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# 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 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 microbicide 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 chlorite (500 ppm), pH 2.8 | 7.5 ± 0.3 | 5.9 ± 0.1 | 5.4 ± 0.8 | 6.0 ± 0.8 |
| | 3% levulinic acid plus 2% SDS, pH 3.0 | <1.7[b] | <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 chlorite (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 ± 0.9 | 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 chlorite (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 chlorite (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 | <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 chlorite (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 | <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. [a] The actual time 0 was delayed by 35 to 45 seconds due to time for sample processing, [b] <1.7 log CFU/cm$^2$, not detected by the direct plating method.

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