INTEGRATED CASSETTE FOR COUNTING LOW-CONCENTRATION LIVE BACTERIA IN FOODS USING 3D STAINING TECHNOLOGY

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ABSTRACT

We have developed a rapid, easy-to-use and accurate live bacteria counter (BC) using the disposable cassette (55 mm x 55 mm x 13 mm), which is composed of tanks containing fluorescent dyes and the micro flow-cell (40 μm x 20 μm). The BC measures live bacteria count through staining live bacteria and detecting these by flow-cytometry technique. We applied 3D staining technology for distinguishing live bacteria from foreign fluorescent particles in foods. And we achieved that the lower detection limits are less than 10^3 (cell/ml) and the correlation factors between the BC and the cultural method are over 0.9.

KEYWORDS: Flow cytometry, Micro flow-cell, Multi staining, Bacteria test, Rapid testing, Food security

INTRODUCTION

As concerns mount over the security of foods, the importance to measure the live bacteria count is increasing. The cultural method is mainly used for measuring live bacteria count. However this method needs labor intensive and 24~48 hours for the incubation. We developed previously the bacteria counter (BC) with the disposable flow-cytometry cassette to rapidly and simply measure live bacteria count [1]. First prototype BC could measure live e-coli count in the concentration from $1.1x10^4$ to $3.3x10^5$ cell/ml, however it cannot distinguish live bacteria from noises of detector and foreign fluorescent substances (vegetable pigments and particles of dye) (Figure.1). It has to distinguish clearly live bacteria in foods and count these as accurately as the cultural method for the practical use [2]. In this paper, we newly developed the procedure and the system for distinguishing live bacteria from foreign fluorescent particles in foods by 3D staining technology in order to improve the detection limit under 10^3 cell/ml. Advanced version BC can count live bacteria accurately from 10^3 to 10^6 cell/ml in vegetables. This will absolve many inspectors from the laboring and time-consuming cultural method for counting live bacteria in foods.

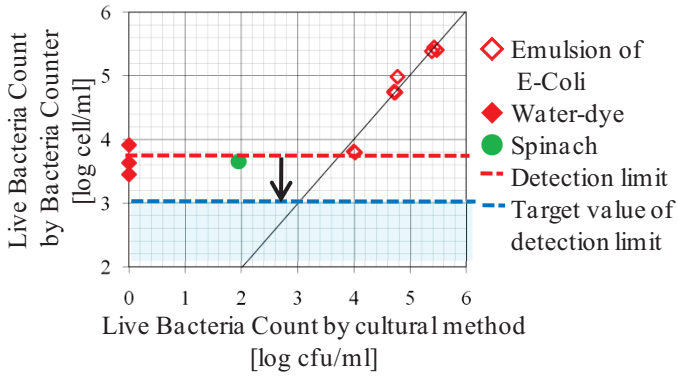
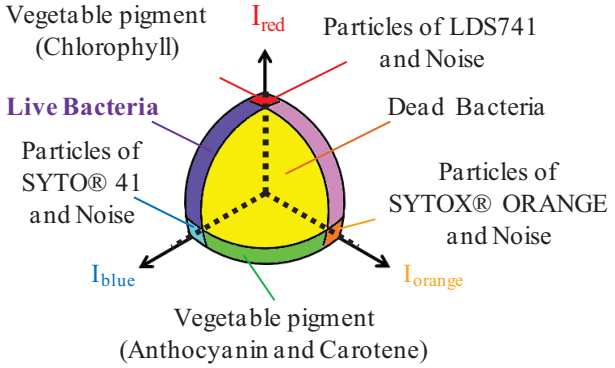


Figure.1: Correlation of live bacteria by the first prototype Bacteria Counter and cultural method
The first type bacteria counter measures more the live bacteria counts in the test sample of low concentration live bacteria than the cultural method.

3D STAINING TECHNOLOGY

We invented the 3D staining technology to distinguish live bacteria from foreign fluorescent substances (particles of fluorescent dyes and vegetable pigments) by fluorescence intensity (Figure.2). Chlorophyll has red and near-infrared fluorescence, meanwhile carotene and anthocyanine have blue and green fluorescence. We select the blue fluorescent dye (SYTO®41, Invitrogen : 450nm fluorescent peak) and the near-infrared fluorescent dye (LDS751: 720nm fluorescent peak) staining live and dead bacteria and the orange fluorescent dye (SYTOX® ORANGE, Invitrogen : 570nm fluorescent peak) staining only dead bacteria. The fluorescence of live bacteria stained with SYTO®41 and LDS751 is easy to be distinguished from one of foreign fluorescent substances by fluorescence intensity in three wavelength regions because only live bacteria emit blue and near-infrared fluorescence but not orange fluorescence .



I_{blue} : Intensity of fluorescence (430-470nm)
I_{orange} : Intensity of fluorescence (550-650nm)
I_{red} : Intensity of fluorescence (680-720nm)

Figure.2: Image of 3D staining technology

BACTERIA COUNTER

The common flow-cytometer enable users to count live bacteria rapidly and accurately, however, this is hard to operate for unskilled users and needs for users to pretreat the test sample (quantifying of the test sample and fluorescent dyes, removing residues of foods and staining bacteria) in advance of measuring live bacteria count (Figure.3). The BC automated the complicated process in the common flow-cytometer to measure live bacteria count in foods. Users simply have to inject the sample into the cassette, set the cassette in the BC and clicking the start button on the PC display and then users can get live bacteria counts in 50 min.

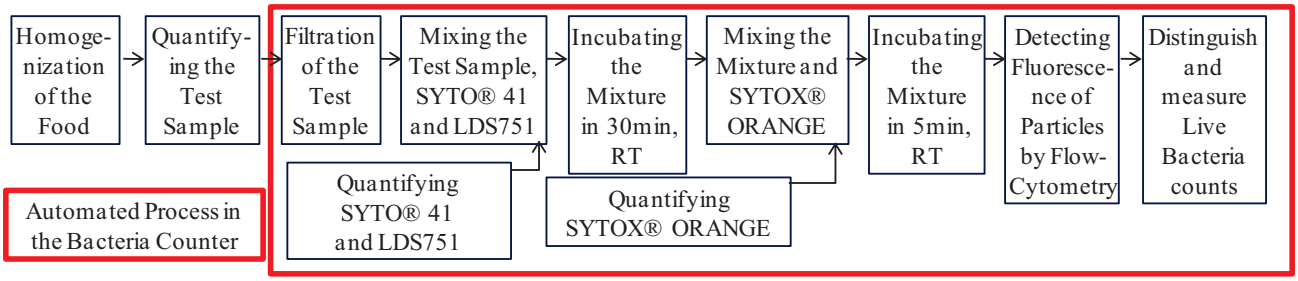


Figure.3: Process of counting live bacteria by common flow-cytometry

Figure.4 shows the picture of the BC and cassettes. The BC is composed of the flowage control unit and the bacterial detection unit. The flowage control unit pneumatically controls the process of the sample treatment and the bacterial detection unit detects fluorescence in three wavelength regions from bacterium or foreign fluorescent substances. The cassette (55mm x 55mm x 15mm) has functions to measure live bacteria count. The injected sample is flowed pneumatically along arrow lines. Live and dead bacteria are stained in staining tanks. And live bacteria stained are counted in the micro flow-cell (40μm x 20μm) by flow-cytometry technique.

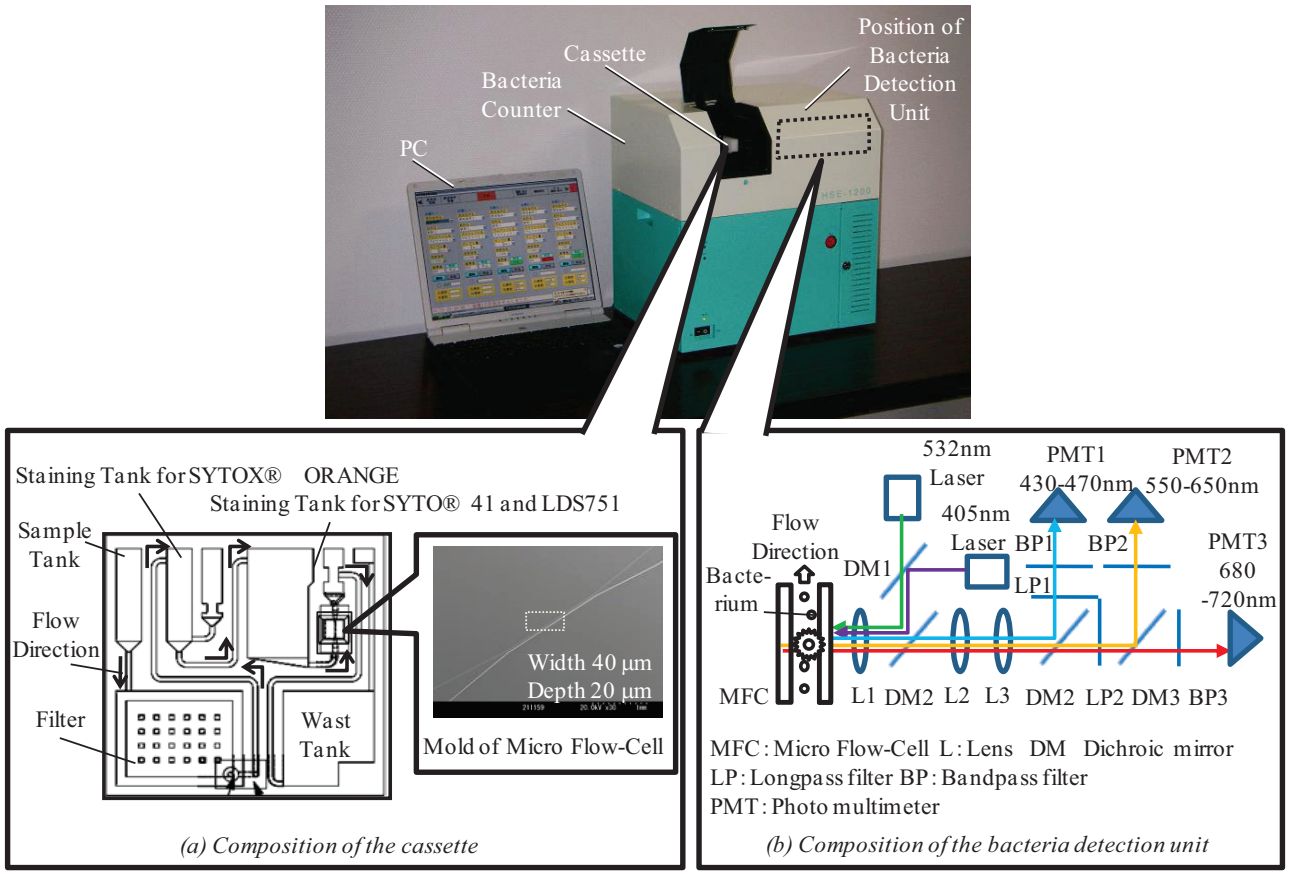


Figure.4: Advanced version Bacteria Counter

(a) *Cassette : The cassette is made of polystyrene, but only the micro flow-cell is composed of double layer of the cover glass (thickness 100 mm) and the grooved PDMS (polydimethylsiloxane) sheet (thickness 500 mm) to reduce the background fluorescence from the material of the micro flow-cell.*

(b) *Bacteria Detection Unit : Bacteria detection unit is composed of the blue laser (405 nm) exciting SYTO®41, the green laser (532nm) exciting SYTOX® ORANGE and LDS751, photo multimeters detecting fluorescence of SYTO®41, SYTOX® ORANGE and LDS751, lenses and optical filters (bandpass filters, longpass filters and dichroic mirrors) separating fluorescence by the difference in wavelength.*

Features of this cassette are the following. All dyes are prepackaged in staining tanks and the waste sample where live bacteria measured is stored in the waste tank for the convenience of use and it uses the narrow straight flow-cell without a sheath-flow enveloping the test sample in a coaxial flow [3] for simplifying the cassette configuration and reducing the production cost.

RESULTS AND DISCUSSION

To evaluate the 3D staining technology to distinguish live bacteria from foreign fluorescent substances (particles of fluorescent dyes and vegetable pigments), we measured live bacteria count in dilution series of the emulsion of e-coli (NT9001) and the homogenized vegetables (spinach, carrot and red cabbage) by the advanced version BC and the cultural method.

Figure.5 shows that the advanced version BC can distinguish more clearly live bacteria from particles of dyes and measure more accurately live bacteria count as compared to the first prototype BC. The measured counts of the water-dye indicates the measurement error because this is the mixed liquid of the water and dyes and doesn't include live bacteria. The measurement error of advanced version BC is less than 10^2 (cell/ml) while one of the first prototype is more than 10^3 (cell/ml), less than 10^4 (cell/ml). The lower detection limit of the advanced version BC is 10^3 (cell/ml) and the correlation factor R^2 between the live bacteria count measured by the advanced version BC and one by the cultural method is high (R^2(e-coli) = 0.97).

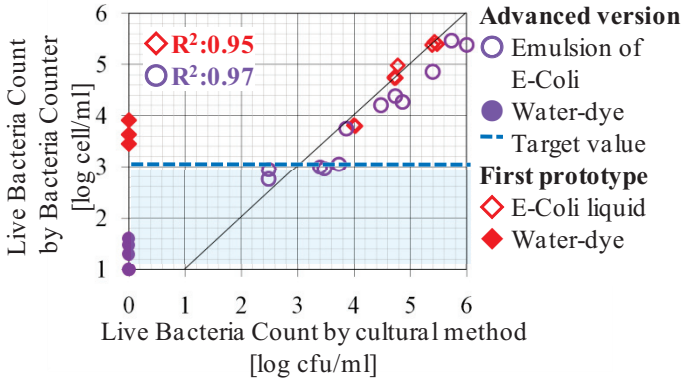


Figure. 5 :
Correlation of live bacteria count in emulsion of e-coli

Figure.6 shows that the advanced version BC can distinguish more clearly live bacteria from vegetable pigments and measure more accurately live bacteria count as compared to the first prototype BC. The live bacteria count in the homogenized spinach by the first prototype BC is 50 times more than one by the cultural method because this can't distinguish live bacteria from pigments of the spinach. Meanwhile one by the advanced version BC and one by the cultural method are almost same. Moreover, the advanced BC can measure accurately live bacteria count in the homogenized carrot and red cabbage. The lower detection limit of the advanced version BC is 10^3 (cell/ml) and the correlation factor R^2 between the live bacteria count measured by the advanced version BC and one by the cultural method is high (R^2(spinach) = 0.96, R^2(carrot) =0.91 and R^2(red cabbage) =0.90).

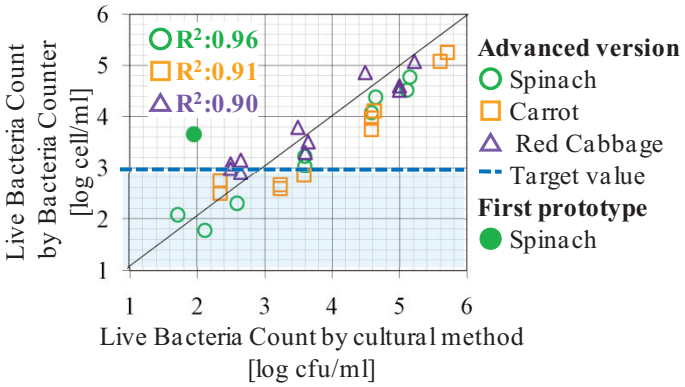


Figure. 6 :
Correlation of live bacteria count in homogenized vegetables

CONCLUSION

As concerns mount over the security for foods, the importance to check the live bacteria counts is increasing. However the cultural method commonly used for measuring live bacteria counts needs labor intensive and the long time for incubation. We have developed a rapid, easy-to-use and accurate live bacteria counter (BC) using the disposable cassette which is composed of tanks containing fluorescent dyes and the micro flow-cell. Applied 3D staining technology for distinguishing live bacteria from foreign fluorescent particles, the BC can measure low-concentration live bacteria accurately. The lower detection limit of the BC is less than 10^3 (cell/ml) and the correlation factor between the BC and the cultural method is over 0.9 in the emulsion of E-Coli and homogenized vegetables. We think the BC has a large potential of changing bacteria counts test for the food .

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