ELSEVIER

Food Microbiology 22 (2005) 595–599

FOOD MICROBIOLOGY

www.elsevier.com/locate/fm

Short communication

Modified Microtiter count method for viable cell counts from pure cultures and food model samples

Sol Kim*,[1], Daniel Y.C. Fung

Food Microbiology Laboratory, Department of Animal Sciences and Industry, Kansas State University, Manhattan, Kansas 66506-1600, USA

Received 4 August 2004; received in revised form 5 November 2004; accepted 5 November 2004

Abstract

A modified Microtiter count method was designed to assess viable cell counts in a rapid, easy, and accurate way. A Spiral plate method was used for comparison with the modified Microtiter count method. There was a strong correlation between the two methods in microbial counts from pure cultures (*Escherichia coli* O157:H7, $R^2 = 0.984$; *Salmonella enterica* serovar Enteritidis, $R^2 = 0.995$; and *Listeria monocytogenes*, $R^2 = 0.994$) and food samples ($R^2 = 0.897$). This study suggests that a modified Microtiter count method can be used to determine viable bacterial cell counts.

Keywords: Microtiter count; Spiral plate; Viable cell counts

1. Introduction

It is necessary to ascertain total viable cell counts in food, water, food contact surfaces, and air near food plants to provide information on food quality, food safety, and potential implications of foodborne pathogens. A conventional standard plate count (SPC) method has been used for assessing viable cell counts for several decades. This method is simple, but time-consuming and labor-intensive. It utilizes a large number of test tubes, pipettes, dilution buffers, sterile plates, and incubation space, requiring much time involved in clean-up and re-sterilization of reusable materials (Fung, 2000). In order to reduce these limitations, alternative approaches have been evaluated, including the Spiral plate system, the Iso-grid system, the Redigel system, and the Petrifilm system (Fung and Chain, 1991). These alternative methods were found to provide accurate aerobic bacterial counts from foods compared with the SPC method (Chain and Fung, 1991). Especially, the Spiral plate method has been extensively used with satisfactory results in viable cell counts from a variety of foods.

Fung and Kraft (1968) first developed a Microtiter method for miniaturization of viable cell count procedures. Subsequently, several reports have been published on this miniaturized system (Fung and LaGrange, 1969; Fung et al., 1976; Casas et al., 1977). The miniaturized technique utilizes Microtiter plates, dilution loops, multiple inoculation devices, and large Petri dishes. The method reduced the volume of reagents and media from about 5–10 ml in a test tube to less than 0.3 ml in wells of a Microtiter plate. Microtiter plates also have been used for a variety of microbiological studies. Fung and colleagues used this technique for carbohydrate tests (Fung and Miller, 1970), IMViC (Indole, Methyl red, Voges-Proskauer, and Citrate) tests (Fung and Miller, 1972), litmus milk tests (Fung and Miller, 1973), and other microbiological studies in relation to a variety of Gram-positive and Gram-negative bacteria of public health significance (Goldschmidt and Fung, 1978). In addition, this system

*Corresponding author. Tel.: +1 765 494 8273; fax: +1 765 494 7953.
E-mail address: kim98@purdue.edu (S. Kim).
[1]Present address: Department of Food Science, Purdue University, 745 Agriculture Mall Drive, West Lafayette, Indiana 47907-2009.

0740-0020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fm.2004.11.011

has been used for identification of fish pathogens (Chen et al., 1976), streptococci, lactobacilli, and Gram-negative rods from animal sources (Jayne-William, 1975, 1976), and anaerobic micro-organisms (Wilkins and Walker, 1975; Wilkins et al., 1975). Kang and Fung (1999) developed a 24-well Microtiter plate method for isolation of *Listeria* spp. from foods. Baldock et al. (1968) used a Microtiter technique to evaluate a large number of bacterial spore samples. Casas et al. (1977) ascertained the accuracy and benefits of the Microtiter count method in enumeration of mesophiles, psychrotrophs, and coliforms in raw and pasteurized milk.

The viable cell count procedure miniaturized by Fung and Kraft (1968) involves 10-fold dilution of samples in a Microtiter plate using a 0.025 ml-calibrated loop. After dilution, the samples are transferred and spot-plated on agar surface by a 0.025 ml-calibrated pipette. After incubation at 32 °C for 15–20 h, colonies in the spots are counted and the number of viable cells in the original sample is calculated. The accepted range of colonies in a spot is 10–100, while the standard range of a SPC method is 25–250 colonies per plate (Fung et al., 1976). The advantages of a Microtiter count method are: (a) reduction of space in operation and incubation; (b) saving of agar plates and Petri dishes; (c) no necessity of pre- and post-experiment manipulations, i.e., preparation of a large number of tubes for dilution, agar, and Petri dishes; and (d) reduction of operational time in terms of making dilutions and plating. Therefore, this method is proposed to be especially advantageous in samples of which cell density is expected to be high. The Microtiter count method was investigated as an AOAC collaborative study and found to be statistically acceptable for making viable cell counts of raw milk, compared with the SPC method (Fung et al., 1976). Nevertheless, the Microtiter count method has not been widely adopted for viable cell counts, but the potential for improvement and thereby wider use clearly exists.

The objective of this study was to evaluate a modified Microtiter count method designed to improve the speed and accuracy of dilutions using a multi-channel micropipette and to deliver more dilute samples on agar surface using a calibrated multiple-point inoculator.

2. Materials and methods

2.1. Cultures and media

Bacterial strains used in this study were obtained from the Food Microbiology Culture Collection at Kansas State University. The strains included *Escherichia coli* O157:H7 ATCC 35150, *Salmonella enterica* serovar Enteritidis USDA-FSCS 15060, and *Listeria monocytogenes* ATCC 19115. They were tested for purity using Gram reactions and biochemical tests: API 20E kit (bioMerieux, Hazelwood, Missouri) for *E. coli* O157:H7 and *S.* Enteritidis, and BBL Crystal kit (Becton Dickinson, Sparks, Maryland) for *L. monocytogenes*. For a pure culture study, pathogen-specific selective agars were used: MacConkey Sorbitol Agar (MSA) for *E. coli* O157:H7, Xylose Lysine Desoxycholate (XLD) for *S.* Enteritidis, and Modified Oxford (MOX) for *L. monocytogenes*. For bacterial enumeration from food samples, Plate Count Agar (PCA) was used. All media were purchased from Becton Dickinson, Sparks, Maryland. The strains were subcultured twice in brain–heart infusion (BHI; Difco Co., Detroit, Michigan) broth medium at 35 °C prior to use.

2.2. Food samples

Meat and seafood products were used as food model samples for this study and purchased at a local grocery in Manhattan, KS, on two different days. They included beefsteak, pork, chicken (drumstick), ground beef, ground pork, ground chicken, ground turkey, Italian sausage, shrimp, and catfish. All food products were stored at 4 °C prior to use. Twenty-five gram portions of each product were placed in plastic stomacher bags including 225 ml of 0.1% sterile peptone-water, and pummeled for 2 min in a Stomacher (Lab Blender 400, A.J. Seward, London, UK). Two samples of each ten different food ($n = 20$) were assayed for viable cell counts of total aerobic bacteria in foods.

2.3. Modified Microtiter count method

All strains used in this study were individually incubated at 35 °C for 0, 3, 6, 12, and 24 h. A 0.3 ml volume of pure culture incubated for 0, 3, 6, 12, and 24 h each or 0.3 ml of diluted food sample was transferred into the first column of a sterile 96-well Microtiter plate (round bottom type, Dynatech Laboratories, Inc., Varginia), using a micropipette. From the second column to the last one, 0.27 ml of 0.1% sterile peptone-water was serially transferred in consecutive wells before dilution using a multi-channel micropipette (Brinkmann Transferpette™, Germany). Serial 10-fold dilution was made using a multi-channel micropipette. The process includes transferring 0.03 ml of the first column into the second column possessing 0.27 ml of 0.1% peptone-water, followed by drawing the solution up and down into the micropipette 3–4 times to mix the diluted samples. The same procedure was performed serially to the highest dilution level of 10^7. It is possible to dilute up to eight samples in a plate (12×8) or 12 samples by 90°-rotating a plate (8×12). A multi-point inoculator (12×8) was dipped into 70% EtOH for about 2–3 s, and then flamed until the alcohol was burned off by following the procedure of Fung et al. (1995). The sterilized inoculator was used to transfer