Analytical Biochemistry 394 (2009) 1–6

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Disposable bioluminescence-based biosensor for detection of bacterial count in food

Jinping Luo [a,b], Xiaohong Liu [a,b], Qing Tian [a,b], Weiwei Yue [a,b], Jing Zeng [c], Guangquan Chen [c], Xinxia Cai [a,b,*]

[a] State Key Laboratory of Transducer Technology, Institute of Electronics, Chinese Academy of Sciences, Beijing 100080, PR China
[b] Graduate School of Chinese Academy of Sciences, Beijing 100080, PR China
[c] Beijing Entry–Exit Inspection and Quarantine Bureau, Beijing 100026, PR China

ARTICLE INFO

Article history:
Received 4 March 2008
Available online 21 May 2009

Keywords:
Disposable biosensor
ATP bioluminescence
Rapid detection
Bacterial count

ABSTRACT

A biosensor for rapid detection of bacterial count based on adenosine 5′-triphosphate (ATP) bioluminescence has been developed. The biosensor is composed of a key sensitive element and a photomultiplier tube used as a detector element. The disposable sensitive element consists of a sampler, a cartridge where intracellular ATP is chemically extracted from bacteria, and a microtube where the extracted ATP reacts with the luciferin–luciferase reagent to produce bioluminescence. The bioluminescence signal is transformed into relevant electrical signal by the detector and further measured with a homemade luminometer. Parameters affecting the amount of the extracted ATP, including the types of ATP extractants, the concentrations of ATP extractant, and the relevant neutralizing reagent, were optimized. Under the optimal experimental conditions, the biosensor showed a linear response to standard bacteria in a concentration range from 10^3 to 10^8 colony-forming units (CFU) per milliliter with a correlation coefficient of 0.925 ($n = 22$) within 5 min. Moreover, the bacterial count of real food samples obtained by the biosensor correlated well with those by the conventional plate count method. The proposed biosensor, with characteristics of low cost, easy operation, and fast response, provides potential application to rapid evaluation of bacterial contamination in the food industry, environment monitoring, and other fields.

To effectively ensure food quality and safety, the hazard analysis and critical control point (HACCP)[1] system, which focuses on prevention and control and is advocated for every stage in the food chain from primary producer to final consumer, has been introduced to the food industry [1–3]. In most critical control points, the detection of bacterial count is the most frequently performed item to monitor bacterial contamination. Conventionally, the bacterial count can be detected by the culturing method, which is very effective but unsatisfactory for the HACCP system due to various operations and an incubation time longer than 24 h. Therefore, it is very indispensable for developing a rapid method to detect the bacterial count in food.

During recent years, many methods for rapid detection of bacterial count, including impedimetric detection, adenosine 5′-triphosphate (ATP) bioluminescence, cell counting, and automated immunoassays, have been proposed [4–8]. Among these methods, ATP bioluminescence is very suitable for on-line monitoring of bacterial contamination in the HACCP system due to no need for a culturing step or large equipment to fulfill the measurement. The ATP bioluminescence assay relies on the fact that ATP could reflect the existence of living microorganisms because ATP is a major biological energy source existing in various bacteria with reasonably constant content of approximately 0.47 fg/cell [9]. Catalyzed by firefly luciferase, the intracellular ATP released from bacterial cells reacts with luciferin to produce bioluminescence, and the bioluminescence intensity is proportional to the ATP concentration that is directly related to the amounts of living microorganisms. Since Sharpe and coworkers first applied the ATP bioluminescence assay to measure microorganism in food during the 1970s (see Ref. [10]), there has been increasing research interest in applications of ATP bioluminescence to evaluating bacterial contamination [11–18]. Several commercial ATP bioluminescence systems have been successfully employed as a hygiene monitor in the HACCP system in situ [19]. However, most of them have costly reagent kits and complicated operations in eliminating the interference from nonbacterial ATP; therefore, they are limited in their applications to the whole food industry.

In this work, a disposable biosensor based on the ATP bioluminescence assay was developed. Combined with the chemical method of ATP extraction, the biosensor detects bacterial ATP with a homemade luminometer [20]. The performance of the biosensor

* Corresponding author. Address: State Key Laboratory of Transducer Technology, Institute of Electronics, Chinese Academy of Sciences, Beijing 100080, PR China. Fax: +86 10 58887172.
E-mail address: xxcai@mail.ie.ac.cn (X. Cai).

[1] *Abbreviations used:* HACCP, hazard analysis and critical control point; ATP, adenosine 5′-triphosphate; TCA, trichloroacetic acid; CTAB, cetyltrimethyl ammonium bromide; SDS, sodium dodecyl sulfate; β-CD, β-cyclodextrin; BRA, bacterial releasing reagent; BSA, bovine serum albumin; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; PMT, photomultiplier tube; CFU, colony-forming units; RLU, relative light units; CV, coefficient of variation.

0003-2697/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2009.05.021

and its application to real food samples were investigated. The results demonstrate that our proposed biosensor can detect the bacterial count in food within 5 min.

Materials and methods

Reagents and specimens

Firefly luciferase (14.9 mg/ml, EC 1.13.12.7) and d-luciferin were purchased from Promega (USA). ATP, apyrase (342 U/mg, EC 232-569-8), and magnesium acetate were obtained from Sigma (USA). Trichloroacetic acid (TCA), cetyltrimethyl ammonium bromide (CTAB), sodium dodecyl sulfate (SDS), and β-cyclodextrin (β-CD) were obtained from Guoyao Chemical (China), and a commercial bacterial releasing reagent (BRA) was obtained from Hygiena (UK). Other chemicals, such as bovine serum albumin (BSA), dithiothreitol (DTT), and ethylenediaminetetraacetic acid (EDTA), were of analytical grade and used without further purification. Most solutions were prepared with Tris–acetate buffer (25 mM, pH 7.8) except the ATP extractant solutions prepared with 2 mM EDTA and 25 mM Tris–acetate (pH 7.8). The luciferin–luciferase solution contained 0.075 mg/ml luciferase, 0.25 mg/ml d-luciferin, 2.5 mM EDTA, 25 mM Mg^{2+}, 2.5 mg/ml BSA, 2.5 mM DTT, and 25 mM Tris–acetate (pH 7.8) and was used as the luminescence reagent. All of the solutions were stored at 4 °C prior to use.

Escherichia coli (ATCC 44113), *Staphylococcus aureus* (ATCC 26001), and beef juice, a type of condiment that is an extractive from beef after decoction with spice and solidification, were provided by the Food Safety Testing Center of the Beijing Entry–Exit Inspection and Quarantine Bureau (China). For experimental use, *E. coli* and *S. aureus* were cultured overnight at 35 °C in buffered peptone water with continuous shaking (250 rpm). Beef juice (25 g) was mixed with 225 ml of NaCl (0.09%, w/w) in a bag filter and homogenized using a BagMixer (Interscience, France). Then the beef juice solution contaminated with the above bacteria cultures was further diluted 10-fold consecutively with the NaCl solution to form a series of test food samples with different bacteria concentrations.

Construction of bioluminescence-based biosensor

As shown in Fig. 1, the proposed bioluminescence-based biosensor contains two parts: a homemade sensitive element and a photomultiplier tube (PMT, module H5773-02, Hamamatsu, Japan) used as detector element. They are enveloped in a dark chamber to isolate light outside. The sensitive element is the key part to produce a measurable bioluminescence, and it is disposable for the sake of low cost and easy operation. The sensitive element is designed on the basis of the bioluminescence ATP assay combined with a chemical method of ATP extraction. It is composed of a sampler, a cartridge, and a microtube. The three parts are linked up through a screw thread in a coaxial suite mode. The sampler consists of a handle, a localizing ring to restrict the sampler, a long screw, and a swab for sampling. To eliminate any free ATP or somatic ATP in samples, the swab is wetted in advance with Tris–acetate containing Triton X-100 (0.03%, w/v) and apyrase (4 U/ml). In the bottom of the cartridge, ATP extractant (150 μl) and the neutralizing reagent (150 μl) are sealed and separated by aluminum foil. The luminescence reagent stored in the microtube can react with ATP to produce bioluminescence. All of the above parts of the sensitive element are made from material compatible with food, such as propylene and polycarbonate, using a fabrication method called injection molding technology. In addition, the microtube is required to be transparent for bioluminescence detection.

The detection principle of the biosensor is can be introduced simply as follows. Once a sample gets to the sampler through swabbing or dropping, the free ATP or somatic ATP in the sample is hydrolyzed. As the sampler is down-spinned continuously, the intracellular ATP is released from bacterial cells by the ATP extractant in a few minutes and the residual extractant is subsequently consumed by the neutralizing reagent. When the lowest aluminum foil is torn, most of the solution containing the intracellular ATP drops into the microtube and the ATP quickly reacts with the luminescence reagent to produce bioluminescence. Then the bioluminescence signal is translated to relevant electrical signal by the PMT and further detected. Because the bioluminescence intensity is proportional to the concentration of the intracellular ATP, the bacterial count of the sample can be easily quantified by measuring the bioluminescence with a portable luminometer. Therefore, the proposed biosensor is appropriate for being applied to rapid detection of bacterial count in many fields, especially in situ.

Optimization of experimental conditions

The ATP extraction conditions affecting the sensitivity of the biosensor were optimized in terms of the concentrations of the

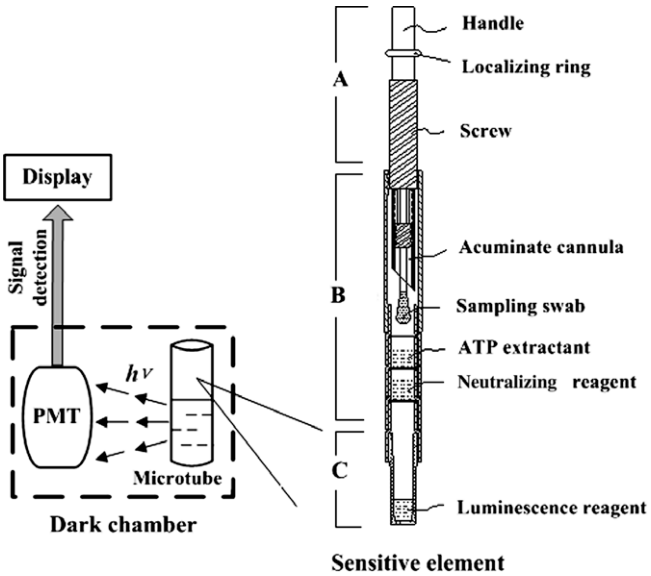

Fig. 1. Block diagram of the bioluminescence-based biosensor designed for rapid detection of bacterial count: (A) sampler; (B) cartridge; (C) microtube.

ATP extractant and the neutralizing reagent. The *E. coli* culture (50 μl) was mixed with 150 μl of the ATP extractant in the microtube for 2 min. After the sequential addition of the neutralizing reagent (150 μl) and the luminescence reagent (100 μl) into the mixture, the ATP extracted from the bacteria reacted with the luminescence agent to produce bioluminescence. The bioluminescence was obtained on a Hitachi F4500 fluorescence spectrophotometer with emission at 550 nm. Through comparing the intensities of the bioluminescence under different ATP extraction conditions, the concentrations of the ATP extractant and the neutralizing reagent were then optimized.

Cultivable plate count

The bacteria culture or food sample was serially diluted by 10-fold with the NaCl solution. For aerobic colony counts, 1-ml aliquots of the diluted sample were pour-plated onto tryptic soy agar (Beijing Luqiao, China) in duplicate and then incubated in an Incucell instrument (MMM, Germany) at 35 °C. After incubation for 24 h, the colonies developed on the plate were counted automatically by an aCOLyte apparatus (Synbiosis, UK) and the result was expressed in colony-forming units (CFU) per milliliter.

Photometry analysis

Under the optimal conditions, the disposable biosensor was prepared and sterilized prior to use. The biosensor was used to detect microbial count in samples with the homemade luminometer. *E. coli, S. aureus,* and real food (beef juice) were chosen as models for demonstrating the application of the proposed biosensor. The detection procedure involved three steps. First, 50 μl of the test sample was added to the wet swab of the sampler, and the sampler was enclosed in the cartridge within 1.5 min. Then the sampler was continuously down-spinned until the swab pierced the first aluminum foil and immersed in the upper solution of the cartridge. After 2 min of quiet placement, the sampler was down-spinned continuously until its localizing ring touched the top of the cartridge. The intracellular ATP was released from bacterial cells by the ATP extractant, and the residual extractant was subsequently consumed by the neutralizing reagent. Finally, the biosensor was shaken twice and immediately put into the vessel of the homemade luminometer. The intensity of the bioluminescence produced from the reaction between the ATP and the luminescence reagent was measured by the luminometer. The result was soon obtained in the form of relative light units (RLU) or CFU per milliliter according to detection demand.

Results and discussion

Choice of ATP extraction method

The method for extracting ATP from living cells has significant influence on the performance of the bioluminescence-based biosensor. Recently, various methods for ATP extraction have been developed, including continuous dc voltage [21,22], microwave [23], ultrasound [24], organic solvent [25], strong acid [26–28], and surfactant [29]. In the current study, acid and surfactant were examined as ATP extractants because the residual extractant could be easily consumed to eliminate the inhibition to firefly luciferase. In addition, this simple extraction method is compatible with the minimized biosensor.

In the experiment, four common reagents (150 μl) were added to the culture of *E. coli* or *S. aureus* (50 μl) to lyse bacterial cells, and the bacterial counts of the solutions were measured by the plate count method. The values of lysing efficiency η are estimated by Eq. (1):

$$\eta = \frac{(C_0 - C)}{C_0} \times 100\%, \tag{1}$$

where C_0 and C are the bacterial count of the solutions before and after lysis, respectively, and η is the lysing efficiency of each reagent to standard bacteria.

The results are given in Table 1. Compared with the solution lysed by SDS, no visible bacteria colony was observed in the plates of the solution lysed by TCA, CTAB, or BRA, indicating that TCA, CTAB, and BRA completely lysed the bacteria, whereas SDS partially did so. Because the intracellular ATP is easily dissociated from those lysed bacterial cells, both TCA and CTAB could be used to extract ATP from bacterial cells. The problem is that the residual TCA and CTAB inhibit the luciferase after ATP extraction. The residual TCA can be diluted with a buffer or neutralized with an alkali. However, the diluting process will complicate the detection and the alkali will decrease the luciferase activity. When CTAB is used to extract microbial ATP, the residual CTAB can be easily neutralized by β-CD to form an inclusion compound [30,31]. In addition, β-CD will not inhibit the luciferase because it is inert with enzymes and intracellular ATP. Hence, we selected CTAB as the proper ATP extractant and β-CD as the relevant neutralizing reagent for all experiments.

Optimization of ATP extraction conditions

The concentration of the ATP extractant shows a strong effect on the response of the biosensor. The effect of the concentration of CTAB on the bioluminescence responses was studied over the concentration range of 0 to 15 mM, whereas the β-CD concentration was 5 mM. The results are shown in Fig. 2. It is obvious that the bioluminescence intensity of the solution with CTAB is far higher than that of the solution without CTAB, demonstrating that the bacterial ATP is effectively extracted from cells by CTAB. At the constant concentration of β-CD, the bioluminescence intensity increases by increasing the concentration of CTAB but decreases dramatically at a concentration of CTAB above 5 mM. This is because the residual CTAB is not completely consumed by β-CD at the higher concentration of CTAB and the excessive CTAB begins to inactivate the luciferase, resulting in the sharp decrease of the

Table 1
Lysing efficiency of four ATP extractants to the standard bacteria culture.

Reagent	Culture of *E. coli*			Culture of *S. aureus*		
	$10^{-7} \times C_0$ (CFU/ml)	$10^{-7} \times C$ (CFU/ml)	η (%)	$10^{-7} \times C_0$ (CFU/ml)	$10^{-7} \times C$ (CFU/ml)	η (%)
TCA	10.2	—	100.0	8.20	—	100.0
CTAB	10.2	—	100.0	8.20	—	100.0
SDS	10.2	1.20	88.2	8.20	1.24	84.9
BRA	10.2	—	100.0	8.20	—	100.0

Note: —, no visible microbe colony in plates of each diluted solution after being incubated overnight.

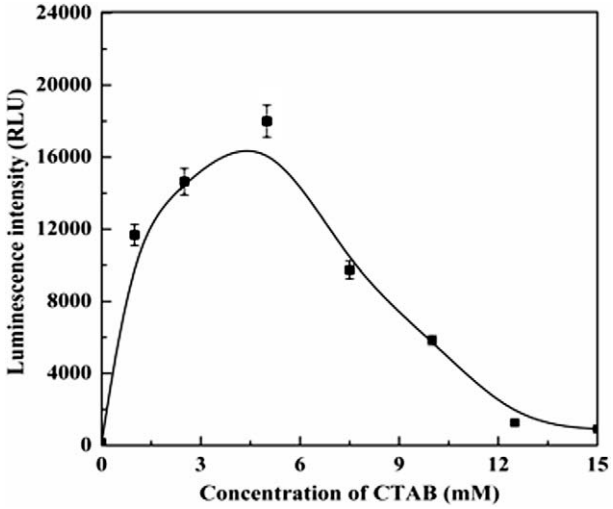

Fig. 2. Effect of concentration of CTAB as ATP extractant on intracellular ATP bioluminescence response. The bacterial count of detected *E. coli* suspension was 8.99×10^6 CFU/ml. The concentration of β-CD was 5 mM. The ATP extraction time was 2 min.

detected bioluminescence. Thus, 5 mM CTAB was used to extract bacterial ATP in the following studies.

To improve the sensitivity of the biosensor, the experiment was further performed to optimize the concentration of β-CD, where the β-CD concentration changed from 0 to 15 mM at the optimal concentration of CTAB. Fig. 3 shows the effect of the β-CD concentration on the bioluminescence responses. It can be seen that the bioluminescence intensity increases with the increase of β-CD concentration, approaching a maximum response at the concentration of 7.5 mM. But the bioluminescence intensity begins to decrease when the β-CD concentration is above 5 mM, probably because part of luciferin is consumed by excessive β-CD to form luciferin/β-CD complex [32]. Therefore, the optimal concentrations of CTAB and β-CD were 5 and 7.5 mM, respectively.

Calibration curve of the biosensor

To calibrate the proposed biosensor, the bioluminescence intensities of various standard bacteria suspensions were examined by the homemade portable luminometer. The experimental results are presented in Fig. 4. According to Figs. 4A and B, the calibration curves show that the disposable biosensor has a linear response to *E. coli* and *S. aureus*, respectively, at a concentration range from 10^3 to 10^8 CFU/ml. The regression equations derived in a log–log relationship are given as

$$\log(I_1) = 0.398 \log(C_1) + 1.760 \quad (2)$$

$$\log(I_2) = 0.376 \log(C_2) + 1.996, \quad (3)$$

where I_1 and I_2 are the bioluminescence intensity of the biosensor responding to *E. coli* and *S. aureus*, respectively, and C_1 and C_2 are the concentrations of the two above-mentioned bacteria obtained by the plate count method, respectively. It is obvious that the bioluminescence intensity is directly proportional to the amount of standard bacteria in a linear calibration range from 10^3 to 10^8 CFU/ml with a correlation coefficient higher than 0.920 ($n \geqslant 11$). The test time for the whole procedure, including the ATP extraction, was less than 5 min. The results indicate that the biosensor can be used for rapid evaluation of bacterial count in standard specimens.

Actually, real food samples are complex because they usually include gram-negative and gram-positive bacteria. Either equation derived from the detection results of a single bacterium is inapposite to describe actual bacterial counts in food samples due to the difference of ATP content in various bacteria [33]. Therefore, a regressive line was obtained by statistically analyzing all of the test data regardless of the type of standard bacteria (Fig. 4C). The equation for description of the regressive line is

$$\log(I) = 0.384 \log(C) + 1.892, \quad (4)$$

where I is the bioluminescence intensity of the biosensor and C is the bacterial count obtained by the plate count method. The empirical formula obtained from the regressive equation was used for practical application. After further analysis of the bioluminescence intensity by the program of the homemade luminometer, the bacterial counts in test samples were directly shown on the display screen of the luminometer.

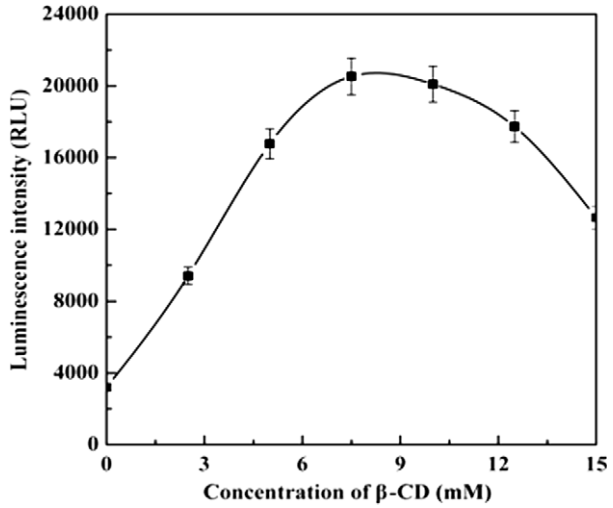

Fig. 3. Effect of concentration of β-CD as the neutralizing reagent on intracellular ATP bioluminescence response. The bacterial count of detected *E. coli* suspension was 3.48×10^7 CFU/ml. The concentration of CTAB was 5 mM. The ATP extraction time was 2 min.

Reproducibility of the biosensor

To evaluate the reproducibility of the biosensor, 14 biosensors were used for detection of *E. coli* suspension with a concentration of 1.37×10^7 CFU/ml premeasured by the plate count method. As shown in Fig. 5, the biosensor exhibits satisfactory reproducibility for detecting bacterial count with a coefficient of variation (CV) of 5.92% calculated by the I_A of 14 individual biosensors.

Analysis of food sample

A series of beef juice solutions were taken as test examples to verify the application of the proposed biosensor to detecting the bacterial count in real food samples. The samples were measured by the biosensor and the standard plate count method simultaneously. Fig. 6 presents the correlation curve between the experimental results of the above two methods. By means of linear regression, a good linear relationship was obtained between the bacterial counts examined by the two methods. The derived equation for describing the log–log relationship is given as below.

$$\log(C_B) = 1.234 \log(C_P) - 1.975, \quad (5)$$

where C_B and C_P are the bacterial counts obtained by the biosensor and the plate count method, respectively. It revealed that the data obtained from the biosensor correlated well with those obtained

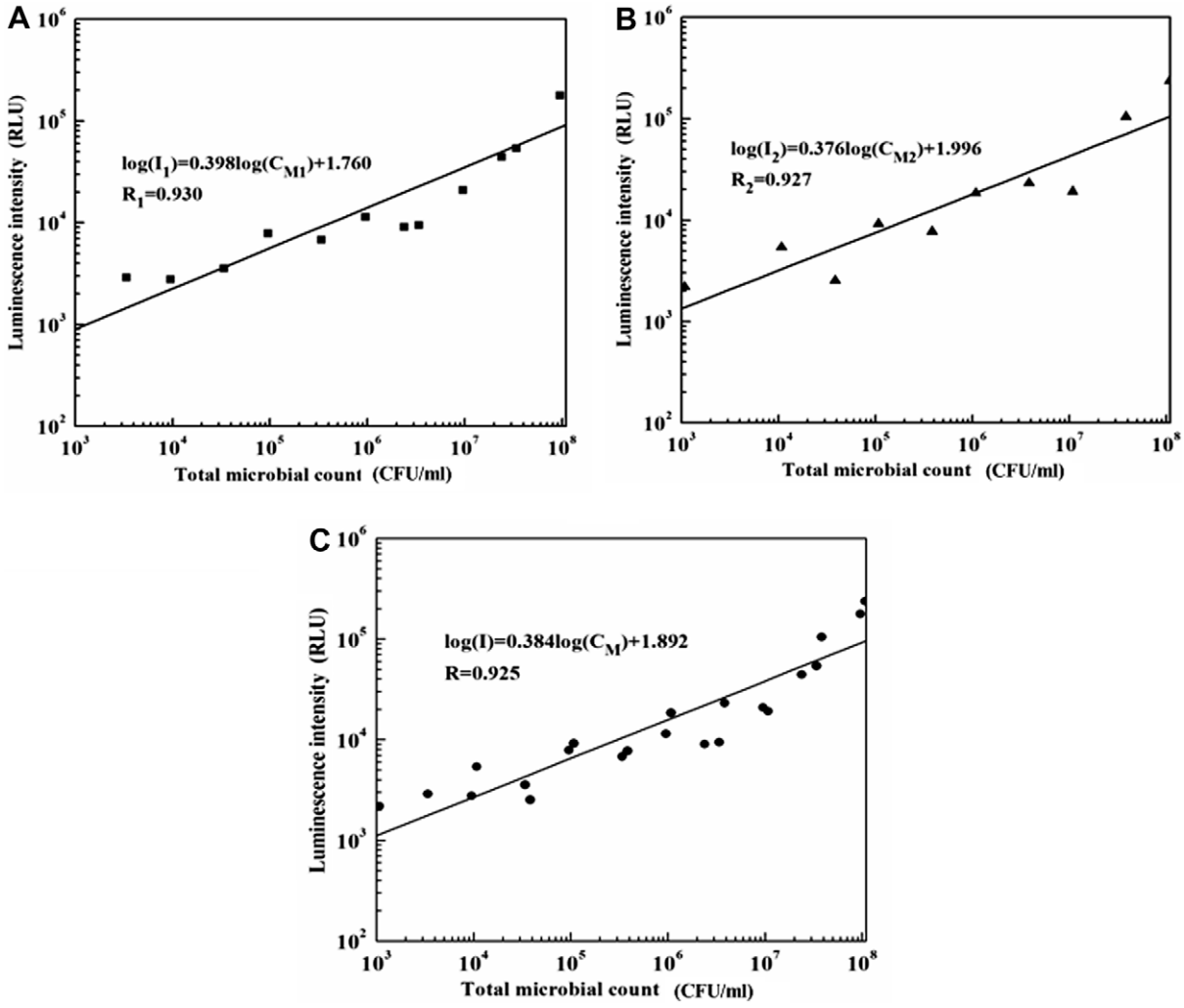

Fig. 4. Calibration curves of the biosensors for *E. coli* (A), *S. aureus* (B), and total bacterial count (C). The bacteria concentrations of the test suspensions ranged from 10^3 to10^8 CFU/ml. The ATP extraction time was 2 min, and the response time to ATP was less than 1 min.

from the plate count method in a linear calibration range from 10^3 to 10^8 CFU/ml with a correlation coefficient of 0.964. The preliminary results illustrate that the biosensor is capable of rapid detection of the bacterial count in food.

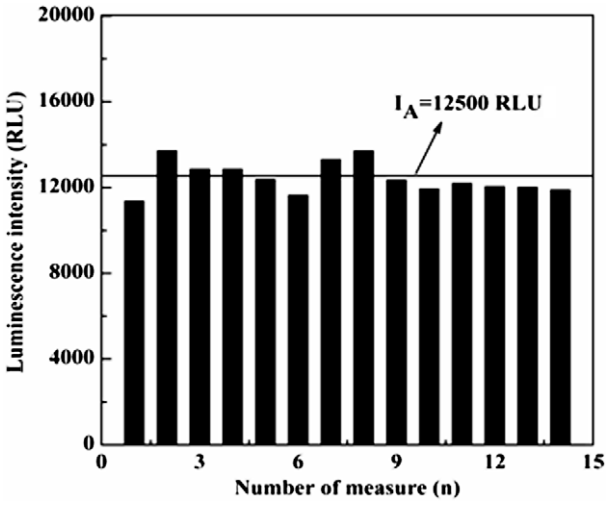

Fig. 5. Reproducibility of the biosensor for detecting 1.37×10^7 CFU/ml *E. coli* suspension. The ATP extraction time was 2 min, and the response time to ATP was less than 1 min. The experiments were repeated 14 times.

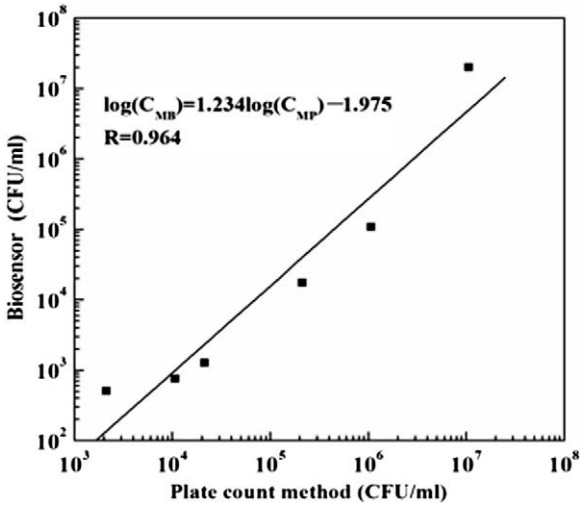

Fig. 6. Correlation curve between the bacterial count of real food samples detected by the biosensor and that detected by the plant count method. The bacterial counts of the test samples ranged from 10^3 to 10^8 CFU/ml.

Conclusion

Based on the bioluminescence ATP assay, a disposable biosensor for rapid detection of bacterial count has been developed. The biosensor is composed of a key sensitive element and a PMT used as detector element. The disposable sensitive element was designed in an integrated configuration involving the sampler, the ATP extraction, and the ATP detection, and each part can be obtained from industrial mass production. The optimized biosensor possesses characteristics of rapid response, wide detection range, and acceptable accuracy in detecting standard bacteria. In addition, the bacterial counts of various beef juice samples obtained from the biosensor correlated well with those from the plate count method, proving the ability of the biosensor in rapid detection of bacterial count in food. Our ongoing studies will continue to apply the biosensor to detecting other food and to evaluating bacterial contamination on surfaces in the food industry.

Acknowledgments

This work was supported by the Beijing Municipal Science and Technology Project (Z0006302040191), the Innovative Program of the Chinese Academy of Sciences (KGCX2-YW-111-2), the Major National Science and Technology Program (2008Z10003003-02), and the Hi-Tech Research and Development Program of China (2007AA03Z428).

References

[1] R.L. Buchanan, HACCP: a re-emerging approach to food safety, Trends Food Sci. Technol. 1 (1990) 104–106.
[2] S. Notermans, M.H. Zwietering, G.C. Mead, The HACCP concept: Identification of potentially hazardous micro-organisms, Food Microbiol. 11 (1994) 203–214.
[3] K. Ropkins, A.J. Beck, Evaluation of worldwide approaches to the use of HACCP to control food safety, Trends Food Sci. Technol. 11 (2000) 10–21.
[4] R. Firstenberg-Eden, J. Zindulis, Electrochemical changes in media due to microbial growth, J. Microbiol. Methods 2 (1984) 103–115.
[5] T. Kleiss, J. Albrecht, T. Putallaz, J.L. Cordier, Impedance measurement of the microbial flora in dairy-based desserts, J. Microbiol. Methods 22 (1995) 131–137.
[6] E.D. Boer, R.R. Beumer, Methodology for detection and typing of foodborne microorganisms, Int. J. Food Microbiol. 50 (1999) 119–130.
[7] D. Champiat, N. Matas, B. Monfort, H. Fraass, Applications of biochemiluminescence to HACCP, Luminescence 16 (2001) 193–198.
[8] J.M. Hawronskyj, J. Holah, ATP: a universal hygiene monitor, Trends Food Sci. Technol. 8 (1997) 79–84.
[9] A. Lundin, in: P.E. Stanley, B.J. McCarthy, R. Smither (Eds.), Rapid Methods in Microbiology, Blackwell Scientific, Oxford, UK, 1989, pp. 11–27.
[10] M. Griffiths, The role of ATP bioluminescence in the food industry: new light on old problems, Food Technol. 6 (1996) 62–69.
[11] C. Bell, C.D. Bowles, M.J.K. Toszeghy, P. Neaves, Development of a hygiene standard for raw milk based on the Lumac ATP bioluminescence method, Intl. Dairy J. 6 (1996) 709–713.
[12] J. Niza-Ribeiro, A.C. Louzã, P. Santos, M. Lima, Monitoring the microbiological quality of raw milk through the use of an ATP bioluminescence method, Food Control 11 (2000) 209–216.
[13] B.H. Shu, D.L. Sun, S.L. Wang, Rapid bioluminescence technique to detect bacteria in meat, Chin. J. Public Health 9 (2003) 483–484.
[14] D.A. Bautista, D.W. Sprung, S. Barbut, M.W. Griffiths, A sampling regime based on an ATP bioluminescence assay to assess the quality of poultry carcasses at critical control points during processing, Food Res. Int. 30 (1997) 803–806.
[15] S.J. Dexter, M. Cámara, M. Davies, K.M. Shakesheff, Development of a bioluminescent ATP assay to quantify mammalian and bacterial cell number from a mixed population, Biomaterials 24 (2003) 27–34.
[16] G.D. Hayes, A.J. Scallan, J.H.F. Wong, Applying statistical process control to monitor and evaluate the hazard analysis critical control point hygiene data, Food Control 8 (1997) 173–176.
[17] T. Sakakibara, S. Murakami, K. Imai, Enumeration of bacterial cell numbers by amplified firefly bioluminescence without cultivation, Anal. Biochem. 312 (2003) 48–56.
[18] D.J. Squirrell, R.L. Price, M.J. Murphy, Rapid and specific detection of bacteria using bioluminescence, Anal. Chim. Acta 457 (2002) 109–114.
[19] K.O. Colquhoun, S. Timms, C.R. Fricker, A simple method for the comparison of commercially available ATP hygiene-monitoring systems, J. Food Protect. 61 (1998) 499–501.
[20] A.Y. Zhou, J.P. Luo, W.W. Yue, B.S. He, Q.D. Yang, X.X. Cai, Development of handheld ATP detecting system based on bioluminescence, Chin. J. Sens. Actuat. 21 (2008) 543–546.
[21] D.W. Lee, Y.H. Cho, A continuous electrical cell lysis device using a low dc voltage for a cell transport and rupture, Sens. Actuat. B 124 (2007) 84–89.
[22] H.Y. Wang, A.K. Bhunia, C. Lu, A microfluidic flow-through device for high throughput electrical lysis of bacterial cells based on continuous dc voltage, Biosens. Bioelectron. 22 (2006) 582–588.
[23] T.S. Tsai, A microwave method for the extraction of cellular ATP, J. Biochem. Biophys. Methods 13 (1986) 343–346.
[24] K.A. Law, J.P. Derrick, S.P.J. Higson, Initial investigations into the ultrasonic lysis of microbial cells for the release of adenosine triphosphate, Anal. Biochem. 317 (2003) 266–267.
[25] N.A. Romanova, L.Y. Brovko, N.N. Ugarova, Comparative assessment of methods of intracellular ATP extraction from different types of microorganisms for bioluminescent determination of microbial cells, Appl. Biochem. Microbiol. 33 (1997) 306–311.
[26] W. Janaszek, J. Aleksandrowicz, D. Sitkiewicz, The use of the firefly bioluminescent reaction for the rapid detection and counting of mycobacterium BCG, J. Biol. Stand. 15 (1987) 11–16.
[27] A. Ishida, T. Yoshikawa, T. Nakazawa, T. Kamidate, Enhanced firefly bioluminescence assay of ATP in the presence of ATP extractants by using diethylaminoethyl–dextran, Anal. Biochem. 305 (2002) 236–241.
[28] M. Contin, M.D. Nobili, P.C. Brookes, Comparison of two methods for extraction of ATP from soil, Soil Biol. Biochem. 27 (1995) 1371–1376.
[29] A. Dukhovich, M.J. Rodriguez, L.G. Gaona, A. Levashov, N. Ugarova, Interaction of long-chain choline derivatives with firefly luciferase and their use as reagents for the extraction of intracellular ATP from microorganisms, Anal. Chim. Acta 303 (1995) 85–90.
[30] I. Satake, S. Yoshida, K. Hayakawa, T. Maeda, Y. Kusumoto, Conductometric determination of the association constants of β-cyclodextrin with amphiphilic ions, Bull. Chem. Soc. Jpn. 59 (1986) 3991–3993.
[31] R. Palepu, J.E. Richardson, Binding constants of β-cyclodextrin/surfactant inclusion by conductivity measurements, Langmuir 5 (1989) 218–221.
[32] A. Lundin, J. Anson, P. Kau, ATP extractants neutralised by cyclodextrins, Proc. Int. Symp. Bioluminesc. Chemiluminesc. 8 (1994) 399–402.
[33] N. Hattori, T. Sakakibara, N. Kajiyama, T. Igarashi, M. Maeda, S. Murakami, Enhanced microbial biomass assay using mutant luciferase resistant to benzalkonium chloride, Anal. Biochem. 319 (2003) 287–295.