THE EFFECT OF DISINFECTANTS ON *LISTERIA MONOCYTOGENES* ADHERENT TO MEAT BIOFILM

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Background

Listeria monocytogenes is the major problem concerning the safety of ready-to-eat (RTE) cooked meat products. When L. monocytogenes occurs in RTE cooked meat products, it is due to after-contamination when the products are handled and packed, because L.monocytogenes occurs in the environment. The occurrence in the environment can be persistent, because L. monocytogenes has the ability to form biofilms on food-processing surfaces. Biofilm is composed of meat residues and microbial polymers formed during bacterial growth. Bacteria adherent to a biofilm is highly protected against chemical compounds such as disinfectants.
It is very important to be aware of the ability of the disinfectants to penetrate a biofilm, so that the right disinfectant can be chosen when problems with biofilm arise in the production plant. It is important that the disinfectants are evaluated in an experimental set-up closely related to the environment in the production plant.
Fatemi et al.(1999) and Krysinski et al. (1992) measured the effect of disinfectants and sanitizers on L. monocytogenes cells adherent to a biofilm attached to stainless steel. Both methods included a biofilm produced in tryptone soya broth (TSB). When using the laboratory broth, the nutrients are different from the nutrients available for the microorganisms in the production plant. Krysinski et al. (1992) enumerated surviving L. monocytogenes by the plate count method after dislodging the cells from the stainless steel. Fatemi et al. (1999) enumerated viable L. monocytogenes in the biofilm by the direct agar overlay method.
The present study introduces a method to create a L. monocytogenes biofilm composed of meat residues normally present on the surfaces of equipment in meat production plants and describes a method to measure the bacterial counts on stainless steel using a Malthus apparatus.

Objectives

The objective of this study is to test the ability of five different disinfectants to inactivate *L. monocytogenes* cells adherent to a biofilm under simulated production site conditions.

Materials and methods

L. monocytogenes DMRICC 3001, originally isolated from the meat environment, was grown in BHI broth at 30°C.
Biofilm was formed on stainless steel coupons (1cm x 2.5cm) by immersing the coupons in a meat sausage/water emulsion (1:4) inoculated with 1% *L. monocytogenes* and incubated at 30°C for 6 days. The coupons were then rinsed with sterile water to remove unattached cells and meat.
Detergents: 4% Deptal FM2, alkaline detergent (NaOH, KOH), Hypred and 5%, P3-topax, 66, sanitizer (NaOH: 5-15%, sodium hypochlorite: <5% active chlorite, alkylaminoxide: <5%), Ecolab.
Cleaning methods: Before disinfection, coupons with biofilm were cleaned in the alkaline detergent or the sanitizer by scrubbing for one minute with a cloth soaked in one of the detergents or by soaking the coupons in the detergent solution for 15 minutes. After cleaning the coupons were rinsed in 10 ml sterile water.
Disinfectants: Deptal Mycoside S (QAC) (Didecyldemethylammoniumchloride) 0.3% for 20 minutes (Hypred), Betane Plus (QAC) (QAC compounds: 5-15%, poly(hexamethylen)biganid, HCl:<5%) 1% for 10 minutes (Ecolab), Desinfect Maxi (QAC) (N,N-didecyl-N,N-dimethylammoniumchloride:5-15%, propan-1-nol:<5%) 1% for 5 minutes (Novodan), sodium hypochlorite (12.4% active chlorite), 0.16% for 5 minutes (Novadan), P3 oxysan (Acetic acid: 15-30%, H_2O_2:5-15%, alkylsulfonate: 5-15%, peraceticacid: <5%, phosphonacid: <1%) 0.1% for 5 minutes (Ecolab).
Disinfection: The coupons were disinfected by soaking in the disinfectants. The concentration and time treatment were chosen from the respective data sheets from the chemical manufacturer. After disinfection, the coupons were rinsed with 10 ml sterile water and air-dried for 5 minutes. Untreated coupons were soaked

in sterile phosphate buffer (pH 7.2) for 5 minutes. All coupons were soaked in a neutralizing liquid for 5 minutes before they were analyzed in the Malthus apparatus
Growth of *L. monocytogenes* was determined by measuring the time until *Listeria monocytogenes* started producing CO_2 in the Malthus apparatus. The correlation between time and cfu/g biofilm was calculated to be: X (log cfu/g)=÷0.29 xY(time until growth) + 9.32 (R=0.99).

Results and discussion

Cleaning with the sanitizer followed by disinfection with pH neutrale quaternary ammonium chloride compounds (QACs) resulted in a complete inactivation of L. monocytogenes adherent to the meat biofilm (see figure 1). There was no difference between the two cleaning methods. QAC is a large four-chain molecule with a central N-atom. The chemcial composition of the three investigated QACs varied in two of the sidechains, but this did not affect the ability of the QACs to inactivate L. monocytogenes. The most important properties for the QAC's ability to penetrate biofilms are resistance to organic matter and low surface tension. The cleaning method could not be evaluated because of the complete inactivation of L. monocytogenes.
The same results were seen when cleaning with an alkaline detergent before disinfection with QACs (figure 2).
Cleaning with the sanitizer by scrubbing followed by disinfection with sodium hypochlorite reduced the count of *L. monocytogenes* by 3.5 log cfu/g compared to untreated coupons (figure 1). Cleaning by soaking the coupons in the sanitizer resulted in a 1 log reduction compared to untreated coupons (figure 1). This indicates that a mechanical treatment is an important factor in reducing bacterial counts.
Cleaning with the alkaline detergent by scrubbing, followed by disinfection resulted in a 1.7 log reduction compared to untreated coupons (figure 2). This is a lower reduction compared to cleaning with the sanitizer, the reason being that the sanitizer contained disinfectant (hypochlorite), which meant that the coupons were disinfected twice.
The lower inactivation of *L. monocytogenes* resulting from disinfection with hypochlorite compared to QACs can be explained by the fact that sodium hypochlorite is very sensitive to the presence of organic matter, especially protein.
Disinfection with peroxide after cleaning with the sanitizer resulted in a 1.5 log reduction of *L. monocytogenes* compared to untreated coupons (figure 1). The cleaning method (soaking or scrubbing) had no influence on the inactivation of *L. monocytogenes*.
When cleaning with the alkaline detergent by scrubbing followed by disinfection with peroxide, the reduction of *L.monocytogenes* was 1.5 log cfu/g, which is similar to cleaning with the sanitizer followed by disinfection.
Soaking in an alkaline detergent, followed by disinfection with peroxide only reduced the count of *L. monocytogenes* by log 0.5 cfu/g compared to the untreated coupons. When scrubbing before disinfection with peroxide, a minor increase in log reduction was seen. This could be explained by the fact that some of the organic matter was removed and the scrubbing opened the biofilm, which meant that the peroxide could penetrate the biofilm more easily. The main explanation for peroxides low capability to inactivate *L. monocytogenes* is that peroxide is very sensitive to the presence of organic matter.
In the present study in a strong biofilm the effect of sodium hypochlorite and peroxide is almost the same, and the effect of the QACs is much higher than that of sodium hypochlorite and peroxide. The result for peroxide differed from the results of Fatemi et al. (1992), who reported a better effect of peroxide compared to hypochlorite in TSB biofilm covered with a layer of milk. Kysinski et al. (1999) reported a high effect of peroxide and one neutral QAC and a low effect of hypochlorite on *L. monocytogenes* culture attached to stainless steel in a TSB biofilm. Hypochlorite generally has a low ability to inactivate bacteria in biofilm. The ability of peroxide to penetrate and inactivate bacteria biofilms seems to vary according to the chemical matrix of the biofilm.
The results of these experiments demonstrate how important it is to be aware of the disinfectant's ability to inactivate bacteria adherent to biofilm. The disinfectants used in this study are normally capable of inactivating planktonic cells completely (LeChevallier et al.,1988). The knowledge from this study is useful when high bacterial counts are detected after cleaning and disinfection, since could indicate problems with biofilm in the production site. In such a situation, it is important to choose the right disinfectant to solve the problem.

Conclusions

From the experiments, the following conclusions can be made:
QACs can completely inactivate L. monocytogenes adherent to a strong meat biofilm with approximately log 7 cfu/g.
Sodium hypochlorite and peroxide have a low ability to inactivate L. monocytogenes in a strong meat biofilm.

References

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Figure 1. Log *Listeria monocytogenes*/g after cleaning with sanitizer followed by disinfection with 3 different QACs, sodium hypochlorite and peroxide (n=4).

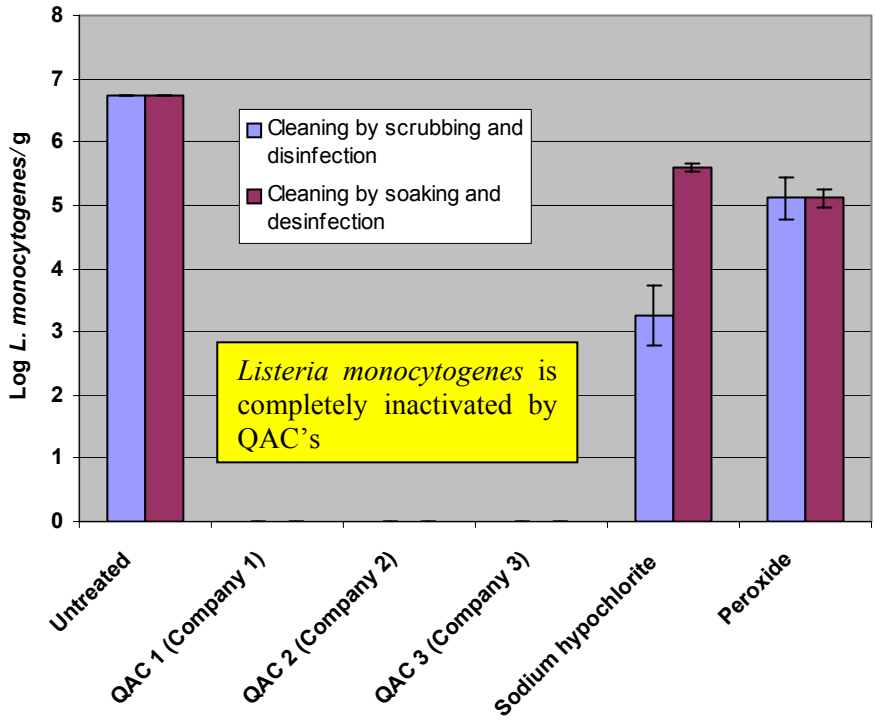

Figure 2: Log *L. monocytogenes/g* after cleaning with the alkaline detergent followed by disinfection with 3 different QACs, sodium hypochlorite and peroxide (n=4).