O6-1 **Alginate film with bacteriocins as surface sanitizer against *Listeria* for the food industry.**

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INTRODUCTION AND OBJECTIVES

In the food processing industry different microorganisms protect themselves from adverse environmental conditions by secreting exopolisaccharides that allow them to adhere to different surfaces and multiply within structures called "biofilms". Among the bacteria that produce biofilms is the foodborne pathogen *Listeria monocytogenes*, ubiquitous in nature, able to grow at low pH, in the presence of high sodium chloride concentrations and at refrigeration temperatures. *Listeria* will grow and produce biofilms on different surfaces within the food industry, in particular in places with high humidity and in the presence of food residues. Inside these biofilms *Listeria* is more resistant to cleaning and sanitizing agents in particular in floor drains (floor gutters), where biofilms grow and seldom are removed during cleaning. Common sanitizers do not penetrate biofilms and are therefore not effective for removal of the pathogen.
A novel approach is the formulation of a bacteriocin based sanitizer with proven activity on *in vitro* grown biofilms of *L. monocytogenes*. The objective of this study was, to evaluate an alginate based surface sanitizer, under industrial conditions, on naturally developed biofilms, in gutters of a salmon processing plant.

MATERIAL AND METHODS

The active compounds of the *Listeria* biocontroler were a bacteriocin like substances (BLS) from three lactic acid bacteria (*Carnobacterium piscicola* ATCC PTA 9380, *C. piscicola* ATCC PTA 9381 and *Enterococcus mundtii* ATCC PTA 9382), plus Nisin (Danisco), at a concentration of 1000 UI/mL. The LAB strains were grown in D-MRS and MRS broth respectively, for BLS production and pasteurized, to inactivate the producer strains, without affecting de BLS activity. The heat treated fermentates (FTT) were incorporated into the alginate based sterile matrix. For preparation of the biocontroler the alginate matrix was poured over a mesh type fabric, which was the support for the film. Calcium chloride was used as coagulant. The biocontrolers were dried at 25 ºC to a water activity of A_w 0.75±0.2. Figure 1 shows the gel with the mesh type support.

In vitro activity of the films was tested by the plate antagonism test, with a pool of five *L. monocytogenes* strains as indicator microorganisms.

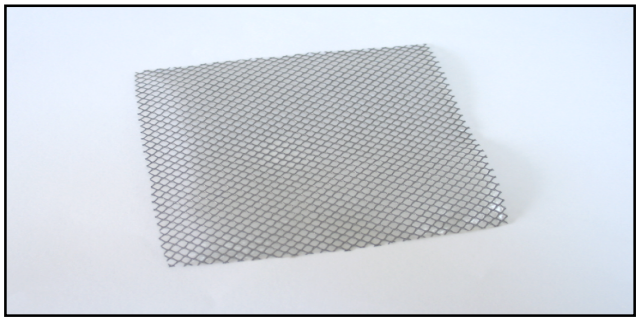

Figure 1. Alginate film as surface sanitizer for floor drains in the food industry.

The biocontroler was tested in floor drains of a salmon processing industry. Previously the presence of *Listeria* was confirmed on these locations. The sanitizer film was attached to the side of floor drain to ensure adequate contact with the surfaces. The experimental design for sampling is shown in Table 1. Control sample 1 and 2 were taken at time zero and test and control sample 3 after 48 h of contact. *Listeria* detection was done by traditional microbiological methods.

Table 1. Microbial sampling design for floor drains with the surface sanitizer and control sites.

Biofilm	Test	Control 2	Control 3
Listeria control	Alginate film with sanitizer	*Listeria* control	Alginate film without sanitizer

Figure 2 is a view from the top, inside the floor drain. The arrow indicates the location of the sanitizer film, closely attached to the side of the drain.

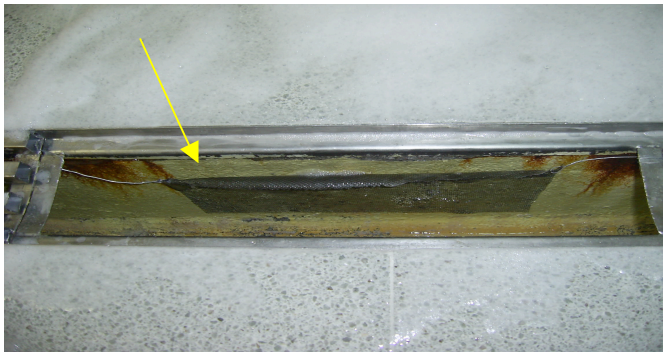

Figure 2. Location of sanitizer film inside floor drain.

The results are informed as presence or absence of *L. monocytogenes* at the different sampling sites.

RESULTS AND DISCUSSION

The antilisteria activity of the heat treated fermentate (FTT) was of 204.800 AU/mL. Figure 3 shows the doubling dilution results of the FTT, plus nisin on the lawn of a pool of five strains of *L. monocytogenes*. These results indicate a high antilisteria activity of the active ingredient that was incorporated into the alginate matrix.

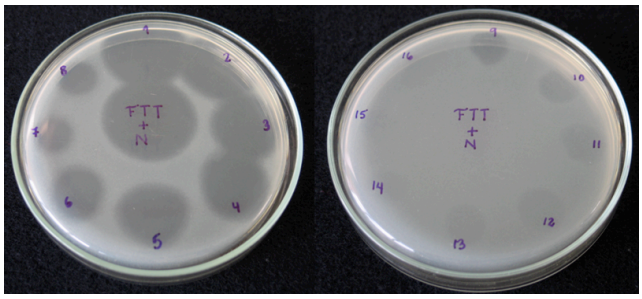

Figure 3. Plate antagonism of the FTT used as active ingredient of the surface sanitizer, (204.800 AU/mL).

After the film was formed, different drying periods were tested. The objective was, to reduce the Aw of the film without loosing activity, in order to achieve a more stable product against bacterial and fungal growth. As shown in Table 2 drying time was important, but also variations among alginates were found. For the purposes of this study, best results were obtained with alginate C, which was used in the sanitizer film.

Table 2. Water activity (Aw) of alginates films from different suppliers after drying at 25°C.

Time (h)	A	B	C
1.0	0.85 ±0.01	0.88 ± 0.01	0.88 ± 0.01
1.5	0.84 ±0.01	0.83± 0.03	0.83± 0.01
2.0	0.83 ±0.04	0.77 ± 0.01	0.75 ± 0.04

The antilisteria activity of the sanitizer film was checked before use. Figure 4 shows the inhibitory zones from three samples showing a homogeneous distribution of the active ingredients within the film.

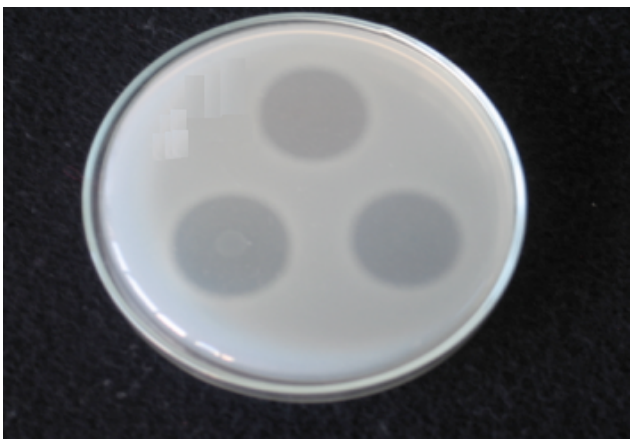

Figure 4. Inhibitory activity of the sanitizer film on a lawn of L. monocytogenes.

Previous laboratory studies on biofilms of *L. monocytogenes* on stainless steel, rubber and teflon coupons, had shown strong inhibition of the bioencapsulated sanitizer, applied during 48 h. In order to prove the effectiveness of the sanitizer film, it was necessary to make field trials, with natural grown *Listeria* biofilm.

The trials with the sanitizer in the food plant were carried out over a two month period. A total of seven times the sanitizers were placed in floor drains, at different locations within the salmon processing plant. The first challenge was to design a frame that would hold the sanitizer film and allow good contact with the wall, where the *Listeria* biofilm was present. Results indicated that 4 out of 7 times, in 57% of the samples, *Listeria* was eliminated from the test surface while in the control samples the pathogen was still present (Table 1 and 3). These results are satisfactory since we could prove that *Listeria* can be eliminated with the sanitizer film when adequate contact is achieved.

Table 3 . Expected results for the sanitizer on a natural developed *Listeria* biofilm

Control 1	Test	Control 2	Control 3
Listeria PRESENT	*Listeria* NOT DETECTED	*Listeria* PRESENT	*Listeria* PRESENT

CONCLUSIONS

An alginate based sanitizer film was designed to control *Listeria* in floor drains of the food processing industry.
In vitro tests showed inhibitory action against a pool of five different strains of *L. monocytogenes*.

The alginate based sanitizer was tested in a food processing industry where positive results were obtained on natural grown biofilms. *Listeria* was not detected in 57% of sides treated with the sanitizer, while the bacterium was still present in the control.

The sanitizer proved effective, but new forms of application must be studied to assure its continuous effectiveness in floor drains.

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