



Effect of swabbing techniques on the efficiency of bacterial recovery from food contact surfaces



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ABSTRACT

Four types of swab (cotton, gauze, polyurethane foam (PU foam) and cellulose sponge) were used to recover four food-borne pathogens (*Salmonella* Typhimurium, *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*) from stainless steel and polyester urethane (old and new) surfaces under wet and dry surface conditions. Characteristics of swabs and swab surfaces were analyzed. The cellulose sponge swab showed the highest bacterial release efficiency, followed by the PU foam, gauze and cotton swabs. The bacterial Gram type affected the efficiency of bacterial recovery on dry surfaces, but the surface type had no apparent effect on the swab efficiency. Swabbing on wet surfaces using PU foam or cellulose sponge yielded a higher efficiency than with gauze or cotton swabs. Swabbing on dry surfaces with cellulose sponge and cotton swabs showed the highest and lowest swab efficiency, respectively. Swabbing on a dry surface decreased the efficiency of all swab types to 30%. For recovery from bacterial biofilms, the swab efficiency was 40% lower than those of wet surfaces. The cellulose sponge and PU foam swabs had a higher percentage recovery of biofilm than gauze and cotton swabs. Thus, the swab type and surface condition can affect the swab efficiency, and choosing the appropriate type of swab for the surface condition will increase the swab efficiency.

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1. Introduction

The Thai Food export industry has the potential to increase as a result of the world market's demand, especially for frozen chicken meat and frozen processed chicken products (Department of International Trade Promotion, 2011). During processing, contamination with foodborne pathogens, such as *Listeria monocytogenes*, *Salmonella* Typhimurium, *Escherichia coli* and *Staphylococcus aureus*, causes the food to become unsafe for consumers and may lead to economic loss of the country due to product recalls and loss of importer trust (Keeratipibul, Oupaichit, & Techaruwichit, 2009; Thongraat, Kusum, & Bangtrakulnont, 1993).

Pathogen contamination in food products can be mainly caused by improper thermal processing, due to insufficient heating or poor

heat distribution, and post-contamination after thermal processing. Normally, the processing products after proper heating steps should not have any bacterial contamination. However, pathogen contamination is still frequently found in the finished products, indicating post-contamination after the heating processes. The pathogens can contaminate the finished products via direct- and/or indirect-contact of the food and contaminated surfaces of processing environments, such as equipment or machines, including the surfaces that are distant from the products, such as the floor and walls in the processing areas. The pathogens are often found attached on the food-contact surfaces of processing equipment, such as knives, cutting boards and the conveyor belts of dicing machines, and these are typically made of stainless steel, plastic and polyester urethane (PSU), respectively. The attachment of pathogens on these surfaces is caused from an initial seeding of bacterial cells adhering on the surfaces, which then excrete a slimy, glue-like substance of polysaccharides combined with water, nutrients, inorganic debris and other residues, to form a biofilm as

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they replicate and colonize the surface. Especially on porous surfaces (such as damaged or old plastic conveyor belts) or in the niches where it is difficult to access, the bacteria may persist and form a biofilm (Poulsen, 1999), after which routine cleaning may not eliminate them. To ensure the safety of the finished food product, it is necessary to control the cleanliness of the environmental surfaces in the processing areas. Swabbing is the most common technique used for monitoring pathogen contamination on surfaces and determining the sufficiency of the cleaning performance.

A common quantitative technique assessing bacterial contamination on environmental surfaces is based on traditional swabbing, using a plastic or wooden shaft with a cotton bud at the end. The principle is that the bacteria are transferred from the contact surface onto the swab, and subsequently from the swab into the enumeration medium (Moore & Griffith, 2002b). However, a significantly lower proportion of bacteria have been reported to be recovered from cotton swabs compared with the number of bacteria on the surfaces. An improper swabbing material or method may consequentially compromise the identification of contamination sources in the processing environment. Consequentially, the materials used for swabbing have been developed considerably so as to enhance the swab efficiency and ease of use in the food-processing factory.

The objective of this study was to identify factors of the swab technique, such as swab type, surface type (including surface roughness), surface condition and bacteria species, which impact on the proportion of bacteria recovered from food-contact surfaces. The results can subsequently be used to improve the bacterial pathogen-detecting efficiency in the food industry in the future.

2. Material and methods

2.1. Analysis of food contact surfaces and swab characteristics

Light microscopy (120 \times ; Nikon DXM1200F; Nikon, Tokyo, Japan) was used to visualize the surface characteristics of four different swab types of cotton, gauze, polyurethane (PU) foam and cellulose sponge (the last two from 3M Thailand Ltd., Bangkok, Thailand). The surface textures of three surface types (stainless steel 304, and new and 5-y old used PSU coupons) were three dimensionally imaged and analyzed for their surface roughness using Surfcom[®] 1400 (Tokyo Seimitsu Co., Ltd, Tokyo, Japan). The roughness analysis was replicated three times to calculate the average roughness value (Ra).

2.2. Analysis of bacterial release from different swabs by direct inoculation

The cotton, gauze, cellulose sponge and PU foam swabs were each inoculated with a suspension of 10^5 colony forming units (CFU) of *S. Typhimurium*, *S. aureus*, *E. coli* and *L. monocytogenes* (one per swab) in buffered 0.1% peptone water (BPW). The swab buds were cut from the stick, soaked in bacteria-free BPW and then vortexed for 1 min to release the bacteria from the swab buds. The BPW was used as control sample. Then, the amount of bacteria (log CFU/ml) in the solution was determined by the spread plate method using violet red bile agar with MUG (Becton Dickinson, Sparks, MD, USA), Baird-parker agar (Becton Dickinson, Sparks, MD, USA), xylose lysine deoxycholate agar (Becton Dickinson, Sparks, MD, USA) and PALCAM agar (Becton Dickinson, Sparks, MD, USA) plates as the selective agar for *E. coli*, *S. aureus*, *S. Typhimurium* and *L. monocytogenes*, respectively. The forming colonies were counted after 24–48 h of incubation at 30 °C for *L. monocytogenes* and 37 °C for the other three pathogens. Each assay was performed in

triplicate. The percentage bacterial release from the swabs was based on the average number of bacteria released compared with the average initial number of bacteria inoculated onto the swab buds.

2.3. Analysis of bacterial recovery from food contact surfaces by different swabs

The method was modified from the study of Moore and Griffith (2002a). Sterile coupons (10 \times 10 cm) of stainless steel 304, and 5-y old used and new PSU were inoculated with 100 μ L of 10^6 CFU/ml of the respective bacterial culture (*S. Typhimurium*, *S. aureus*, *E. coli* and *L. monocytogenes*), while the 30 \times 30 cm sterile coupons were inoculated with 1 ml of 10^5 CFU/ml of the cultures. Each inoculum (cell suspension) was spread evenly over the coupon surface with a sterile bent rod. Then, the swabs were immediately used to swab the inoculated coupons while the surface was still wet. The inoculated 10 \times 10 cm coupons were swabbed by pre-moistened cotton or PU foam swabs, while the 30 \times 30 cm coupons were swabbed by pre-moistened gauze and cellulose sponge swabs to collect the bacteria cells from the surfaces. In addition, after inoculation, the coupons were dried at room temperature in a safety cabinet for 1 h. Fresh swabs were used to rub the coupons when the surfaces were completely dried. All the swabs were then soaked in BPW, vortexed for 1 min and enumerated for the amount of bacteria (log CFU/coupon) by the spread plate method on the respective agar plates. The percentage bacterial recovery from each surface was assessed by comparing the average number of bacteria recovered from the surface with the average initial number of bacteria inoculated onto the surface.

2.3.1. Analysis of the swab efficiency for biofilms

The experiment was modified from the study of Chaturongkasumrit, Takahashi, Keeratipibul, Kuda, and Kimura (2011), where 200 μ l of 10^8 CFU/ml cultures of *S. Typhimurium*, *S. aureus*, *E. coli* and *L. monocytogenes* were inoculated on sterile coupons of 5 \times 5 cm of stainless steel 304, and 5-y old used and new PSU. The cultures (20 ml) were grown in modified Welshimer's broth for *L. monocytogenes* (Chaturongkasumrit et al., 2011), M63 broth for *S. Typhimurium* and *S. aureus* (Ausubel et al., 1994) and M9 broth for *E. coli* (Oh, Jo, Yang, & Park, 2007). The inoculated coupons in the cultures were then incubated at 30 °C for *L. monocytogenes* or 37 °C for the other three pathogens for 24 h to form biofilms. The coupons were then rinsed with 20 ml sterile distilled water three times and rubbed with the respective swab (cotton, gauze, PU foam or cellulose sponge) in different directions. The swabs were then soaked in BPW, vortexed for 1 min and the amount of bacteria in the BPW determined by the respective spread plate method. Moreover, the residual biofilms on the rubbed coupons were transferred into BPW using a scraper and determined for the amount of bacteria by the respective spread plate method. The swab efficiency was calculated by the average amount of bacteria in the suspension from the swabs divided by the sum of the average amount of bacteria in the suspension from the swabs plus the average amount of residual bacteria recovered from the scraped coupons.

2.3.2. Statistical analysis

To compare means of swab efficiency, the SPSS version 17.0 program was used for analysis of variance (ANOVA) and Duncan's multiple range test (DMRT).

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