Biofilm Formation of Foodborne Pathogens and their Control in Food Processing Facilities

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Abstract

Microorganism can form biofilm to adapt various hard-to-live conditions for long-term survival. Foodborne pathogens can seed in the biofilm to escape the regular sanitation practice, especially in floor drains. Food processing facilities generally use chlorine-based, QACs-based, or phenolic-based sanitizers in their sanitation practice. The advantage of these sanitizers is cheap and effective and the disadvantage is its efficacy can be reduced by a lot of factors and easy to produce resistance. New novel bactericide as an alternative is needed when efficacy of these traditional sanitizers is substantially reduced. For control of Listeria monocytogenes in floor drain contamination, especially for ready-to-eat food processing plant the procedure of biocontrol is needed because floor drain is too deep to reach. The product of biocontrol contains beneficial bacteria and these beneficial bacteria can live in the biofilm at harsh environment and these beneficial bacteria consistently produces various bacteriocins to kill L. monocytogenes and ensures floor drain without the contamination of L. monocytogenes for long-term.

Keywords: Foodborne pathogens; Biofilm; Chemical control; Biocontrol

Introduction

For survival under harsh environment, such as bad nutrition, irregular heat, and various chemicals, bacteria can form biofilms composed of single or multi layers of microorganisms embedded in their own extracellular polymeric substances (EPS) which associated with a solid surface [1-5]. Studies demonstrated that most foodborne pathogens, especially Listeria monocytogenes, Salmonella, and Shiga toxin-producing Escherichia coli, can survive in various parts, including equipment, belts, floor drains at food processing facilities though improvements in plant layout, equipment design, and procedures for cleaning and sanitizing [4,6,7]. Generally, these pathogens when existed in biofilms are more resistant to general cleaning and sanitation procedures than their planktonic counterparts [1-4].

L. monocytogenes is problem for ready-to-eat (RTE) food during processing at a modern low temperature processing plant [1]. The floor drains have been identified as the main contamination source. Researches have documented that L. monocytogenes can survived long-term in floor drain and the genotype of L. monocytogenes isolate responsible for recent foodborne outbreak has exactly matched with the isolate of L. monocytogenes isolated many year ago.

Two methods are used for elimination of L. monocytogenes in floor drain. They include chemical method and biological method [8-14]. Among them chemical method, especially chlorine-base chemicals is most commonly used method because it is effective, rapid and can repeat many times until all samples collected from floor drain turn negative. Disadvantage is that L. monocytogenes may survive in biofilm that can attach the drain for quite long distance and can be recovered quickly and even become chemical-resistant strain if same chemical is continuously used. Recently a novel new bactericide was developed and it has excellent biofilm-removal capability [8-10, 15-18] (Table 1). As listed in the table its biofilm removal ability is much better than acidified sodium chlorite 500 ppm, pH 2.8 [13]. According to the Food Code 2009, sanitation is defined as application of heat or chemicals on cleaned food contact surfaces in order to get a 5-log reduction of representative pathogens. Results of antimicrobial activity revealed this microbicide has substantial ability to penetrate the EPS barrier through surface protein denature and provide sufficient concentration and exposure time to kill all of the cells in the biofilm [8-10,14,17]. Currently this microbicide has been commercialized by HealthPro Brands, Inc. (Fit-L, Mason, OH) and its bactericidal effect and safety has been applied in medical fields for removal of dental biofilm [17].

The other method is biocontrol for elimination of L. monocytogenes in floor drain. Their purposes is to inoculate or spray harmless bacteria, which have the ability to survive the harsh environment, penetrate the existed biofilm, occupy the original biofilm and develop its own biofilm, and continuously produce the metabolites to kill the existed L. monocytogenes on the floor drain. Thus, the protection from contamination of L. monocytogenes will last much longer time, especially for ready-to-eat food processing facilities.

At present, this new technology is developed and its efficacy for reduction or elimination of L. monocytogenes in floor drains in food processing plant and RTE food processing plant is thoroughly evaluated [11,12,14]. Two competitive exclusion bacteria (CE), Lactococcus lactis subsp. lactis (#C-1-92; a Nisin A and B producer) and Enterococcus durans (#152; a L50 A and B producer) were identified. These two CE isolates were combined and evaluated to control Listeria sp. in floor drains of a RTE poultry processing plant. Results revealed that treating the floor drains with CE four times in one week eliminated detectable Listeria sp. from 5 of 6 drains and the drains remained free of detectable Listeria sp. for 13 weeks following the first treatments were applied [14]. These studies indicate that CE can effectively reduce Listeria contamination in biofilms and in flow
drains of a plant producing RTE poultry products. Its advantage over chemical treatment is its application can provide the food processing facilities a log-term protection from the contamination of *L. monocytogenes*.

| Coupon material          | Chemical treatment                  | Salmonella Enteritidis count (log10 CFU/cm²) at min: |
|--------------------------|-------------------------------------|-----------------------------------------------|
|                          |                                     | 0a                                      1                     5          20         |
| Stainless steel          | PBS, pH 7.2                          | 8.0 ± 0.6                                 | 8.4 ± 0.2 | 8.6 ± 0.2 | 8.1 ± 0.5  |
|                          | Acidified sodium chloride (500 ppm), pH 2.8 | 7.5 ± 0.3                             | 7.9 ± 0.1 | 7.4 ± 0.8 | 7.0 ± 0.8  |
|                          | 3% levulinic acid plus 2% SDS, pH 3.0 | <1.7<sup>a</sup>                      | <1.7      | <1.7      | <1.7       |
| Polyvinyl chloride       | PBS, pH 7.2                          | 8.8 ± 0.1                                 | 9.0 ± 0.5 | 8.8 ± 0.1 | 8.3 ± 0.4  |
|                          | Acidified sodium chloride (500 ppm), pH 2.8 | 6.9 ± 0.3                             | 5.5 ± 0.8 | 5.3 ± 0.2 | 2.9 ± 0.1  |
|                          | 3% levulinic acid plus 2% SDS, pH 3.0 | 2.3 ± 0.7                                    | 1.7 ± 0.0 | 2.2 ± 0.1 | <1.7       |
| Nitrile rubber           | PBS, pH 7.2                          | 7.8 ± 0.9                                 | 8.0 ± 0.6 | 7.7 ± 0.9 | 7.7 ± 0.6  |
|                          | Acidified sodium chloride (500 ppm), pH 2.8 | 7.2 ± 0.5                             | 5.2 ± 0.1 | 2.6 ± 0.3 | <1.7       |
|                          | 3% levulinic acid plus 2% SDS, pH 3.0 | 4.1 ± 0.7                                    | 1.7 ± 0.2 | <1.7      | <1.7       |
| Glass                    | PBS, pH 7.2                          | 8.2 ± 0.7                                 | 8.7 ± 0.2 | 8.4 ± 0.5 | 8.4 ± 0.5  |
|                          | Acidified sodium chloride (500 ppm), pH 2.8 | 6.8 ± 0.5                             | 3.3 ± 0.3 | 1.7 ± 0.1 | <1.7       |
|                          | 3% levulinic acid plus 2% SDS, pH 3.0 | <1.7<sup>a</sup>                      | <1.7      | <1.7      | <1.7       |
| Ultra-high molecular weight polyethylene | PBS, pH 7.2                          | 8.4 ± 0.1                                 | 8.0 ± 0.4 | 8.4 ± 0.2 | 8.4 ± 0.2  |
|                          | Acidified sodium chloride (500 ppm), pH 2.8 | 6.8 ± 0.7                             | 6.1 ± 0.1 | 1.7 ± 0.1 | <1.7       |
|                          | 3% levulinic acid plus 2% SDS, pH 3.0 | <1.7<sup>a</sup>                      | <1.7      | <1.7      | <1.7       |

Table 1: Inactivation of *Salmonella* Enteritidis in biofilms by a liquid preparation of 3% levulinic acid plus 2% sodium dodecyl sulfate (SDS) at 21°C for different exposure times. <sup>a</sup>The actual time 0 was delayed by 35 to 45 seconds due to time for sample processing. <sup>b</sup><1.7 log CFU/cm², not detected by the direct plating method.

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