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Adhesive microflora on stainless steel coupons in seafood processing plant

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Abstract: Adhering microflora profile is essential for the development and design of cleaning and sanitizing procedures, which is a part of the HACCP system. The profile provides a complete view of the target microorganisms that can potentially contaminate food products. The aim of this study is, therefore, focused on examination of microbial population adhered to the equipment surfaces at seafood processing plant. Adhering microflora was evaluated by installing the stainless steel coupons on the surface of equipment. Seventy stainless steel coupons were attached on 7 different locations at seafood processing plant and then fourteen coupons were collected at 1, 2, 3, 5 and 9 weeks interval. The population of bacteria adhered on samples were enumerated using swab test and spread plate method on different selective microbiological media. The total viable count and *Pseudomonas spp*. population found on the stainless steel coupons were ranged from 10^1 to 10^6cfu/cm^2. The results also indicated that mature biofilm might form at some locations after three weeks. After 5 weeks, the highest viable bacterial population (10^6cfu/cm^2) was observed on the stainless steel coupons attached in the trolley used for carrying the soup. These finding supported that the appropriate cleaning and sanitizing procedure should be strictly applied to assure safety in food processing plant.

Keywords: Biofilm Formation, Microflora, Stainless Steel Coupon, Seafood Processing

1. Introduction

A major goal for the food processing industry is production of good food both in terms of quality and safety of the consumer [1, 2]. To reach this goal, microorganisms associated with food spoilage and food borne disease should be controlled [1]. During processing and preservation techniques, a part of them are eliminated. However, the efficient cleaning and disinfecting procedures are the main requirements because many food pathogenic and spoilage bacteria are able to attach to food contacting surfaces [3, 4, 5]. Moreover, they might still remain viable even after cleaning and disinfection which might lead to food contamination [2, 4, 6-8]. Among many reasons causing food contamination, microbial biofilm is important. A biofilm can be broadly defined as a microbiologically derived sessile community characterized by cells that are attached to a substratum or interface or to each other. Biofilms are embedded in a matrix of extracellular polymeric substances (EPS) that they have produced [9, 10]. In real food processing environments, biofilm communities are inhabited by numerous different species. Mixed-species biofilms are usually more stable than mono-species biofilms. Cell-to-cell interactions have been demonstrated to play a key role in biofilm formation, biofilm structure, as well as in the resistance of biofilm community members against antimicrobial treatments [4, 11-13]. That is the reason why cells in biofilm are significantly resistant to sanitizers compared with the same cells being planktonic [4, 8, 14]. They also enhance the capacity to survive under stresses conditions that are commonly encountered within food processing [3, 15, 16].

Many studies have been attempted to investigate the behavior of mono-species biofilms, both of spoilage bacteria and specific pathogens such as *Pseudomonas spp*. and *Listeria monocytogenes; Salmonella spp.; Staphylococcus spp*. [11, 17-21]. However, limited research has been published related to the microbiology of food processing surfaces even though microbial contamination in the processing environment is an important parameter. The microorganisms present are likely a mixture of many species [4, 22] and some of these microorganisms produce exopolymers that can facilitate the retention of pathogenic microorganism [5]. Understanding the characteristics of adhered microflora on food processing equipment would provide valuable information in designing the cleaning and disinfecting procedures.

Therefore, the objective of this study was to investigate the biofilm formation in seafood processing plant by the population of microflora adhered on stainless steel coupons at different interval of 9 week study. The results of this study will help in understanding the time required for biofilm-formation in food processing plant; which will consequently help in improving the effectiveness of the cleaning and disinfecting procedures.

2. Material and Methods

2.1. Processing Plant and Product Manufacturing

Samples were taken from the surface equipment at a high care area in a seafood processing plant which produced ready to eat food. Experiment was carried out during October 2013 to April 2014.

In the food processing plant, a cooker first cooked and then the content was transferred through blue conveyor for cooling down. Once cooled, the products were passed through white conveyer to a container for adding herbs. Products were subsequently frozen before adding soup and frozen again. After that, they were transferred to packaging section.

At the end of each production day, a complete cleaning and disinfecting procedure was carried out. Cleaning was performed using low-pressure and foam cleaners. Alkaline detergent, superp foam was used as cleaning agent, and then spectrum (Quaternary ammonium compounds) was used for disinfecting.

2.2. Stainless Steel Coupons Preparation and Installation

Stainless steel (type 304, no. 2B finish), which is extensively used in the manufacturing of food processing equipment [23], was used in this study. Based on the result from preliminary experiment, seven locations (Fig.1) in the food processing plant were determined for installing stainless steel coupons. Before installation, stainless steel (SS) coupons (2×5 cm) were soaked overnight in commercial detergent solution, degreased with 70% ethanol, thoroughly rinsed with tap water and then with distilled water, air-dried and sterilized by autoclaving for 15min at 121°C [23, 24]. After that, sterilized SS coupons were transferred to the food processing plant (FPP). At FPP, SS coupons were attached at 7 different locations near food contact surfaces by rubber silicone sealant (SP-N192, Sparko, Thailand) [25]. Seventy SS coupons samples were kept in the FPP for 1 week, 2 weeks, 3 weeks, 5 weeks and 9 weeks. The sanitary procedures include fresh-water rinsing every 3 hours, and completed clean in the end of working day consist of water rinsing, cleaning with alkaline detergent and disinfecting with quaternary ammonium compounds (QACs).

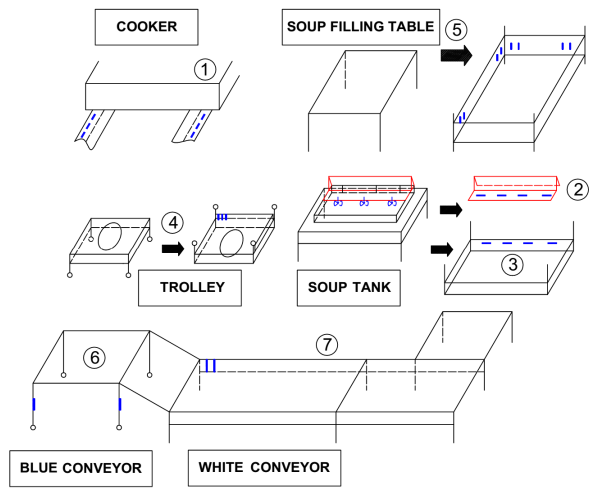

Fig. 1. *Diagram of locations where stainless steel coupons (▬) were installed on the equipment at food processing plant.*

2.3. Microbiological Analysis

At each sampling period, fourteen SS coupons were aseptically detached and placed into plastic petri dishes, sealed and placed in cooling box, and then transported to the laboratory. Samples for microbiological analysis were taken from the entire surface of each coupon twice with cotton swab. Before swab test, SS coupon was rinsed twice with 10 ml distilled water, then swabbed thoroughly on the side without glue; after that the swab heads were broken off into a glass tube. The tube containing the cotton swab blended with 10 ml of sterile physiological (0.85%) salt water with 0.1% peptone for 60s [1]. Serial dilutions were prepared for each sample and spread duplicate onto Tryptic soy agar (TSA) (Difco) and Citrimide agar (Difco). The plates of TSA and Citrimide agar were incubated at 37°C for 1 day for testing total viable count and *Pseudomonas spp*.

3. Results and Discussion

The populations of total viable count (TVC) and *Pseudomonas spp*. at different sampling locations and at different sampling times are presented in Table 1 and Table 2.

It is observed that microbial adhesion appeared in many inspected locations and the microbial population ranged from 10^1–10^6 cfu/cm^2. The similar experimental findings was also reported by Birna *et al.* [26] at shrimp factory, where bacteria population in the range of 10 to 10^4 cfu/cm^2 were obtained at two sampling times, 2 months and 4 months. The result indicated that it might be unlikely to develop large biofilm structures with multilayer bacteria in food processing environments, for examples above 10^8 cfu/cm^2, because the regular use of sanitizer and low temperature in working area limit the extensive biofilm formation [26]. Similarly, another research in ice-cream plant reported that SS coupons placed near food contact surface did not contain high levels of microorganisms in short time; the highest number was 6.5 × 10^3cfu/cm^2 after 8 h at conveyor belt [27]. However, these adherent microorganisms could still contribute to the contamination of food products. Increasing sampling time from 1 to 5 weeks increased the number of adhered microorganisms on SS coupons; the highest bacteria population was detected at trolley (8.2 × 10^6cfu/cm^2). It was observed that biofilm formed after three weeks at some locations such as guide of cooker, trolley, soup filling table and the bottom of soup tank with bacterial population ranged from 2.3 × 10^5 to 4.0 × 10^5cfu/cm^2; indicating that the cleaning procedure was improper. In addition, regular daily cleaning and disinfection procedures was carried out, sampling and analysis over long period (from 1 to 9 weeks) demonstrated that mature biofilm could be formed after 3 weeks, especially in locations where high nutrient was present that was necessary for the growth of organisms. Biofilm formation on equipment surfaces increases the biotransfer potential that can be described as the ability of any microorganisms present on equipment surfaces both before and after the cleaning procedures to contaminate a product during processing.

Table 1. *Population of adhered microflora enumerated as Total viable count (CFU/cm²) at different locations and different sampling times*

Sampling time					
Coupon locations	**1 week**	**2 weeks**	**3 weeks**	**5 weeks**	**9 weeks**
Guide of cooker # 1	1.0 x 10^1	3.0 x 10^1	2.3 x 10^5	1.7 x 10^5	6.3 x 10^3
Guide of cooker # 2	1.0 x 10^1	<10	8.4 x 10^2	<10	1.0 x 10^1
Soup tank -top # 1	<10	1.0 x 10^1	1.5 x 10^1	1.3 x 10^2	1.6 x 10^2
Soup tank -top # 2	<10	<10	<10	1.0 x 10^1	6.0 x 10^2
Soup tank -bottom# 1	<10	<10	5.0 x 10^1	<10	2.0 x 10^2
Soup tank- bottom # 2	<10	<10	3.1 x 10^5	<10	8.8 x 10^3
Trolley # 1	6.4 x 10^4	4.4 x 10^3	4.0 x 10^5	8.2 x 10^6	6.0 x 10^3
Trolley # 2	6.8 x 10^3	<10	1.3 x 10^3	5.5 x 10^3	1.6 x 10^4
Soup filling table # 1	<10	<10	3.6 x 10^5	1.8 x 10^4	<10
Soup filling table# 2	<10	8.5 x 10^1	1.0 x 10^4	3.1 x 10^3	<10
Blue conveyor # 1	7.9 x 10^2	3.3 x 10^5	<10	<10	<10
Blue conveyor # 2	4.5 x 10^1	<10	<10	2.5 x 10^1	2.0 x 10^1
White conveyor # 1	<10	<10	1.7 x 10^4	<10	<10
White conveyor # 2	<10	<10	<10	1.6 x 10^3	<10

Table 2. *Population of Pseudomonas spp. (CFU/cm²) at different locations and different sampling times.*

Sampling times					
Locations	**1 week**	**2 weeks**	**3 weeks**	**5 weeks**	**9 weeks**
Guide of cooker # 1	<10	<10	1.4 x 10^2	4.0 x 10^3	5.7 x 10^2
Guide of cooker # 2	<10	<10	3.6 x 10^2	<10	<10
Soup tank -top # 1	<10	<10	<10	<10	<10
Soup tank -top # 2	<10	<10	<10	<10	9.5 x 10^1
Soup tank -bottom# 1	<10	<10	6.0 x 10^1	<10	1.5 x 10^1
Soup tank- bottom # 2	<10	<10	1.5 x 10^5	<10	4.1 x 10^3
Trolley # 1	2.1 x 10^3	3.4 x 10^3	1.9 x 10^5	2.3 x 10^6	1.0 x 10^3
Trolley # 2	2.0 x 10^2	<10	3.5 x 10^2	4.3 x 10^3	7.3 x 10^3
Soup filling table # 1	<10	<10	2.1 x 10^5	6.8 x 10^3	<10
Soup filling table# 2	<10	<10	2.4 x 10^3	6.0 x 10^2	<10
Blue conveyor # 1	3.0 x 10^1	1.6 x 10^5	<10	<10	<10
Blue conveyor # 2	<10	<10	<10	1.5 x 10^1	<10
White conveyor # 1	<10	<10	1.3 x 10^4	<10	<10
White conveyor # 2	<10	<10	<10	1.3 x 10^3	<10

Similar to the result of TVC, large population of *Pseudomonas spp*. (Table 2) were found on the surface of SS coupons at trolley #1, soup tank (bottom) and soup filling table as an evident to support the biofilm formation. *Pseudomonas* has been found to adhere easily to the surfaces and is excellent biofilm formers as reported in several studies [28, 29].

4. Conclusions

The present study results demonstrated that the processing equipments of food industries harbor a microbial ecosystem after production and daily cleaning procedure. Population of microflora adhesion on stainless steel coupon at food processing plant revealed that inappropriate cleaning process leads to the formation of mature biofilms after 3 weeks. Therefore, to prevent the detached or dispersed microorganism from biofilm in processing environment, effective the cleaning procedure should be developed and extensive cleaning of equipment should be done in every two weeks to inhibit biofilm formation. Moreover, extensive cleaning should be done at locations that come in contact with high nutrient content.

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