnight.

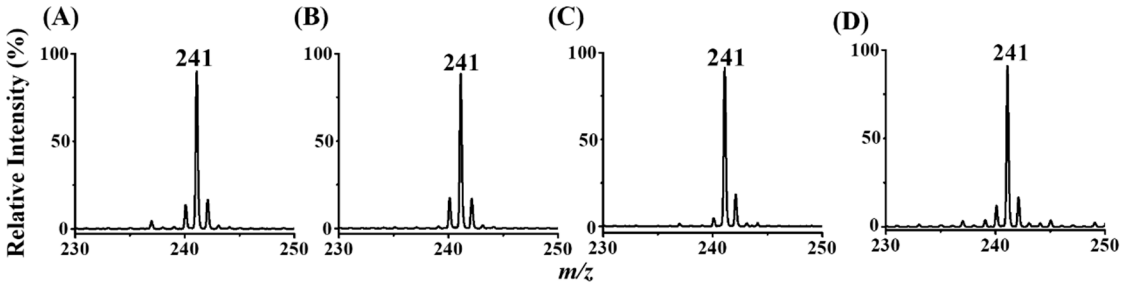

Figure S18. (A, B, C, and D) C-API mass spectra of the 4 replicated samples obtained by using the MALCY-probe to trap target species from the orange juice samples (3 mL) without spiked with *E. coli* O157:H7 followed by bacterial peroxidase reaction with the addition H_2O_2 (100 mM, 1 μL) and TMB (1 mg mL^{-1}, 1 μL) for another 15 min prior to MS analysis.

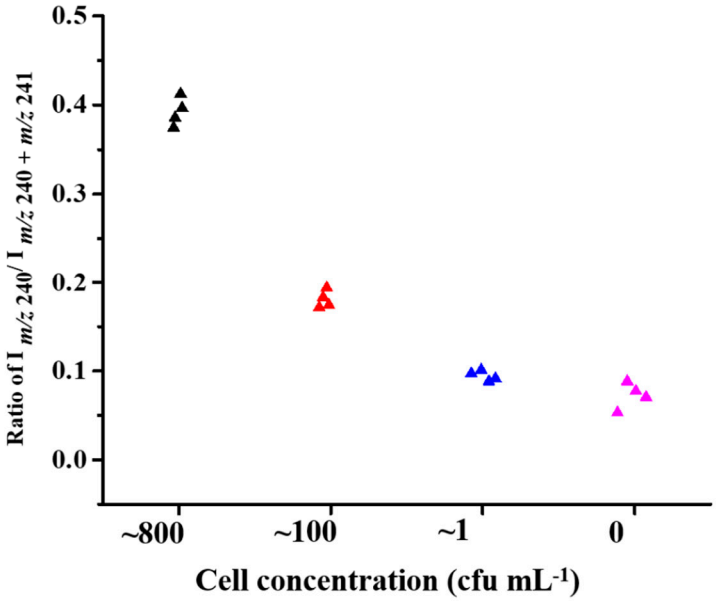

Figure S19. Examination of the variations of the ratio of the ion intensity at *m*/*z* 240 to the sum of the ion intensity at *m*/*z* 240 and 241 versus the bacterial concentration obtained from the results shown in Figure S15 (black triangles), S16 (red triangles), S17 (blue triangles), and S18 (pink triangles).

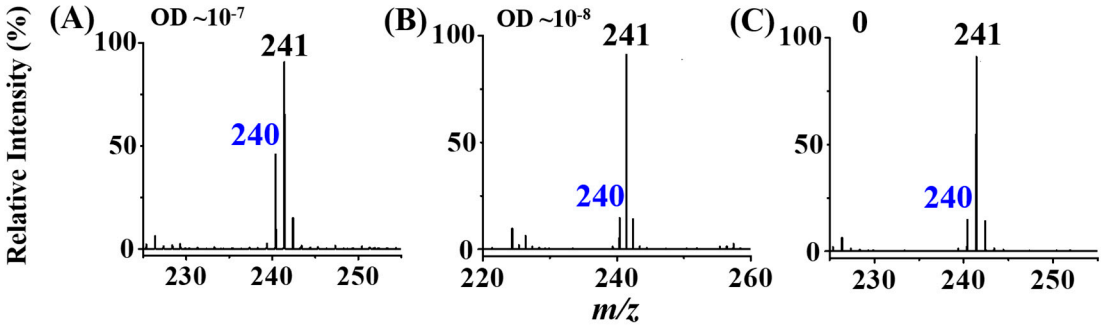

Figure S20. C-API mass spectra of the artificial urine samples (3 mL) spiked with *E. coli* J96 at the concentrations of OD_{600} of (A) ~10^{-7}, (B) ~10^{-8}, and (C) 0 prepared in PBS buffer (10 mM, pH 7.5) obtained after enriched by MALCY-probe followed by releasing the bacteria trapped on the probe to ammonium acetate buffer (pH 4.5, 8 μL) under shaking for 15 min, followed by addition H_2O_2 (100 mM, 1 μL), and TMB (1 mg mL^{-1}, 1 μL) and standing for another 15 min prior to MS analysis. The resultant samples were 100-fold diluted with ethanol/deionized water (2:1, v/v) prior to C-API-MS analysis. One droplet of the resultant samples (5 μL) were used for MS analysis.

References

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