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Survival of *Listeria monocytogenes* on conveyor belt material with/without antimicrobial additives

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**Running title:** Survival of *Listeria monocytogenes* on conveyor belt

**Keywords:** *Listeria monocytogenes*, survival, conveyor belt, antimicrobial additives

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ABSTRACT

Survival of *L. monocytogenes* on conveyor belt material with and without antimicrobial additives was investigated. In these experiments, also the effect of food debris (meat, fish and vegetables) and temperature (10, 25 and 37°C) was evaluated. The pathogen survived best at 10°C, followed by 25°C and 37°C on both conveyor belt materials. The reduction rate of the pathogen on conveyor belts with antimicrobial additives in the first six hours at 10°C (0.6 log unit) was significantly higher than that on the surfaces without the additives, or in presence of food residue (0.2-0.3 log unit). At high temperature (37°C) and low humidity (20%), a rapid decrease in numbers of the pathogen on both conveyor belt surfaces was observed. Under these conditions, an effect of antimicrobial substances could not be noticed. However, at ambient (25°C) and low (10°C) temperature and high humidity (60-75%), a rapid decrease in cell counts was observed. These results demonstrated that temperature and relative humidity play an important role in the reduction of the bacteria on the surfaces. Antimicrobial conveyor belts reduced numbers of *L. monocytogenes* faster than normal conveyor belts. However, this effect is limited on dry surfaces, where a reduction took place at lower rate than that in the first six hours when the surfaces were still wet. Moreover, the presence of food debris neutralized the effect of antimicrobials. The results suggested that the antimicrobial additives in the conveyor belt could help to reduce numbers of microorganism in particular at low temperature and in an absence of food residues.
1. Introduction

Contamination of *Listeria monocytogenes* on ready-to-eat cooked meat products most likely occurs after thermal processing. Many studies report that *L. monocytogenes* attaches to various types of surfaces in food processing plants (Beresford et al., 2001; Lunden et al., 2002) such as stainless steel, glass, polypropylene and rubber (Mafú et al., 1990; Wong, 1998). The bacteria survive and grow on spots that are difficult to clean and disinfect due to their poor designs, such as slicing machines, conveyor belts (Lin et al., 2006), and narrow openings between surfaces and apparatus (Tompkin et al., 1992). Of the many processing surfaces, conveyor belts are of great concern in food processing because during transfer of food products the belts are often contaminated with high numbers of microorganisms including foodborne pathogens such as *L. monocytogenes* (Thévanot et al., 2005) and *Escherichia coli* O157:H7 (Keeratipibul et al., 2009). Inadequate cleaning and disinfection procedures can cause accumulation of bacteria on surfaces and formation of biofilms, which are difficult to control or remove by conventional cleaning methods (Chmielewski and Frank, 2003; Smoot and Pierson, 1998). However, recontamination from contaminated surfaces to food products can be prevented by hygienically designed apparatus, cleaning and disinfection and zoning (Anonymous, 1991). At present, it is possible to add antimicrobial additives (e.g. triclosan, silver) to surface materials to control the survival or growth of microorganisms on the surfaces. Triclosan and silver are GRAS (generally recognized as safe) and approved by the U.S. Food and Drug Administration for their safety (Jones et al. 2000) and are currently used in medical devices, food processing, and building materials (Feng et al., 2000). Several studies have been published about the effect of antimicrobial additives in surface materials used in the food processing industry against microorganisms, e.g. antimicrobials incorporated in cutting boards, food containers (Wijnhoven et al., 2009) and the inner liner in refrigerators (Kampmann et al., 2008). However, few studies have investigated survival of microorganisms...
on antimicrobial surfaces in comparison to normal surfaces in practical situations of low and ambient temperatures or with food residues.

In this study, the survival of three strains of *L. monocytogenes* was studied on conveyor belt materials with and without antimicrobial additives at different temperatures and in the presence and absence of food residues.

2. Materials and methods

2.1 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) obtained from the Food and Consumer Product Safety Authority (Zutphen, the Netherlands) were used in this study. Stationary-phase cultures of all strains were stored in 1 ml cryo vials (Greiner Bio-one, Frickenhausen, Germany) with 25% (v/v) glycerol (Fluka-Chemica, Buchs, Switzerland) and glass beads (2 mm, Emergo, Landsmeer, the Netherlands) at -20°C. For each experiment, one glass bead of each strain was transferred to tryptone soy agar plates (TSA, Oxoid, UK), which were incubated at 30°C for 24 h. A single colony was picked, inoculated into 10 ml brain heart infusion broth (BHI; Difco, Becton Dickinson, Maryland, USA) and incubated for 72 h at 10°C or for 24 h at 25°C and 37°C.

2.2 Conveyor belt material (US Patent 6994209)

Two types of food grade conveyor belt materials were used in this study. The control conveyor belt material without antimicrobial additives (CM-) (FNB-6EZCT, Habasit Netherlands BV, Nijkerk, the Netherlands) was composed of polyester fabric impregnated with thermoplastic polyurethane (TPU). The conveyor belt material with antimicrobial additives (CM+) (FNB-6EZCT+H14, Habasit Netherlands BV, Nijkerk, the Netherlands) had
the same composition and was impregnated with thermoplastic polyurethane (TPU) and the antimicrobial compound HyGUARD®. According to the US patent 6994209 (Cediel et al., 2006), antimicrobial substances used are silver zeolite, aluminum oxide, calcium oxide, magnesium oxide, zinc pyrithione, oxybisphenoxarsine or a combination of those. The conveyor belt surfaces were cut from the roll in sheets of 10 x 10 cm.

2.3 Food debris

Raw food (minced meat, smoked salmon and chopped lettuce) were bought from a local supermarket, kept in a refrigerator (< 7°C) and used within 24 h. To prepare a 10% suspension of the food debris, 10 g of each selected food was aseptically placed in Stomacher® bags and 90 ml peptone saline solution (PSS: 8.5 g/l NaCl (Merck, Darmstadt, Germany) and 1 g/l Neutralised Bacteriological Peptone (Oxoid, Basingstoke, England)) was added. The food was then homogenized in a stomacher (Stomacher® 400 Circulator, England) for 1 min at 260 rpm.

2.4 Artificial contamination of the surfaces

The conveyor belt sheets were washed with anionic-active detergent, rinsed with hot water (70-80°C) and air dried. Before each experiment, the belt surfaces were disinfected with 70% ethanol for 15 min and air dried for 15 min in a laminar flow cabinet. Appropriate serial dilutions of the overnight cultures were prepared in PSS. For the selected final dilutions (approximately 10^6 CFU/ml), PSS containing 0.1% Tween 80 (Merck, Hohenbrunn, Germany) was used to lower the surface tension in order to obtain an even spread of bacteria on the surfaces. The influence of food debris on the antimicrobial action was investigated by preparing the final dilution of the test cultures in a 10% suspension of the food products (with 0.1% Tween 80). From these tubes with and without food debris, 250 μl was spread over 100 cm² of the belt surfaces by using a cotton swab (Sterile wooden/cotton
swab, LP Italiana SPA, Milano, Italy). The conveyor belt sheets were then placed at 10°C, 25°C and 37°C for 72 h. Relative humidity (Rh) was measured at each storage temperature with a digital hygro-thermometer (VWR International BV, Amsterdam, the Netherlands).

2.5 Enumeration of test strains from the conveyor belt sheets

The conveyor belt surfaces were sampled after 0, 6, 24, 48 and 72 h by rubbing 10 x 10 cm areas with sterile cotton swabs (EUROTUBO® collection swab, Delta lab, Rubi, Spain) stored in 5 ml of buffered peptone water (BPW) according to ISO 18593:2004 (Anonymous, 2004). The swabs were reinserted in the BPW, vortexed and resuscitated for one hour. After that, 2 x 1 ml of the swab solution was added to 2 tubes with 2 ml peptone saline solution (PSS). The contents of these tubes (3 ml) were plated onto two Petrifilm™ Environmental Listeria plates (3M Company, St.Paul, MN, USA.). All plates were incubated at 37°C for 24-30 h, and the numbers of recovered L. monocytogenes were determined and transformed into log CFU per 100 cm².

Reduction rates of L. monocytogenes (log CFU/100 cm²/h) were calculated by dividing the reduction in cells counts on the surfaces in times by lapse times (h), which were 6 h from the first six hours and 66 h from 6 to 72 h.

2.6 Statistical analysis

The results of triplicate experiments were combined. The significance of difference in survival of L. monocytogenes (P < 0.05) on types of the surfaces, Listeria strains, food residues, and temperatures was determined with ANOVA and a general linear model using SPSS for Windows 98/NT/2000 release 15. A balance of input (initial concentration of inoculum) and output (number of bacteria on the tested surfaces before and after sampling) was calculated.
3. Results and discussion

As can be seen in Fig. 1 (A, B and C), a rapid decrease of 2.5 - 4 log CFU/100 cm² of the test strains on the test surfaces was observed during the first six hours. Thereafter, the reduction was only 0.1 log unit in 6 h. These results were similar with those of Kusumaningrum et al. (2003) and may be attributed to the stress conditions during the rapidly decreasing water activity. Despite the rather high standard deviations found in our experiments, it becomes clear from Fig. 1 (A-B) that at lower temperatures (25 and 10°C), the cells were significantly faster inactivated on the antimicrobial belt surfaces compared to the normal belt surfaces ($P<0.05$). The high reduction rate during the first 6 h demonstrated that the antimicrobial additives in the test surfaces were more active in wet than dry conditions. From the moment the surface was dry, the remaining cells were protected by the low water activity (McEldowney and Fletcher, 1988). Another explanation could be the fact that in a broth culture, a fracture of the population might be resistant against detrimental influences such as low pH, low water activity, and low concentration of preservatives or biocides (Wesche et al., 2009). A temperature close to the optimum growth temperature of the microorganism gave a more rapid inactivation and no extra effect of the antimicrobial belt could be demonstrated (Fig.1C). At 37°C, the surfaces were dry within one hour, whereas these took 2 and 4 hours to dry at 25 and 10°C, respectively. This emphasizes the benefit of thorough drying of surfaces directly after cleaning and disinfection.

The ability of bacteria to survive on food contact surfaces has been reported previously (Bremer et al., 2001; Wilks et al., 2006; Wong, 1998) and is influenced by bacterial strain, temperature, time, humidity, availability of nutrients, pH, presence of inhibitors and surface materials (Allan et al., 2004; Beumer and Hazeleger, 2003). All three $L.\ monocytogenes$ strains used in this study showed the same pattern in survival on the two surfaces. The recovery of the test organisms from the surfaces was approximately 70-75%, whereas 15%
was lost during spreading of the test organism and a small proportion of the cells (10%) remained on the surfaces after sampling (data not shown).

During the experiments we measured the Rh; however, it was not a set variable. At high temperature (37°C), the Rh was low at around 20%. This combination of high temperature and low Rh, together with the fast drying of the surfaces, resulted in a rapid decrease in numbers on both types of the conveyor belt surfaces (Fig. 1C). This phenomenon was also described in a rapid reduction of *Pseudomonas fluorescens* at 35°C on antimicrobial-containing inner liners in refrigerators (Kampmann et al. 2008). At ambient (25°C) and low (10°C) temperature, the Rh in our experiments showed high values (60-75%). These combinations resulted in a better survival of the target organism on the belt surfaces, probably because more time was needed to obtain a completely dry surface (2-4 h). Furthermore, it is known that *L. monocytogenes* can survive in stress conditions such as at the low temperature (2-4°C) (Rocourt and Cossart, 1991) or in low water activity (0.92) (Petran and Zottlola, 1989) or in less oxygen in vacuum package (Walker et al., 1990). Regarding to survival of *L. monocytogenes* at low temperature, it is due to the fact that changes in temperature results in changes in membrane composition to maintain the optimum fluidity for growth at low temperature (Beales, 2004). This is an important aspect, since these conditions can be found in many food production areas. Similar results were found in a study of antibacterial substances in a kitchen, where the bacterial counts on antimicrobial surfaces were significantly lower than those on conventional surfaces (Sadako et al., 2001).

Presence of 10% food debris (minced meat, smoked salmon and chopped lettuce) decreased the antimicrobial effect and protected the cells. In table 1, the results obtained at 10°C are presented. On the antimicrobial belt, a decrease in numbers of the test strains on the antimicrobial belts (log 0.63/100 cm² per h) was faster than that on the normal belts (log 0.38/100 cm² per h). In the presence of food debris, however, even in the low concentrations
(10%) used in this experiment, the decrease is reduced to only log 0.2-0.3/100 cm² per h and no statistically significant difference (P>0.05) was found in the survival of *L. monocytogenes* on both types of the materials.

In comparison to lettuce, *Listeria* survived better in the presence of meat and fish residues. This may be attributed to a higher concentration of protein and fat in these products (Vorst et al. 2006). Moreover, amino acids in meat and fish can bind to the antimicrobial additives released from the surfaces, and in this way enhances the survival of the pathogen (Liau et al., 1997). Similar results were found in several studies, where milk and chicken or meat suspensions provided a protective effect on *Salmonella enteritidis* and *Campylobacter jejuni* on stainless steel surfaces (Kusumaningrum et al., 2003), on *L. monocytogenes* and *Yersinia enterocolitica* on a mortar (Allan et al., 2004) and on *L. monocytogenes* and *Salmonella typhimurium* on stainless steel and buna-N rubber (Helke and Wong, 1994).

It is not fully clear which antimicrobial additives were present in the test surfaces in our study. The US Patent (Cedeil et al., 2006) indicated that the additives used in the conveyor belt might be silver zeolite, aluminum oxide, calcium oxide, magnesium oxide, zinc pyrithione or oxybisphenoxarsine. Some of these substances demonstrated an antimicrobial effect against *E. coli* and *L. monocytogenes* on surfaces (Cowan et al., 2003) and in suspensions (Chang et al., 2008). Relevant data obtained from the literatures demonstrated the effect of those antimicrobial additives in surface materials (Kampman et al. 2008, Cowan et al., 2003). However, the antibacterial effect was considerably greater in suspension (Chang et al., 2008) than that on surfaces (Cowan et al., 2003). Furthermore, longer contact time and lower temperature also resulted in a greater reduction. Studies on the antimicrobial effect of silver-containing inner liners in refrigerators demonstrated that reduction of bacterial numbers on the silver-containing surfaces at 35°C was one log unit faster than at 5°C, when compared 24 h with 72 h (Kampman et al. 2008). Our results agreed with the previous study, in which
the test at 10°C required a longer contact time (72 h) than at 37°C (24 h) to achieve a similar reduction after exposure to the antimicrobial surfaces. However, it becomes clear from the previous studies that even for the same antimicrobial agent results may vary considerably (Cowan et al., 2003, Chang et al., 2008). With the set up of the testing procedure, choice of test strain, concentration of the antimicrobial agent in the material, temperature, relative humidity, suspension-/carrier tests, the reducing effect of the antimicrobial agents will be influenced.

In conclusion, the presented results demonstrate that the antimicrobial additives in the conveyor belt material can help to reduce survival of microorganism on the surfaces. However, the effectiveness of the antimicrobial additives was reduced in presence of food debris. Although the additives in the surfaces are not known, the results were comparable with previous studies. Additional studies are required to examine the effectiveness of the antimicrobial conveyor belt on the other microorganisms such as *E. coli* and *Salmonella* spp.

**Acknowledgements**

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**References**


Fig. 1. Survival of *L. monocytogenes* on conveyor belt material with (◊: CM+) or without (▲: CM-) incorporated antimicrobial additives at 10°C (A), 25°C (B) and 37°C (C). (Means of three *L. monocytogenes* strains ± standard deviation (n = 3), DL: detection limit.)
Reduction rates of *L. monocytogenes* (log CFU/100 cm$^2$/h, mean of three *L. monocytogenes* strains ± standard deviation, n=3) on conveyor belt material with (CM+) and without (CM-) antimicrobial additives in presence of 10% food debris stored at 10°C for 72 h.

<table>
<thead>
<tr>
<th>Type of food debris</th>
<th>CM+ 0-6 h</th>
<th>CM+ 6-72 h</th>
<th>CM- 0-6 h</th>
<th>CM- 6-72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.63 ± 0.02$^a$</td>
<td>0.02 ± 0.00</td>
<td>0.38 ± 0.06$^b$</td>
<td>0.03 ± 0.00</td>
</tr>
<tr>
<td>Minced meat</td>
<td>0.22 ± 0.05$^c$</td>
<td>0.03 ± 0.01</td>
<td>0.26 ± 0.03$^c$</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Smoked salmon</td>
<td>0.23 ± 0.01$^c$</td>
<td>0.03 ± 0.01</td>
<td>0.26 ± 0.03$^c$</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Chopped lettuce</td>
<td>0.33 ± 0.04$^c$</td>
<td>0.03 ± 0.01</td>
<td>0.31 ± 0.11$^c$</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

$^a$ Different letters in the same row or column are significantly different ($P = 0.05$).

$^b$ Reduction rates were calculated at two different time intervals (first 6 h and 6-72 h).