Journal of Microbiological Methods 76 (2009) 169–173

Contents lists available at ScienceDirect

Journal of Microbiological Methods

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

A novel method and simple apparatus for the detection of thermophilic *Campylobacter* spp. in chicken meat products

Sueptrakool Wisessombat [a], Kanokwan Kittiniyom [b], Potjanee Srimanote [c], Wijit Wonglumsom [b], Supayang P. Voravuthikunchai [a,*]

[a] Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
[b] Department of Clinical Microbiology, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand
[c] Faculty of Allied Health Science, Thammasart University, Patomthanee 12121, Thailand

ARTICLE INFO

Article history:
Received 8 August 2008
Received in revised form 3 October 2008
Accepted 7 October 2008
Available online 28 October 2008

Keywords:
Apparatus
Thermophilic *Campylobacter*
Chicken

ABSTRACT

Conventional procedures for isolation of thermophilic *Campylobacter* spp. from chicken are complex, labor intensive, and time-consuming. The objective of this study was to create a novel *Campylobacter* culturing apparatus. A main concept of the device was based on the ability of *Campylobacter* to pass through a 0.45 μm pore size filter in viscous media. Preliminary study demonstrated that only viable *Campylobacter* moved through the membrane filter and could multiply in the enrichment culture. *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* in the chicken samples were detected at cell concentrations as low as 10 cfu/g, after 24 h incubation at 42 °C. In total, 84 retail chicken samples were comparatively studied using both conventional method and apparatus. Sixteen samples (19.05%) were positive by the apparatus method; 14 (16.66%) of these positive samples contained *C. coli* and 2 (2.38%) contained *C. jejuni*. With the conventional method, 7 (8.33%) samples were positive 7 (8.33%) with *C. coli*. In conclusion, the apparatus detected more positive samples than did the conventional culture method.

1. Introduction

Thermotolerant *Campylobacter* spp, those species that grow at 42 °C, *C. jejuni* and *C. coli*, are most frequently isolated from diarrhoeal patients (Friedman et al., 2000). Poultry has been recognized as the primary reservoir of *Campylobacter* and play an important role in the transmission of *Campylobacter* enteritis to humans (Humphrey et al., 2007; Pearson et al., 2000). Other species associated with human illness include *C. lari* and *C. upsaliensis* (Allos, 2001; Lastovica and Skirrow, 2000).

Traditional methods for the isolation and identification of *Campylobacter* from food products require enrichment for 48 h and subculturing to selective agar, followed by phenotypic identification. This method takes up to 5 days in total to obtain a result (Corry et al., 1995). Many attempts are being made to develop more effective detection methods. This time-consuming and labor intensive procedure was replaced by PCR which is a rapid specific nucleic acid amplification method for the detection of food-borne pathogens. Adaptation of PCR assays were made by performing hybridization using blotting of PCR products (Gonzalez et al., 1997; van Doorn et al., 1997), PCR enzyme-linked immunosorbent assay procedure (Lawson et al., 1999; O'Sullivan et al., 2000), and real-time PCR (Lund et al., 2004; Nogva et al., 2000; Sails et al., 2003). However, these methods are very expensive and therefore not a feasible option for use in developing countries (Corry et al., 1995). In addition, all of these methods were unable to establish the viability of the organism.

In our study, the development of *Campylobacter* culturing apparatus on the basis of the motility ability of the organism for the detection of viable thermotolerant *Campylobacter* spp. is established. The system could permit more rapid and reliable detection of the pathogen. This system will be extremely useful for food manufactures. Furthermore, pathogen identification can provide important epidemiologic information that can aid in preventing further spread of the disease. Conventional cultural method was tested for a comparative study on a panel of bacterial strains isolated from foods.

2. Materials and methods

2.1. Bacterial strains

Reference strains including *C. jejuni* ATCC 33291, *C. jejuni* ATCC 81176, *C. coli* MUMT 18407, *C. coli* MUMT 18630, *C. lari* ATCC 43675, and *C. upsaliensis* DMST 19055 were used for the validation of the developed method. All *Campylobacter* spp. were cultured on charcoal cefoperazone desoxycholate agar (CCDA) (Oxoid, Basingstoke, UK). The plates were incubated at 42 °C for 24 h at microaerobic atmosphere with 5% O_2, 10% CO_2, and 85% N_2.*Campylobacter* were maintained in brain heart infusion broth (Difco, Detroit, Mich) supplemented with 15% glycerol at −70 °C.

* Corresponding author. Tel./fax: +66 7 444 66 61.
E-mail address: supayang.v@psu.ac.th (S.P. Voravuthikunchai).

0167-7012/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.mimet.2008.10.007

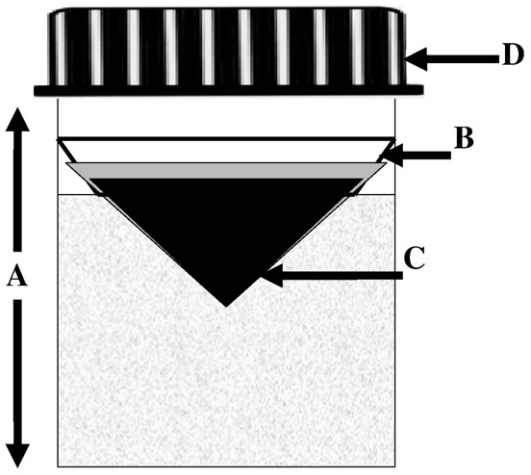

Fig. 1. Diagram of *Campylobacter* apparatus. Enrichment chamber (NL1201-0100; outside diameter, 62 mm; internal diameter, 53 mm; length, 70 mm) (Nalgene Co., Rochester, N.Y.) containing 90 ml of Bolton broth (A). A polypropylene funnel (NL4250-0055; maximum diameter, 60 mm; minimum diameter, 35 mm; length, 20 mm) (Nalgene) placed over the chamber (B). A cone shaped nitrocellulose membrane filter (0.45 μm) coating with Bolton broth containing 0.5% agar, then filled with 2 ml of Bolton broth without antibiotics (C). A lid was loosely fitted on the container (D).

2.2. Isolation of Campylobacter from food

A conventional method for the isolation of *Campylobacter* from food was performed according to Bacteriological Analytical Manual established by the US Food and Drug Authority (U.S. FDA, 2001). A total of 84 chicken samples from different portions including breasts, legs, and wings were tested. For analysis, 25 g slices were cut aseptically from the surface of the sample, at least 10 cm^2 of the skin was included. The sample was placed in a stomacher bag with 100 ml of Bolton broth (Oxoid) supplemented with 10 mg/l of amphotericin B, 20 mg/l of trimethoprim, 20 mg/l of vancomycin, and 32 mg/l of cefoperazone (Sigma-Aldrich, St. Louis, USA) and pummeled in a stomacher (Mayo, HG400V, Italy) for 1 min at 200 rpm. The isolation of *Campylobacter* spp. was carried out according to the procedure in the previous report (Hunt et al., 1998). In brief, the sample in the enrichment broth was incubated at 37 °C for 4 h, followed by further incubation at 42 °C for 24 h. After 24 h, the sample was streaked on CCDA supplemented with 10 mg/l of amphotericin B and 32 mg/l of cefoperazone (Sigma-Aldrich). The identification of *Campylobacter* was carried out by the observation on colony characteristics, microscopic morphology, and biochemical tests including oxidase reaction, catalase production, ability to hydrolyze sodium hippurate, and nalidixic acid sensitivity (Nachamkin, 2003).

2.3. Campylobacter enrichment apparatus

The apparatus was designed based on the ability of *Campylobacter* to move through a 0.45 μm pore size in a viscous condition. The apparatus was composed of two compartments connecting by a filter-containing division (Fig. 1). One compartment was for the inoculation of food samples while the other compartment was filled with *Campylobacter* enrichment broth containing oxygen-reducing agent. This apparatus was a closed system for the isolation of *Campylobacter* without gas-generating sachet.

Nitrocellulose membrane filter with 0.45 μm pore size (Millipore, Watford, UK) folded into a cone shape was autoclaved. The sterile filter paper was submerged for 1 min in Bolton broth containing 0.5% agar (Difco, Detroit, USA) at 50 °C to 60 °C. The filter paper was removed from the medium and placed in an upright position on a sterile petridish for 30 min to produce a soft-agar-coated membrane filter.

2.4. Evaluation of selectivity of Campylobacter enrichment apparatus

Each of the reference strains of the *Campylobacter* was inoculated into CCDA and incubated at 42 °C for 24 h. After incubation, a loopful of bacterial was taken and suspended in 1 ml of buffer peptone water (BPW). Ten milliliters of viable inoculum were added to 90 ml of Bolton broth in a sterile polypropylene plastic container to give the final cell numbers at 10–10^4 cfu/ml. For a non viable inoculum control, the bacterial cells were resuspended in 1 ml of saline containing 200 ppm sodium hypochlorite and incubated at room temperature for 4 h to kill all viable cells (Hayashi et al., 2006). Subsequently, the cell suspension was washed three times with saline and finally resuspended in 10 ml of BPW. A sterile polypropylene funnel with soft-agar-coated membrane filter was placed over the container. Two milliliters of Bolton broth was added into the cone, then a lid was loosely fitted over the container. The containers were then incubated at 37 °C and 42 °C for 6, 12, 18, and 24 h. To determine the number of viable *Campylobacter*, 1 ml of the culture in the cone was determined by a drop plate method. One hundred microliter of the bacterial suspension in each serial dilution was pipetted into a tube containing 900 μl of sterile BPW. From each dilution, 10 μl of the samples were transferred to CCDA supplemented with antibiotics. Plates were allowed to dry and incubated at 42 °C for 18–24 h. All measurements were performed in triplicate and all experiments were performed twice. The mean and standard error were calculated from the combined measurements.

2.5. Inoculated food

Inoculum preparation was carried out as described previously. Twenty five grams of chicken with or without 10 ml of the viable

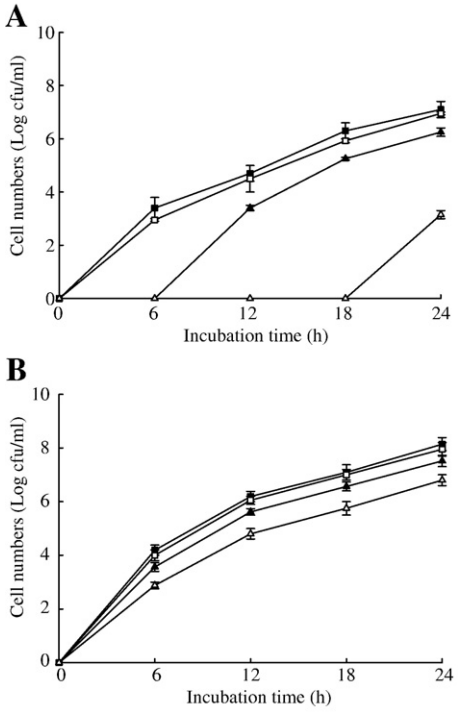

Fig. 2. The effect of temperature on the growth of *C. jejuni* strain ATCC 33291. The initial bacterial concentration of each inoculum was 10^4 (■), 10^3 (□), 10^2 (▲), and 10 (△) cfu/ml. After the inoculation of *C. jejuni* into the enrichment chamber, the apparatus was incubated at 37 °C (A), and 42 °C (B). Viable counts were determined on CCDA agar. The plates were incubated at 42 °C for 24 h under microaerobic conditions. All measurements were performed in triplicate and all experiments were performed twice. The mean and standard error were calculated from the combined measurements.