Inactivation of *Listeria monocytogenes* by Disinfectants and Bacteriophages in Suspension and Stainless Steel Carrier Tests

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MS 14-151: Received 31 March 2014/Accepted 16 July 2014

**ABSTRACT**

To simulate food contact surfaces with pits or cracks, stainless steel plates with grooves (depths between 0.2 and 5 mm) were constructed. These plates were artificially contaminated with *Listeria monocytogenes* in clean conditions, with organic soiling, or after 14 days of biofilm formation after which inactivation of the pathogen by Suma Tab D4 (sodium dichloroisocyanurate, 240 and 300 mg/liter), Suma Bac D10 (quaternary ammonium compound, 740 mg/liter), and bacteriophage suspension (Listex P100) was determined. Both chemical disinfectants performed well in suspension tests and in clean carrier tests according to the European standard with a reduction of more than 5 and 4 log units, respectively, of *L. monocytogenes* was observed in the shallow grooves compared with the deeper grooves. Furthermore, presence of food residues and biofilm reduced the effect of the disinfectants especially in the deep grooves, which was dependent on type of food substrate. Bacteriophages showed the best antimicrobial effect compared with the chemical disinfectants (sodium dichloroisocyanurate and quaternary ammonium compound) in most cases in the shallow grooves, but not in the deep grooves. The chlorine based disinfectants were usually less effective than quaternary ammonium compound. The results clearly demonstrate that surfaces with grooves influenced the antimicrobial effect of the chemical disinfectants and bacteriophages because the pathogen is protected in the deep grooves. The use of bacteriophages to inactivate pathogens on surfaces could be helpful in limited cases; however, use of large quantities in practice may be costly and phage-resistant strains may develop.

*Listeria monocytogenes*, a ubiquitous pathogenic bacterium, is widely found in the natural environment including plants, soil, sewage, water, and also in human and animal feces (27, 29, 67). Due to this widespread occurrence in nature, the pathogen can enter food processing plants via several vectors, such as personnel, equipment, raw materials, pest animals, and process waste (67). The pathogen is able to adhere to and form biofilms on several surface materials, such as stainless steel, rubber, and plastic, which are used in food processing facilities (11, 14, 49). Furthermore, the bacterium has been frequently isolated from wet and cold spots (23) as well as from surfaces and equipment that are difficult to clean (18, 33, 48).

In food processing plants, cleaning and disinfection are the most important steps to eliminate dirt and microorganisms that accumulate on food-contact surfaces, equipment, walls, and floors. Improperly cleaned and disinfected surfaces can lead to cross-contamination when food products come into contact with the surfaces or when dirt and microorganisms are dispersed during processing. Additionally, biofilms can be formed on surfaces, particularly in pits or cracks that are difficult to clean. Once the biofilm is formed, it is problematic to remove the pathogens by normal cleaning and disinfecting procedures (8, 54) because abraded surfaces can provide protection to firmly attached bacteria. Moreover, microorganisms inside soil are protected against disinfectants (2, 9).

Various disinfectants, such as peracetic acid, quaternary ammonium compounds (Qacs), hypochloride, sodium dichloroisocyanurate (SDC), and iodofores have demonstrated their efficacy against *L. monocytogenes* in both suspension (12, 24, 59, 69) and surface tests (2, 10, 41), where the antimicrobial activity of the disinfectants was more effective in suspension than in surface tests. In a suspension, the disinfectants come into contact with free-floating cells, whereas on a surface the disinfectants can reach the organisms only from one side (28, 36, 63). Moreover, the effectiveness of the disinfectants will be reduced when the disinfectants are inactivated by or bind to organic matter present on the surface resulting in less disinfectant coming into contact with the microorganism (32, 68).

Apart from chemical disinfectants, bacteriophages (viruses that infect bacteria) have been used to control bacteria in all stages of food production from preharvest to finished products (22, 44, 47, 52, 57). Although some studies on the efficiency of bacteriophages in removing bacterial biofilm cells have been performed with *Escherichia coli* (26), *L. monocytogenes* (38), and *Pseudomonas*
fluorescens (60), still, little information is available on the efficiency of bacteriophages on the inactivation of pathogens in biofilms (43, 65, 66).

The effectiveness of disinfectants against bacteria on smooth surfaces such as stainless steel coupons or chips (1, 7, 12, 32, 34), buna-N rubber (58, 64), Teflon (55), polyester fabrics, and polyurethane surfaces of conveyor belt (64) has been well documented; however, only few studies have been performed on surfaces with pits or grooves. The purpose of this study was to simulate the practical situation where a Listeria biofilm is formed in cracks in the equipment and to investigate the effect of two commercial disinfectants, based on chlorine and Qacs and a Listeria bacteriophage product against L. monocytogenes in suspension, and in carrier tests with flat and rutted (grooved) surfaces.

**MATERIALS AND METHODS**

**Bacterial strains.** L. monocytogenes LF 38 (serotype 1/2a isolated from cooked ham), LF 36 (serotype 1/2b isolated from cooked sausage), and LF 29 (serotype 4e isolated from cooked sliced sausage) were obtained from The Netherlands Food and Consumer Product Safety Authority (Wageningen, The Netherlands). Stationary-phase cultures of all strains (24 h, 30°C) were stored in 1-ml cryo vials (Greiner Bio-one, Frickenhausen, Germany) with 25% (vol/vol) glycerol (Fluka-Chemica, Buchs, Switzerland) in brain heart infusion (BHI) broth (Difco, BD, Sparks, MD) and 2-mm glass beads (Emergo, Landsmeer, The Netherlands) at −20°C. For each experiment, one glass bead of each strain was inoculated to a separate tryptone soy agar plate (Oxoid, Basingstoke, England), and then incubated at 30°C for 24 h. A single colony was picked, inoculated into BHI broth and incubated at 30°C for 24 h. Test suspensions were prepared by mixing cultures of the three L. monocytogenes strains, where 2 ml of overnight cultures of each strain (BHI broth, 30°C for 24 h) were combined to give a 6-ml mixed culture of L. monocytogenes, which was serially diluted in peptone saline solution (PSS: 8.5 g/liter NaCl; Merck, Darmstadt, Germany) with 250 ml of demi water, after adjustment of the pH to 7.2 with 1 M HCl. The phage suspensions were freshly prepared before use to prevent deterioration during storage. The concentration of the phages was determined by a double agar overlay method according to Carey-Smith et al. (19) using BHI agar (BHI plus Agar technical; Agar no. 3: 12 and 4 g/liter for bottom and top layer, respectively; Oxoid) and Listeria innocua (serovar S; Micreos Food Safety BV) as the indicator organisms. The plates were incubated at 30°C for 24 h and plaques were counted.

**Test surfaces.** Two types of surfaces were used in this study: stainless steel coupons (AISI type 304 standard; ODS, Barenrecht, The Netherlands) with a total surface (both sides) of 3.8 cm², and stainless steel plates (AISI type 304 standard; ODS) measuring 12 by 10 by 1 cm in which two identical sets of grooves (5-, 2-, 1-, 0.5-, 0.2-mm depth, 1-mm width, and 10-cm length) were cut (Fig. 1).

**Disinfectants.** Suma Tab D4 (SDC; Diversey Inc., Utrecht, The Netherlands) and Suma Bac D10 (Qac; Diversey Inc.) were used in this experiment at concentrations of 240 mg/liter for SDC (pH 6) and 740 mg/liter for Qac (pH 10.1 to 10.3) according to the manufacturer’s recommendations. Sterile water was used as a control in the experiments. The disinfectants were freshly prepared shortly before use on the day of the experiments to prevent deterioration during storage.

**Neutralizers.** For the chemical disinfectants, inactivation liquid was used as a neutralizer (6) containing 1 g/liter L-histidine (Merck), 3 g/liter lecithin (VWR International, Poole, England), 5 g/liter sodium thiosulfate pentahydrate (Na₂S₂O₃·5H₂O; VWR International), 30 ml/liter Tween 80 (Merck), and 10 ml/liter phosphate buffer. The phosphate buffer was prepared by dissolving 34 g of potassium dihydrogen phosphate (KH₂PO₄; Merck) in 500 ml of demi water, after adjustment of the pH to 7.2 with 1 M sodium hydroxide (NaOH; VWR Prolabo, Lutterworth, England), the volume was filled with 1 liter of demi water. There were 9 ml of inactivation liquid that were dispensed into test tubes and sterilized at 121°C for 15 minutes.

For Listeria bacteriophages, a dead cell suspension of L. innocua (serovar S), was used as a neutralizer (37), which was prepared by adding 1 ml of an overnight culture (BHI broth, 30°C, 24 h) of L. innocua to 9 ml of PSS, which were subsequently sterilized for 15 min at 121°C.

**Preparation of food residues.** Modified atmosphere–packed cooked ham, vacuum-packed smoked salmon, and chopped endives were bought from a local supermarket, kept in a refrigerator at 4 to 7°C and used within 24 h. To prepare a 10%
FIGURE 2. Diagram shows all tests performed in this study. The suspension and carriers tests on smooth surface were performed with or without food residues (w/ or w/o) according to the European standard EN 1040:2005. Tests on the surface with grooves were performed with L. monocytogenes cells on wet and dry conditions in presence and absence of food residues and 2-week food biofilm. The disinfectants used were SDC (240 mg/liter), and Qac (740 mg/liter), while sterile water was used as a control.

Suspension tests (case 1). The tests were performed according to the European standard EN 1040:2005 (6). An overnight culture of L. monocytogenes with or without 10% food residue was added to each disinfectant at recommended concentrations at room temperature for 5 min of contact time. The tests were performed in triplicate for each disinfectant on different days. Figure 2 shows a diagram of all tests that were performed in this study.

Carrier tests. The tests were performed on smooth surfaces (case 2) and surfaces with grooves (case 3). Before the experiments started, the stainless steel coupons and plates with grooves were soaked overnight in anionic-active detergent (Drei; Procter & Gamble Netherlands BV, Rotterdam, The Netherlands). Thereafter, both surfaces were rinsed with hot water (70 to 80°C) and air dried. The coupons were put in a glass container covered with aluminum foil, while the plates were wrapped in paper and then all were sterilized at 121°C for 15 min.

Carrier tests on smooth surfaces (case 2) (4). To inoculate the stainless steel coupons, 10 μl of the test suspensions (~10^9 CFU/ml resulting in ~6.4 log CFU/cm²) were put on one side of the coupon and allowed to air dry for 1 h in a biological safety cabinet. After inoculation of the stainless steel coupons, these were placed in a 12-well plate (Greiner Bio-One BV, Alphen a/d Rijn, The Netherlands) containing 1 ml of SDC (240 mg/liter) or Qac (740 mg/liter) and left at 1-, 2-, and 5-min contact time at room temperature. The experiment was repeated three times on different days for each disinfectant.

Carrier tests on surfaces with grooves (case 3.1). To inoculate the surface with grooves (case 3.1), 10 ml of test suspension (~10^7 CFU/ml, resulting in ~6 log CFU/cm²) with or without 10% food suspension were put on the plates and evenly spread over the surfaces to fully fill the grooves by using a sterile sponge. The plates were then kept at room temperature for 15 min and 24 h to simulate wet and dry conditions, respectively. After inoculation of the plates, 240 mg/liter SDC or 740 mg/liter Qac were sprayed on the plates (5 ml) by using a sterile spray bottle (perfume spray bottle; Etes, Wageningen, The Netherlands) and left to achieve 5-, 10-, 15- or 20-min contact time at room temperature. The experiment was repeated two times on different days for each disinfectant.

Carrier tests on surfaces with grooves in presence of a biofilm (case 3.2). To mimic the situation in a food processing plant with biofilm formation in cracked equipment, a biofilm was grown by daily inoculating a diluted overnight culture of the three L. monocytogenes strains with each food residue (3 ml, ~10^6 CFU/ml) in the grooves for 7 consecutive days. Thereafter, only food suspension (3 ml) was added daily to the grooves for another 7 days to allow further development of the biofilm. During this period, the plates were stored at 15°C in a closed plastic box to maintain stable humidity (~50% relative humidity, measured by a digital hygrothermometer; VWR International BV, Amsterdam, The Netherlands). The disinfectant tests were carried out as described in the previous paragraph, for 20-min contact time.

For the bacteriophage test, 5 ml of a 10^9 PFU/ml phage suspension were sprayed over the biofilm plates. Thereafter, the plates were put in sterile plastic bags (Aseptic vacuum bags, 400 b10 mm; Hevel, Zaandam, The Netherlands) to maintain humidity and left for 30-min contact time.

The tests on surfaces with grooves were performed in duplicate on different days for each disinfectant and for bacteriophages.

Effect of disinfectants on suspended biofilm cells. The 2-week biofilm cells were taken out of each groove by scraping the grooves with sterile extra-thin toothpicks (Jordan; Intec BV, Utrecht, The Netherlands). The cells were mixed with 9 ml PSS, and tested with disinfectants as described in the suspension test.

Microbiological analysis. In the suspension test (case 1), the reaction was terminated after the required contact time by
transferring 1 ml of the suspensions to 9 ml of neutralizer for 1 min. Of these suspensions, 100 μl was then plated on Agar *Listeria* according to Ottaviani and Agosti (ALOA; BioMérieux SA, Marcy l’Étoile, France) using a spiral plater (Eddy Jet; IUL Instrument, SA, Barcelona, Spain). For the carrier tests on flat surfaces (case 2), the disinfectant reaction was terminated by transferring the coupons plus disinfectant to a 9-ml tube containing neutralizer and 4.5-g glass beads (1 mm; Emergo, Landsmeer, The Netherlands). As a blank, inoculated coupons were transferred to 10 ml of PSS plus glass beads. The cells were removed from the coupons by vortexing the tubes for 30 s. Thereafter, 1 ml of the suspension was poured plated in tryptone soy agar.

For the carrier tests on surfaces with grooves (case 3.1), samples were taken using cotton swabs and cotton threads after the required contact time. Cotton swabs made with extra-thin toothpicks and cotton wool (0.1 g, 100% Hygienic cotton; Etos), and cotton threads (27-cm length, 0.75-mm diameter [0.1 g], Super Durable Glanskatoen no. 8; HWS Markoma BV, Ninove, Belgium) were wet sterilized at 121°C for 15 min. The sterile cotton swabs were used to take from the 2-, 1-, 0.5-, and 0.2-mm grooves by swabbing three times through the grooves. The sterile cotton threads were used to take samples from the 5-mm groove by flossing and scraping the groove for 30 s. After sampling, the cotton swabs and cotton threads were put in 9-ml neutralizer, vortexed, and 100 μl were plated on ALOA plates using a spiral plater.

For the carrier tests with bacteriophages (case 3.2), the cotton threads and cotton swabs were soaked for 1 min in a heat killed suspension of *L. innocua* (10^7 CFU/ml) to bind free phage particles as neutralizer. Subsequent sampling and plating were similar as described above. During the 14 days of growing biofilms, the numbers of *Listeria*, aerobic plate count, and lactic acid bacteria were determined at different time intervals on separate plates using ALOA agar, tryptone soy agar, and De Man, Rogosa, and Sharpe Agar (Lactobacillus broth; Merck; and 1.2% [wt/vol] Agar technical, Agar no. 3; Oxoid), respectively.

**Data and statistical analysis.** The volume of each groove (cubic millimeters) was calculated and transformed to milliliters. The amount of organisms in a groove was then divided by this volume to obtain a concentration in log CFU per milliliter. The value was calculated as log CPU per milliliter of the grooves in order to equally compare the results obtained from the grooves that have different depths.

For statistical analysis, the results of duplicate experiments were combined. The significance of differences (P < 0.05) in numbers of *L. monocytogenes* that were reduced by different types of disinfectants, food residues, and types of surface were determined with analysis of the variance and a general linear model using PASW Statistics 17 (SPSS Inc., Chicago, IL).

**RESULTS AND DISCUSSION**

**Suspension tests (case 1).** Reduction of more than 5 log units of *Listeria* planktonic cells and biofilm cells in suspension was observed after 5-min contact time with 240 mg/liter SDC and 740 mg/liter Qac in all test conditions, where there was no significant difference (P > 0.05) in reduction of the cells between the two disinfectants in absence of food residues. In contrast, the effectiveness of SDC (~1-log reduction in 5 min with 3% ultrahigh-temperature-processed milk) was less than that of Qac (5-log reduction in 1 min with 10% ultrahigh-temperature-processed milk) in presence of food residues as expected (21, 25, 46). The effectiveness of SDC and Qac in absence of food residues was higher than in presence of food residues (data not shown). However, these results comply with the European standard EN1040 (6), which indicates a reduction of 5 log units should be obtained after 5-min contact time for an effective disinfectant.

**Carrier tests on smooth surfaces (case 2).** The results from these tests on the stainless steel coupons showed more than a 5-log reduction after 1-, 2- and 5-min contact time with no significant difference (P > 0.05) between SDC and Qac (data not shown). These results complied with the European standard EN13697 requirement (4) for a reduction of 4 log units on clean surfaces indicating an effective disinfectant in the surface test.

**Carrier tests on surfaces with grooves (case 3).** The shape of the 1-mm wide grooves with depths of 0.2, 0.5, 1, 2, and 5 mm used as test surfaces in this study was beyond the capability of standard sampling methods such as surface contact plates and swabbing techniques. Therefore, several methods, e.g. the use of filter paper, cotton swabs, and flossing threads were evaluated for their effectiveness to recover *Listeria* cells from the grooves. The results showed that the flossing technique with cotton thread led to 70% recovery for the 5-mm groove. For the other grooves, this method could recover only 20%. With cotton swabs prepared from extra-thin toothpicks and cotton wool, a recovery of 70% could be obtained for the remaining grooves (data not shown). Therefore, flossing and swab methods were chosen as sampling methods for the 5-mm groove and the other grooves, respectively.

In these tests, the contact times of disinfectant on carriers were 5, 10, 15 and 20 min. Since the inactivation obtained for the different contact times was not significantly different (P > 0.05), which may be due to exhaustion of the disinfectants (3, 51), only the results from the contact time of 5 min are shown. Furthermore, reduction of *L. monocytogenes* was dependent on the groove depth; the deeper the groove, the lower the reduction. This indicates that the activity of the disinfectants stopped due to the inability to reach the cells in the deeper areas. Only the results of the extremes, the 5- and 0.2-mm grooves, are presented in Figures 3 and 4.

All food residues were tested negative for *L. monocytogenes*. In absence of food residues on a wet surface after exposure to water (control) and the disinfectants, a high reduction of the cells was observed in the 0.2-mm grooves, whereas a similar trend but less reduction was observed in the 5-mm grooves. On the dry surfaces, treatment of the cells in the 0.2-mm groove resulted in levels below the detection limit, which can be partly explained by a high reduction observed already as a result of the drying of the plates for 24 h. For the 5-mm grooves, this initial reduction was much lower and not all listerias were inactivated by the treatment. In most cases, there was no significant difference (P > 0.05) between the control and disinfectants. The results demonstrated that drying of surfaces alone could already largely reduce the cells on the surfaces, especially in the shallow grooves where deeper grooves provided suitable
FIGURE 3. Numbers of L. monocytogenes (log CFU per milliliter) in wet conditions for the 0.2- (a) and 5-mm (b) grooves and dry conditions for the 0.2- (c) and 5-mm (d) grooves, in presence and absence of food residues at initial inoculation (T = 0), before (D 15 min or 24 h for wet and dry conditions, respectively) and after exposure to sterile water (control), SDC (240 mg/liter), and Qac (740 mg/liter) for 5 min. Data are means ± standard deviations of duplicate experiments for each disinfectant (n = 2). Dashed lines and vertical arrows (↓) indicate values lower than the detection limit. DL, detection limit of the method.

FIGURE 4. Numbers of L. monocytogenes biofilm cells (log CFU per milliliter) in presence of food residues in 0.2- (a) and 5-mm (b) grooves at initial inoculation (T = 0), before (D 14), and after exposure to sterile water (control), SDC (240 mg/liter), Qac (740 mg/liter), and bacteriophages (10^9 PFU/ml). Data are means ± standard deviations of duplicate experiments for each disinfectant (n = 2). Dashed lines and vertical arrows (↓) indicate values lower than the detection limit.
conditions to prevent complete drying, protecting some of the cells from the disinfectants. Drying of surfaces has been considered as a significant bactericidal method to maintain low numbers of bacteria in any environment and is dependent on type of organism, temperature, relative humidity, and soiling (39, 45, 53). Furthermore, type of surface and topography have been reported to play an important role on efficiency of disinfectants (30, 40), in particular surfaces with scrapes or defects where soil can accumulate and protect bacteria against cleaning and disinfecting agents (16, 17, 51).

In presence of food residues in the wet 0.2-mm grooves, Listeria was reduced 2 to 3 log dependent on types of food residues (Fig. 3a). After exposure to the disinfectants, a reduction to below the detection limit was observed for all food residues with no significant difference (P > 0.05) between SDC and Qac. In the 5-mm grooves before treatment, less reduction (<2 log CFU/ml) was observed. SDC was significantly (P < 0.05) less effective (<2 log) than Qac (≥ 2 log CFU/ml) for all food residues, except fish. In the dry 0.2-mm grooves already a reduction of >3 log CFU/ml was observed 24 h after inoculation without treatment. After exposure to the disinfectants the numbers were further reduced to below the detection limit. In the 5-mm groove, only a small (<0.5 log CFU/ml, i.e., vegetable, fish, and ham residues) or no reduction (milk residue) was observed immediately before treatment probably because the cells were protected from the drying in the deep grooves. Treatment with SDC and Qac did not inactivate all listerias, with no significant difference (P > 0.05) between the two disinfectants except in the case of ham and vegetable residues, where Qac performed significantly better than SDC in the wet grooves and for vegetables in the dry grooves. Previous studies have reported an inactivation of the antimicrobial activity of disinfectants by organic matter on surfaces showing 1- to 2-log reductions on soiled surfaces compared with >3- to 4-log reductions on clean surfaces (2, 34). This could be due to the chemical compositions such as protein and fat, which can bind with the disinfectants or reduction of penetration into the bacterial layer (32, 68). The chlorine-based disinfectants have been found to be easily inactivated by organic materials (25, 46), whereas Qac was reported to be less inactivated by organic matters (21), which was confirmed in most cases in this study. In general, the test on surfaces is dependent on the surface materials used and viability of the cells dried on the surfaces resulting in inconsistent results when compared with the test in suspensions (15). Under practical conditions high amounts of organic materials may be present resulting in less or no activity of the disinfectant. When the concentration of SDC was increased from 240 to 300 mg/liter, a comparable reduction of the cells to that of Qac was obtained; however, there was no effect on reduction in the deep grooves (data not shown). The results indicated that the disinfectant could not reach all cells in the bottom of the groove, while such grooves (5-mm depth, 1-mm width) might be present in practice (e.g., small spaces between connection parts of slicers). The effectiveness of the disinfectants in the deep areas could be increased by using high pressure spraying (32); however, this method might have adverse effects since it could also spread the bacterial cells to other areas and environments, in particular when high numbers of bacterial cells are accumulated (42).

**Carrier tests on surfaces with grooves in presence of a biofilm (case 3.2).** After 14 days of biofilm growing in 0.2-mm grooves (Fig. 4), the number of cells remained more or less stable, with a decrease of the cells in vegetable and ham biofilms (1.5 and 1.8 log CFU/ml, respectively). In 5-mm grooves; however, growth of biofilm cells was observed in all food residues. A previous study reported that L. monocytogenes has the ability to form biofilms at numbers of 10^4 to 10^7 CFU/cm^2 (34) and adheres to a variety of food contact, surfaces such as plastic, glass, rubber, and stainless steel, particularly in pits or crevices that are difficult to clean (31, 61).

To check if the biofilm cells were more resistant to the disinfectants than the planktonic cells, the biofilm cells were brought into suspension, and then tested. The obtained results, a 5-log reduction in all conditions after 5-min exposure to the disinfectants, indicated no difference in reduction of biofilm cells (case 3.2) compared with planktonic cells (case 1). These results complied with a previous study, where reduction of the suspended biofilm cells was higher than that of biofilm cells attached to surfaces after exposure to disinfectants (50). It has been described that a 10 to 100 times higher concentration of disinfectants may be required to obtain the same reduction of biofilm cells compared with planktonic cells (40, 56) indicating the importance of preventing biofilm formation. For the bacteriophages, an efficacy test was previously performed with L. monocytogenes strains in presence and absence of food residues on flat, stainless steel surfaces. The results demonstrated that rapid inactivation of Listeria cells (~2 to 3 log CFU/cm^2) within 30 min of contact time was obtained (data not shown), which was similar with the results of Soni and Nannapaneni (65), where a reduction of 3.5 log CFU/cm^2 was obtained in the same time with a phage treatment on a 1-week-old L. monocytogenes biofilm on stainless steel coupons.

In the 0.2-mm grooves (Fig. 4a), Qac showed better reduction (2 to >3 log) than SDC (<1.2 to 2.7 log) with inactivation below the detection limit in vegetable biofilms. The phages showed higher reduction (>3 log CFU/ml; P < 0.05) than that of water or the chemical disinfectants in three food biofilms, except for the vegetable biofilms where all cells were inactivated by every disinfectant. In the 5-mm grooves, SDC and Qac did not reduce Listeria better than water alone. However, the phages reduced approximately 1.4 log CFU/ml in ham and fish biofilms, and approximately 0.8 log CFU/ml in milk and vegetable biofilms, which was not significantly different (P > 0.05) from that of the disinfectants, except for ham biofilms. The results indicated that only in the 0.2-mm grooves the phages were likely to be more effective in the biofilms with food matrix than the chemical disinfectants. This was probably due to the fact that the disinfectants and phages did not penetrate
the food/bacterial matrix completely in the deep groove (20, 41). Furthermore, the effectiveness of the disinfectants on *L. monocytogenes* biofilms may be reduced by presence of other bacteria (~6 to 7 log CFU/ml) including lactic acid bacteria (~4 log CFU/ml) in the grooves as well (data not shown). Phage efficacy depends on susceptibility of the bacterial cells to the phages and availability of receptor sites between phages and the bacterial cells (13), which could be blocked by the food matrix (35). In this study, the phage efficacy was enhanced by keeping the biofilm plates in plastic bags after application of the phages to maintain a high humidity that would enhance the diffusion and the chance of contact between *Listeria* cells and phage particles (66).

Strikingly, the biofilm removal by sterile water (control) resulted in a similar reduction of the biofilm cells compared with disinfectants. In the present study, the tests were performed to simulate survival of bacteria in presence or absence of organic matter in pits or cracks on food contact surfaces and to test the effect of disinfection, therefore cleaning of the surfaces before using disinfectants was excluded. So in this case, plates and grooves were dirtier than soiled surfaces in reality, which explains the observed low efficacy of the disinfectants, and the water washed away the biofilm cells from the grooves. Rinsing the biofilm on surfaces by water has been reported to reduce the biofilm cells at about 1 log unit of cells (34).

The results in this study clearly demonstrated that presence of grooves or spaces on surfaces, humidity, and presence of food substrates influenced the antimicrobial effect of disinfectants and bacteriophages. Bacteriophages showed a better antimicrobial effect than the chemical disinfectants (i.e., SDC and Qac), in the shallow grooves but not in the deep grooves. Use of bacteriophages as a biocontrol could only be a promising method in limited cases; however, use of large quantities in practice may be costly and phage-resistant strains may occur (35, 62).

**ACKNOWLEDGMENTS**

The authors thank Diversey Inc. (Utrecht, The Netherlands), who provided the chemical disinfectants for this study. We also thank Micreos Food Safety BV (Wageningen, The Netherlands) for *Listeria* bacteriophage Listex P100 and *L. innocua* (serovar 5). Our great appreciation goes to Feifei Gao for her technical assistance in the phage experiments, and Hilda Nyati for her work on smooth surfaces.

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