RESEARCH IN PROCESS

Evaluation of the Efficacy of Antimicrobial Agents to Prevent the Transfer of *Listeria monocytogenes* from Existing Biofilms to Produce or Processing Surfaces

SUMMARY

Listeria monocytogenes (*Lm*) is a foodborne pathogen that can cause serious illness and even death in susceptible individuals. *Lm* outbreaks have been associated with fruits, sprouts and vegetable row crops. *Lm* can form or be part of biofilms on produce and surfaces in produce harvesting and processing environments. Once established in a biofilm, *Lm* has highly diminished susceptibility to antimicrobial agents and is difficult to eradicate. Cells surviving in such biofilms can detach and be carried to new surfaces where they can start the formation of a new biofilm or become part of an existing biofilm. It is therefore extremely important to prevent the transfer of cells from existing biofilms to previously uncontaminated surfaces on produce or processing equipment. The proposed study will examine the efficacy of antimicrobial agents to inactivate *Lm* released from existing biofilms and prevent the formation of new *Lm*-containing biofilms on produce and equipment surfaces.

OBJECTIVES

1. In laboratory experiments, determine the concentrations of hypochlorite, hydrogen peroxide, peracetic acid and other permissible antimicrobials that are required to prevent the transfer of *Lm* from an existing biofilm to an uncontaminated surface when tap water or water with different organic loads is the transfer medium.
2. Determine if *Lm* released from a biofilm is able to take refuge on a new surface coated with organic materials and bacteria.
3. Validate the results by monitoring the transfer of *Lm* existing as biofilms on leafy greens and fruits and vegetables to uncontaminated produce or equipment surfaces in the presence of antimicrobials. Also monitor the transfer from abiotic surfaces to leaves or produce surfaces.

METHODS

In the first phase of the study, the biofilm formation ability of *Lm* strains in complete and produce-related media is being determined. A collection of 23 *Lm* strains was subjected to biofilm assays using crystal violet and resazurin methodology.

These methods were suitable for assays with cells growing in complete medium, but plating of biofilm-derived cells after sonication treatment was done for biofilms formed in lettuce wash water and filtered lettuce extract.

Using the three selected *Lm* strains, LM390, LMH7650 and Scott A, sensitivity to sodium and calcium hypochlorite was determined for planktonic cells and for cells within biofilms.

RESULTS TO DATE

Using the resazurin assay, differences in the biofilm formation of the *Lm* strains grown in complete medium were observed (Fig. 1). Strain LM390, associated with a disease outbreak involving cantaloupes, was one of the strongest biofilm formers, whereas laboratory strain, Scott A, was one of the weakest. Biofilms developed in lettuce extract or washes produced only weak or no signals with the resazurin assay; therefore, counts of viable cells in 72-h biofilms were established (Fig. 2).

The MIC values for strains LM390 and Scott A biofilm cells were 19.5 and 625 ppm with hypochlorite in PBS or TSBYE, respectively. When the biofilms were exposed to 5 ppm hypochlorite in PBS, inactivation times were 60 and 2 min for Lm390 and Scott A biofilm cells, respectively. At a concentration of 7.5 ppm hypochlorite, no biofilm cells from strains LM390 and Scott A were detected after exposure for 20 and 1 min, respectively.

BENEFITS TO THE INDUSTRY

The research is expected to yield important data on the efficacy of antimicrobial washes on the prevention of transfer of *Lm* from existing biofilms on produce or abiotic surfaces to previously *Lm*-free surfaces on produce or processing equipment. The data can be used by the produce industry and governmental agencies to provide recommendations regarding the use of antimicrobials in the industry with respect to the prevention of spread of *Lm* within facilities and produce items.

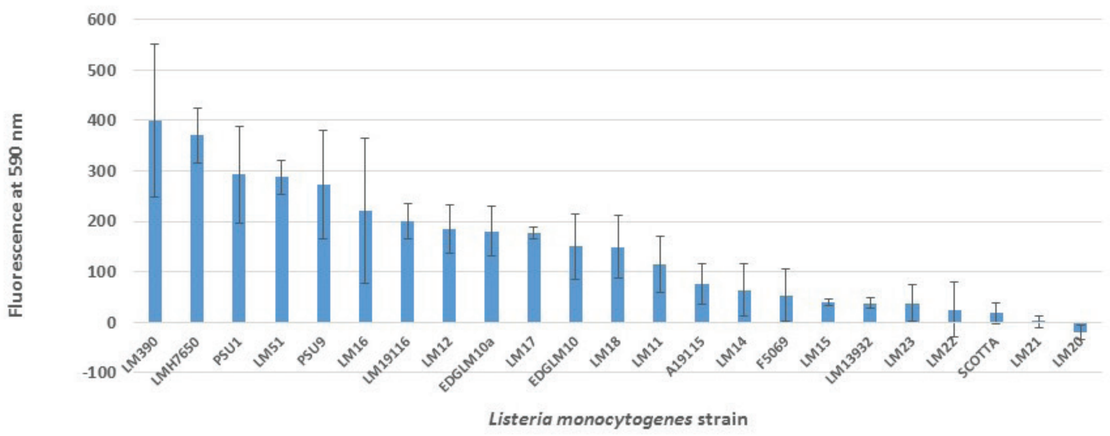

Figure 1. Fluorescence measurements for biofilms exposed to resazurin. Individual strains were grown in TSBYE medium at room temperature, diluted to an OD600 of 0.05 and transferred to microtiter plate wells. Cells were allowed to attach to the wells for 4 h. The medium was removed and replaced by fresh medium. Biolfilm development was allowed to proceed for 48 h at room temperature. Data are for triplicate assays and errors bars indicate standard deviation.

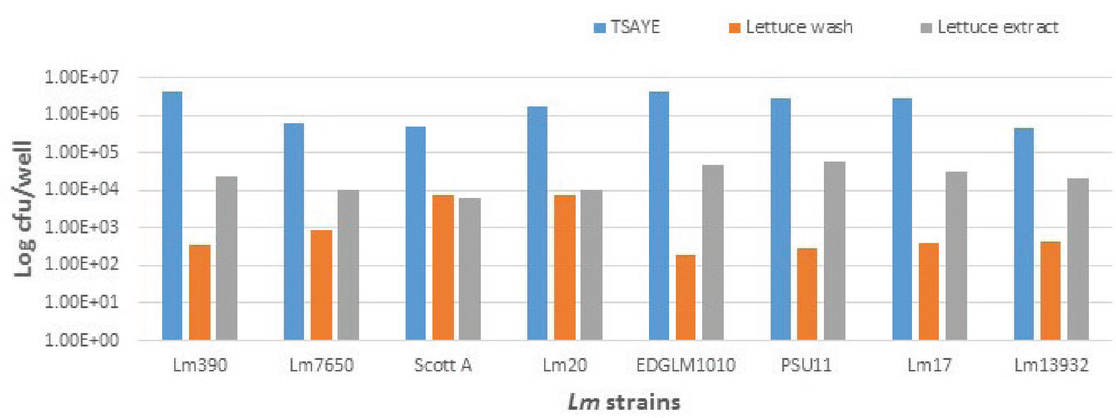

Figure 2. Viable cell count of *Lm* biolfilms grown in complete medium (TSAYE); lettuce wash water and lettuce extract (1:10 w/v). Cells were suspended in the media and allowed to attach to the microtiter plate wells. The medium was replaced with fresh medium at 24 and 48 h. Cell counts were obtained after 72 h incubation at room temperature after sonication of the plates for 30 min.

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