LISTERIA MONOCYTOGENES: BIOFILM IN FOOD PROCESSING

Article *in* Italian Journal of Food Science · January 2012

1 author:

Pierluigi Di Ciccio
Università degli studi di Parma
26 PUBLICATIONS **50** CITATIONS

SEE PROFILE

REVIEW

LISTERIA MONOCYTOGENES: BIOFILMS IN FOOD PROCESSING

P. DI CICCIO[1]*, M. CONTER[1], E. ZANARDI[1], S. GHIDINI[1], A. VERGARA[2], D. PALUDI[2], A.R. FESTINO[2] and A. IANIERI[1]

[1]University of Parma, Dept. of Animal Production, Veterinary Biotechnologies, Food Quality and Safety, 43100 Parma, Italy

[2]University of Teramo, Dept. of Food Science, 64100 Teramo, Italy

*Corrsponding author: pierluigialdo.diciccio@nemo.unipr.it

ABSTRACT

Contamination of food by *Listeria monocytogenes* (*L.m*) frequently occurs in food processing environments, where cells persist due to their ability to attach to surfaces. *L.m* is able to attach to and colonize environmental surfaces by producing a three-dimensional matrix of extracellular polymeric substances (EPS) called biofilm; such structures are dynamic systems. Once established, biofilms can serve as a source of product contamination. Moreover, *L.m* in the biofilm state shows a reduced susceptibility to antimicrobial agents. The present review focuses on *L.m* biofilms in food processing environments. In addition, some aspects of biofilm control and eradication are highlighted.

- Keywords: biofilm, food industry, food safety, *Listeria monocytogenes* -

INTRODUCTION

Listeria monocytogenes is a ubiquitous facultative intracellular bacterium that is potentially pathogenic to humans (FARBER and PETERKIN, 1991). This pathogen is frequently isolated from a variety of food products and also from soil, vegetation, fecal matter, sewage, water, and animal feed (DONNELY, 2001; MOLTZ and MARTIN, 2005; TOMPKIN, 2002). Meat, poultry, dairy, vegetables, and ready to eat (RTE) products have all been implicated as vehicles of listeriosis (CONTER *et al.*, 2009a; EFSA, 2007; TEIXEIRA *et al.*, 2007). In fact, ingestion of food contaminated with *L.m* is the primary route of transmission to humans (DUSSURGET, 2008).

Although the incidence of listeriosis is low, it remains a public health concern because of its high mortality rate (20-30%) (SUTHERLAND *et al.*, 2004). *L.m* strains show heterogeneous levels of virulence (LIU *et al.*, 2007) that are regulated by the presence of pathogen-specific genetic factors, which affect the outcome of human and animal infection (CONTER *et al.*, 2009a; THEVENOT *et al.*, 2006). Additional factors, however, may contribute to the pathogenesis potential (CHATTERJEE *et al.*, 2006). *L.m* is able to colonize surfaces, and particular subtypes may persist in food production plants for several years (PIETTE and IDZIAK, 1991). One reason for this persistence may be the ability of *L.m* to form biofilms (SCHLECH *et al.*, 1983). Biofilms are assemblages of microorganisms that adhere to each other and/or to a surface and are embedded in a matrix of exopolymers (MORRIS and MONIER, 2003). Particularly, biofilms allow microorganisms to persist in the environment and resist desiccation, UV light, and treatment with antimicrobial and sanitizing agents (DI BONAVENTURA *et al.*, 2008).

BIOFILM FORMATION

The term "biofilm" was created to describe the sessile form of microbial life, characterized by adhesion of microorganisms to biotic or abiotic surfaces, with consequent extracellular production of polymeric substances (MUSK *et al.*, 2005). It is now generally accepted that bacteria grow preferentially as biofilms (HALL-STOODLEY and STOODLEY, 2005). In fact, in nature and food systems, microorganisms are attracted to solid surfaces. Initially, microorganisms are simply deposited; later, they get attached, grow, and actively multiply to form a colony of cells (ALLISON and SUTHERLAND, 1987). This mass of cells then becomes large enough to entrap organic and inorganic debris and other microorganisms, leading to the formation of a microbial biofilm. In food processing environments, these biofilms may be a few micrometers or several millimeters thick and contain 90-97% water (STOODLEY *et al.*, 2002). Biofilm development can occur on almost any surface in any environment in which viable microorganisms are present. Biofilm formation is a dynamic process and involves a series of steps. Initial attachment is via weak electrostatic and van der Waals' forces and occurs rapidly; but, within a short time, film growth begins with physical attachment of the cells to the surface by complex polysaccharides (OLIVEIRA *et al.*, 2010). In particular, the first step of biofilm formation is conditioning of the surfaces (HOOD and ZOTTOLA, 1997). This conditioning film favors biofilm formation and alters the physicochemical properties of the surface (e.g., changes in hydrophobicity and electrostatic charges) (DICKSON and KOOMARAIE, 1989). The second step of biofilm formation is the attachment of microorganisms to the conditioned surface. During this stage, bacteria can easily be removed. The irreversible attachment of cells happens in the third step, and the removal of cells requires much stronger forces. During this period, the attached cells also produce additional extracellular polymeric substances (EPS) that help anchor the cells to the surface (COSTERTON *et al.*, 1995). Multilayers of bacterial cells entrapped within the EPS-containing matrices develop within the biofilm. Besides EPS and microbial cells, biofilms are composed of proteins, nucleic acids, and lipids, which provide mechanical stability, surface adhesion, and scaffold formation for the three-dimensional architecture that interconnects and immobilizes biofilm cells (FLEMMING and WINGENDE, 2010). However, diverse polymers are used by different species or strains of the same species. The identification of these matrix components may provide clues for the identification and application of matrix-degrading enzymes that prevent formation and/or activate biofilm dispersion (KARATAN and WATNICK, 2009; LANDINI, 2009). Microorganisms within the biofilm grow in the matrix-enclosed microcolonies interspersed within highly permeable water channels (COSTERTON *et al.*, 1994). At some point after film formation, the attached bacteria or pieces of film are released in order to allow the cells to survive and colonize new niches. This release leads to contamination of the product stream. The vegetative cells may reattach in downstream parts of the plant and initiate biofilm formation, completing the cycle. When planktonic cells attach to a surface, there may be a lag before growth commences as they adapt to the sessile state. The cells released from the film may behave like biofilm cells when they reattach, showing no lag and growing more rapidly than primary cells (RASMUSSEN *et al.*, 2005). The drivers for biofilm formation are the following: defence against harmful conditions; colonization of a favorable niche; utilization of potential benefits of the community; protection from the bulk phase environment, where there may be toxins, antibiotics, detergents, or sanitizers; the possibility of organization through inter-cell

signalling; and transfer of genetic information, including resistance genes (JEFFERSON, 2004).

BIOFILMS IN THE FOOD ENVIRONMENT

Microbial adhesion and biofilms are of great importance for the food industry and occur on a variety of food contact surfaces (OLIVEIRA *et al.*, 2007). Biofilms continue to pose concerns to food manufacturers as they are one of the major reasons for limiting the shelf life and favoring pathogen contamination of food products. Moreover, biofilms form a reservoir of contamination that persists where manufacturing plant cleaning is ineffective (SENCZEK *et al.*, 2000). Depending on the specific systems investigated and the nature of the microorganism, biofilms can display a wide range of phenotypes. *L.m* biofilms, grown under static conditions, generally consist of a homogeneous layer of cells and/or microcolonies, with biofilm cells displaying morphology similar to that of planktonic cells. In contrast, *L.m* biofilms grown under continuous flow conditions consist of spherically shaped microcolonies that are surrounded by a network of knitted chains composed of elongated cells (RIEU *et al.*, 2008).

Studies have shown the capacity of *L.m* to persist in the environment for years. Biofilms form not only on processing environment surfaces but also on food itself, which offers the potential for cross-contamination and post-process contamination (KUMAR and ANAND, 1998). Moreover, environmental surfaces such as floors and walls may also be indirect sources of contamination, e.g., transference to food products by vectors such as air, people, and cleaning systems (GIBSON *et al.*, 1999). In food systems, improperly cleaned and sanitized equipment and airborne microbiota are usually considered important niches of contamination (Fig. 1) (SIMÕES and VIEIRA, 2009). Other common sources involved in biofilm accumulation include floors, waste water pipes, bends in pipes, rubber seals, conveyor belts, stainless steel surfaces, glasses, etc. Buna-N and Teflon seals have also been implicated as important sites for biofilm formation (FRANK and KOFFI, 1990; RODAS-SUAREZ *et al.*, 2006). HERALD and ZOTTOLA (1988) observed that *L.m* can attach to stainless steel via produced attachment fibrils. In meat and dairy processing industries, the presence of *L.m* has been found on equipment and utensil surfaces (LÓPEZ *et al.*, 2008).

Importantly, bacterial biofilms have been consistently described as being more resistant to biocides than planktonic cells (GILBERT *et al.*, 2002; SCENHIR, 2009). The reasons for this decrease in susceptibility or "tolerance" is a biofilm-associated phenotype (ASHBY *et al.*, 1994; BROWN and GILBERT, 1993; DAS *et al.*, 1998), which includes decreased metabolism, quiescence, reduced penetration due to the extracellular polymeric matrix (PAN *et al.*, 2006), and enzymatic biocide inactivation (SCENHIR, 2009).

In nature, biofilms may be composed of a single species or represent a consortium of numerous species. Several types of microorganisms (spoilage or pathogenic) are capable of participating in adhesion processes and biofilm formation. Mixed species biofilm formation of several *Staphylococcus aureus* strains with *L.m* has been demonstrated (RIEU *et al.*, 2008). In addition, some researchers have found that *L.m* grows preferably as a biofilm as part of a consortium of bacterial species (JEONG and FRANK, 1994a-b; KALMOKOFF *et al.*, 2001). Other reports examining longer-term biofilm formation have noticed that *L.m* is a poor organism for cell attachment and biofilm formation, and this has led to suggestions that *L.m* may use a primary colonizing bacterium of a different bacterial species to form a biofilm consortium on a surface (MOLTZ and MARTIN, 2005). CARPENTIER and CHASSAING (2004) and TOMPKIN (2002) showed that the "house flora" of food processing premises has a strong effect on the likelihood of finding *L.m* on inert surfaces. A study on mixed biofilm formation of *L.m* in combination with various secondary species showed that mixed species biofilms were established, and depending on the specific combination, they showed increased, reduced, or no effect on the number of *L.m* cells in the biofilm (CARPENTIER and CHASSAING, 2004). Furthermore, an elaborate study on the formation of mixed species biofilms of *L.m* and *Lactobacillus plantarum* showed that mixed species biofilms have the capacity to be more resistant against disinfectant treatments than a single species biofilm or planktonic cells (VAN DER VEEN and ABEE, 2010). Another interesting study on the adherence of *L.m* to preformed *Lactococcus lactis* biofilms with different architectures, matrices, and cell surface properties showed that *L.m* biofilm formation can be influenced by resident biofilms (HABIMANA *et al.*, 2009). The impact of secondary species on *L.m* settlement, biofilm for-

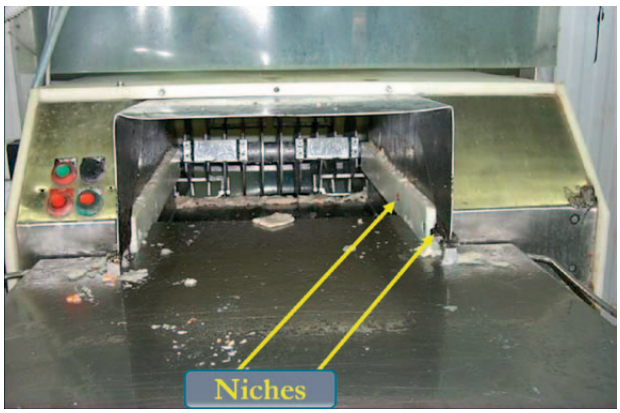

Fig. 1 - Example of possible niches of *L. monocytogenes* in a battering and breading machine.

mation, and persistence in food processing environments remains to be characterized.

Bacterial attachment to surfaces is influenced by the physicochemical properties of both the surface and microorganism, such as temperature and hydrophobicity (GORSKY *et al.*, 2003; HOOD and ZOTTOLA, 1997). It has been suggested that flagellation and motility play a role at the cell level in several stages of biofilm formation (MOENS and VANDERLEYDEN, 1996). Motility has been shown to facilitate attachment to both biotic and abiotic surfaces (BUTLER *et al.*, 1979), since flagella, in addition to being the locomotive organelles of bacterial motility, have also been reported to serve as adhesive structures (MOENS and VANDERLEYDEN, 1996). With regard to food surfaces, many studies have shown the attachment of *L.m* to meat and poultry surfaces (CHUNG *et al.*, 1989; DICKSON and KOOHMARAIE, 1989; THÉNEVOT *et al.*, 2006). However, these studies did not clearly reflect the formation of biofilms with regard to these surfaces.

LISTERIA MONOCYTOGENES AND BIOFILM

L.m can adhere rapidly and firmly to inert surfaces commonly found in the food processing industry. This bacterium has been found to form biofilms on surfaces such as plastic, polypropylene, rubber, stainless steel, and glass and on the interface between two different materials (e.g., plastic and stainless steel) (CHAE *et al.*, 2006; HOOD and ZOTTOLA, 1997; MOLTZ and MARTIN, 2005; SIMÕES *et al.*, 2010). *Listeria* sources in processing plants include conveyor belts, cutters, slicers, bringing and packaging machines, coolers, freezers, floors, and drains (D'ORIO *et al.*, 2007; EKLUND *et al.*, 1995; MIETTINEN *et al.*, 2001).

The mechanisms by which *L.m* survives under harsh physical and chemical stress conditions are, at least in part, due to its ability to form biofilms on surfaces within the food processing environment (CHAE *et al.*, 2006; HOLAH *et al.*, 2004). Several researchers have reported that *L.m* is not capable of forming thick biofilms made up of several layers (9 to 12 Log CFU/cm^2), but rather it adheres to surfaces at levels ranging from 4 to 6 Log CFU/cm^2 (GRAM *et al.*, 2007).

The ability of *L.m* to produce biofilm significantly differs according to the growth temperature and growth surface. For example, *L.m* has the capacity to adhere rapidly to stainless steel surfaces, and this risk is aggravated considering that this bacterium is able to reach an irreversible stage in a few hours (OH and MARSHALL, 1996). The ability to form biofilms at 4°, 12° and 22°C is significantly more pronounced on glass than on polystyrene and stainless steel. Furthermore, at 37°C, both stainless steel and glass allow comparable amounts of biofilm formation, significantly higher than on polystyrene (Figs. 2 and 3) (DI BONAVENTURA *et al.*, 2008). The ability of *L.m* to colonize a surface at low temperatures, used in the food industry to process and store a significant amount of foodstuffs, added to the recent finding that *L.m* cells in biofilms can survive storage at 4° or 10°C for at least 5 days (SMITH *et al.*, 2004), increases the propensity for cross-contamination to foodstuffs.

Recent studies have shown significant differences in biofilm formation on polyvinyl chloride (PVC) (BORUCKY *et al.*, 2003; DJORDJEVIC *et al.*, 2002) or stainless steel (FOLSOM and FRANK, 2006) between different lineages, although the findings were contradictory (CHAE *et al.*, 2006; DJORDJEVIC *et al.*, 2002). DI BONAVENTURA *et al.* (2008) did not find any relationship between phylogeny and the ability to form a biofilm on different surfaces at different temperatures. Furthermore, no differences among environmental and food strains were observed in the experiment. At the same time, differences in biofilm production have been shown (KALMOKOFF *et al.*, 2001; PALMER *et al.*, 2007): serotype 1/2c forms a higher average amount of biofilm than serotype 1/2a on stainless steel and serotypes 1/2a and 4b on glass, at 37°C.

Food contact surfaces have different physicochemical characteristics: hydrophilic (glass, stainless steel) and hydrophobic (polystyrene). Some studies have failed to find a correlation between hydrophobicity and surface attachment. Particularly, CHAE *et al.* (2006) recently found that attachment on glass is independent of hydrophobicity level. Other authors (DI BONAVENTURA *et al.*, 2008) suggested that the hydrophobicity level is correlated with biofilm formation on glass. These conflicting results are probably due to the fact that hydrophobicity can differ between serotypes or strains, and it can change with variation in growth conditions (GIOVANNACCI *et al.*, 2000).

The cell surface is generally considered a significant factor in bacterial attachment to surfaces. Many studies suggest that microbial cell surface charge and hydrophobicity play an important role in the initial steps of microbial adhesion. CHAE *et al.* (2006) demonstrated that high levels of extracellular carbohydrates produced by *L.m* increase their ability to form biofilms, indicating the importance of this characteristic for the biofilm forming ability of a given strain.

Bacterial attachment to surfaces is influenced not only by cell surface and hydrophobicity but also by the presence of particular surface appendages such as flagella and fimbriae. Very little is known about the relationship between motility and biofilm formation and, in any case, only swimming motility has been considered. Recent findings suggest that Gram-positive organisms, including *L.m*, have evolved multiple molecular strategies for the formation of pili on microbial surfaces (HUNG and SCHNEEWIND, 2004). Furthermore, DONS *et al.* (2004) showed

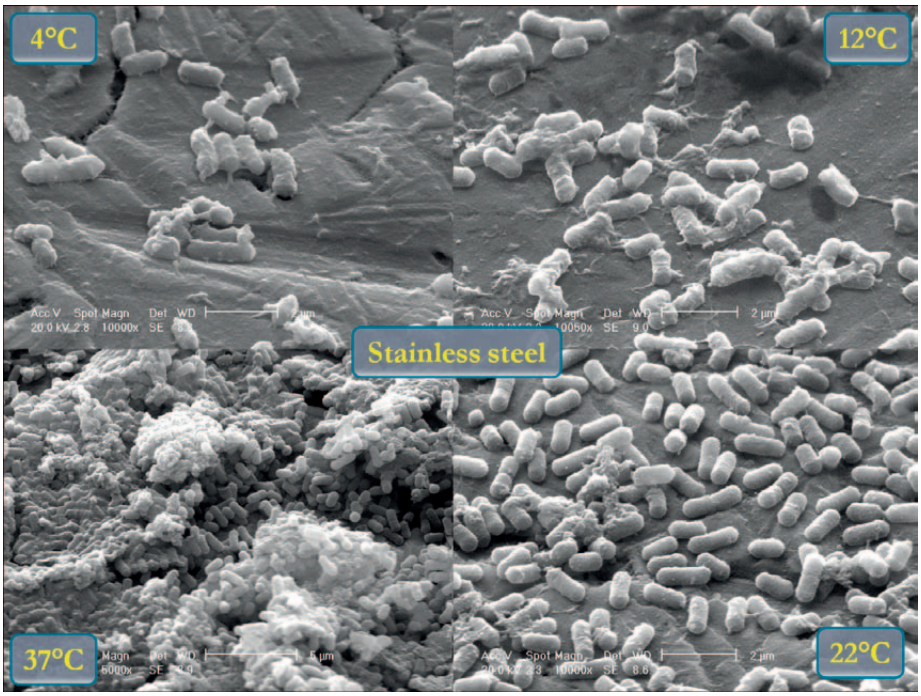

Fig. 2 - Biofilm formation by *L. monocytogenes* on stainless steel at different temperatures.

that motility by swarming is critical in host-cell invasion and virulence of *L.m*. In an attempt to individuate a relationship between *L.m* flagellum-mediated motility (swimming) and biofilm formation, it has been shown that temperature strongly influences flagellum production in *Listeria* spp. (DJORDJEVIC *et al.*, 2002). On the contrary, motility did not show a positive correlation with biofilm-forming ability, regardless of the substratum (DJORDJEVIC *et al.*, 2002). On the whole, functionally active flagella (motility) are probably not required for biofilm formation in *L.m*, and changes in surface structures other than flagella contribute to bacterial attachment to a solid surface.

At both 4° and 12°C, *L.m* produces a rudimentary biofilm consisting only of sparse clusters of cells and minimal amounts of EPS, re-

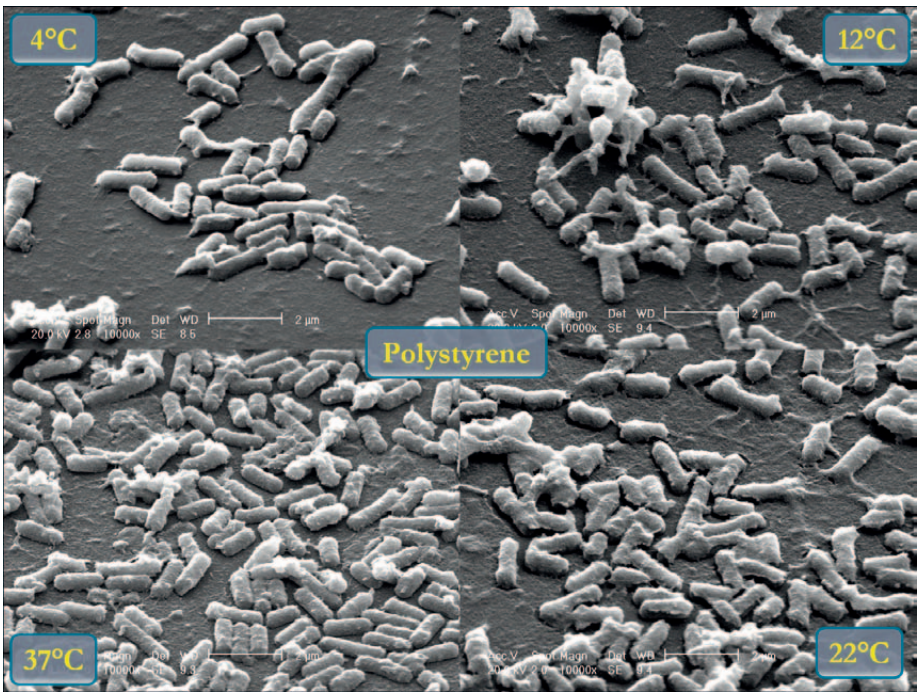

Fig. 3 - Biofilm formation by *L. monocytogenes* on polystyrene at different temperatures.

gardless of the substratum. On the contrary, at 22° and 37°C, the biofilm formed is more complex in terms of cell number and EPS produced. Although only on glass it appeared as a complex three-dimensional architecture, consisting of dense cell aggregates held together by extracellular matrix and surrounded by void areas, probably representing water channels for nutrient circulation and facilitation of waste removal in the biofilm (COSTERTON *et al.*, 1995). The relevant amount of EPS that can be observed in biofilms formed on glass raises a relevant hygienic/sanitary issue, since the chlorine resistance of *L.m* strains may be closely associated with the amount of EPS produced by the biofilm cells (FOLSOM *et al.*, 2006).

The time available for biofilm formation depends on the frequency of cleaning regimes. Product contact surfaces are typically cleaned several times per day, while environmental surfaces such as walls are cleaned once per week. Therefore, there is more time for biofilm formation on environmental surfaces. GIBSON *et al.* (1999) found that although attachment to a variety of surfaces in the food processing environment readily occurred, extensive surface colonization and biofilm formation only occurred on environmental surfaces. Finally, routine cleaning in place (CIP) cycles in food plants may not remove all cells, and the remaining biofilm cells may allow more rapid plant recolonization by a seeding process or by providing a surface to which new cells may readily adhere (MASHALL, 1994). The situation is exacerbated if fouling has occurred, and it is not removed during CIP (HINTON *et al.*, 2002).

BIOFILM AND SANITIZING AGENTS

The elimination of biofilms is a very difficult task because many factors affect detachment. Sanitation, i.e., cleaning and disinfection, is carried out in food processing plants in order to produce safe products with an acceptable shelf life and quality. An effective sanitation program is the major method to control surface contamination. When *L.m* is in the biofilm state, it exhibits an increased tolerance to disinfectants than their free living counterparts; and thick complex biofilms are more difficult to remove than adhered single bacterial cells (KLAEBOE *et al.*, 2006). The recalcitrance of biofilms towards biocide treatment is not in dispute, and the existence of resistance phenotypes induced by various nutrient limitations has been postulated to explain this observation (BROWN and GILBERT, 1993).

SINDE and CERBALLO (2000) found that sanitizers alter the surface properties of materials, and this alteration is directly related to the degree of attachment. Thus, sanitizers have different effects on bacterial adhesion depending on food contact materials. In particular, quaternary ammonium compounds were more effective against *L.m* attachment on stainless steel and rubber than on polytetrafluorethylene (PTFE), for which the effect was very similar. In some studies, it has been reported that *L.m* attached to food contact surfaces exhibited a reduced susceptibility to conventional sanitizers like acid anionic sanitizers and quarternary ammonium compounds (FRANK and KOFFI, 1990). A reasonable explanation for the reduced efficacy of such agents against biofilms is incomplete biofilm penetration by such reactive biocides (HUANG *et al.*, 1995) and the wide variation of environmental conditions that exist on food contact surfaces.

Antimicrobial agents are far more effective against actively growing cells, i.e., the best disinfectants for planktonic cells are not necessarily the most suitable ones for biofilm cells (HOLAH *et al.*, 1990). Caustic chlorine washes can be very effective in removing well-established biofilms by breaking down the polysaccharide matrix, rather than inactivating the microorganisms, probably because high pH favors ion-promoted detachment (COSTERTON *et al.*, 1995). PAN *et al.* (2010) demonstrated that biofilm cells show a reduced susceptibility to a sanitizer. This resistance to a sanitizer was greater on a Teflon substrate than on a stainless steel substrate. Similar results were observed by KRYSINSKI *et al.* (1992), who found that the resistance of *L.m* biofilms on stainless steel was lower than that on polyester or polyester/polyurethane. In addition, BREMER *et al.* (2002) reported that there was a significant difference in the effectiveness of sanitizers against cells attached to stainless steel surfaces than to conveyer belt surfaces (PVC/polyester).

The hydrodynamic conditions under which biofilms grow also have a significant effect on structure (STEWARD and COSTERTON, 2001). Thus, LIU and TAY (2001) found that at relatively high shear stress, as might be found in a food production plant, the biofilm was denser than at lower shear. Therefore, shear stress during biofilm development may profoundly affect the results of disinfection trials and the success of cleaning procedures.

Physical surface phenomena such as increased nutrient concentration at the surface are also often quoted as improving the tolerance to antimicrobial stress of biofilm bacteria. A recent work showed that micro- and nano-emulsions may be effective anti-biofilm agents, although *L.m* films appeared to be resistant (TAKEUCHI *et al.*, 2000). The widespread use of antimicrobial agents such as sanitizers or disinfectants in food processing or equipment cleaning and their effect on antimicrobial resistance is being investigated (CONTER *et al.*, 2009b; SPLENDIANI *et al.*, 2006).

Genetic factors such as the mobility of antibiotic resistance genes found on plasmids and transposons can increase the transfer of anti-

biotic resistance between bacteria (SOMERS and WONG, 2004). The ability of bacteria to adapt to adverse environmental conditions is an important factor in the development of resistance. This is because exposure of the organism to a sublethal level of an antimicrobial agent can lead to adaptation and development of resistance to higher levels of the antimicrobial agent or even cross-resistance to other agents. Although several authors report interactions between the bacterial biofilm physiological state and resistance to antibiotics or biocides, very little information is available on the cross resistance of sessile bacteria to antibiotics and biocides (SCENHIR, 2009). The emergence of resistant bacteria to conventional antimicrobial agents clearly shows that new biofilm control strategies are required (SIMÕES and VIEIRA, 2009).

SOLUTIONS: CONTROL AND REMOVAL OF BIOFILMS

The evaluation of biofilm sanitation procedures should be part of the Hazard Analysis and Critical Control Points (HACCP) development plan in order to control those biofilms prevalent in processing areas (SENCZECK *et al.*, 2000). There are several steps in normal cleaning/sanitation procedures, including cleaning, rinsing, and sanitizing, in that order (GIBSON *et al.*, 1999). The purpose of cleaning is to remove residual materials that may interfere with the sanitation procedure, because the presence of organic material on the biofilms significantly reduces the log kill. This reinforces the importance of adequate cleaning before the use of sanitizers (KRYSINSKY *et al.*, 1992).

Biofilms in the food industry can be eliminated by adopting different strategies such as physical, chemical, and biological methods. The use of enzyme-based detergents as bio-cleaners can serve as a viable option to overcome biofilm problems in the food industry. Due to EPS heterogeneity, a mixture of enzymes may be necessary for sufficient biofilm degradation. However, the use of enzymes in biofilm control is still limited due to the low prices of chemicals used today compared to the costs of enzymes. As a matter of fact, the technology and production of these enzymes and enzyme-based detergents are mostly patent-protected. Moreover, the low commercial accessibility of different enzyme activities limits their current usage. Enzymes and detergents have also been used as synergists to improve disinfectant efficacy (JOHANSEN *et al.*, 1997).

An interesting study on the control of *L.m* in a biofilm by competitive exclusive microorganisms indicated that *L.m* in biofilms can be strongly inhibited (> 5 Log CFU of *L.m*/cm^2) by metabolites of *Enterococcus durans* and *Lactococcus lactis* subsp. *lactis*. These two strains were isolated and screened from biofilms obtained from the floor drains of food processing plants that were free of *L.m* according to records. *Enterococcus durans* and *Lactococcus lactis*, subsp. *lactis* are beneficial organisms and can be used as starter cultures for food fermentation, and they have great potential for controlling *L.m* biofilms in food processing environments (ZHANG *et al.*, 2007).

Studies have shown that some detergents are bactericidal, and some disinfectants may even depolymerize EPS, thus enabling biofilm detachment from surfaces, e.g., oxidants such as chlorine and hydrogen peroxide (JUVEN and PIERSON, 1996). Monolaurin (glycerol monolaurate) was also found to be lethal to *L.m* at low concentrations. In addition, a synergistic interaction between monolaurin and organic acids like acetic acid also caused a pronounced inhibition of *L.m* (NIKOLAEV and PLAKUNOV, 2007). More recently, several authors (GUERRIERI *et al.*, 2009; SORUM and L'ABEE-LUND, 2002) demonstrated that bacteriocin producers showed the best antilisterial potential.

Microbial molecules, commonly used as biopreservatives, such as nisin, lauricidin, reuterin, and pediocin, have been well documented for their biofilm control potential against microorganisms commonly found in dairy processing facilities, including *L.m* (MAHDAVI *et al.*, 2007; ZHANG *et al.*, 2007). Bacteriocin application has also been tested on food packaging materials for the biocontrol of *L.m* on meats (MING *et al.*, 1997). The antagonistic effect was made more effective by decreasing the pH due to lactic acid production in the *Lb. plantarum* biofilm; the outcome was confirmed by the considerable activity in the *Lb. plantarum* biofilm and suspension. Comparing the antilisterial activity of lactic acid bacteria (LAB) biofilms against both planktonic and adherent cells, the *L.m* adherent cells showed a higher resistance. This finding can be explained by the greater resistances that microorganisms display to exogenous agents in biofilms (SAFDAR and ARMSTRONG, 2003).

The use of bacteriophages to control biofilms may provide a natural, highly specific, nontoxic, and feasible approach for controlling several microorganisms involved in biofilm formation (KUDVA *et al.*, 1999). A bacteriophage (*L.m* phage ATCC 23074-B1) was used successfully in *L.m* biofilm inactivation (HIBMA *et al.*, 1997). LU and COLLINS (2007) engineered a bacteriophage to express a biofilm-degrading enzyme. This enzymatic phage had the ability to attack bacterial cells in the biofilm and the biofilm matrix, substantially reducing the biofilm cell counts (more than 99.9% removal). The technology for this process has not yet been successfully developed, and relatively little information is available regarding the action of bacteriophages on biofilms (STOODLEY *et al.*, 2002).

More recently, the use of chlorine dioxide (CD) as a decontamination agent has demonstrated its high effectiveness against a wide variety of

microorganisms. It is a strong oxidizing agent with several advantages, such as the formation of low toxic disinfection by-products, effectiveness at low concentrations, low reaction time, ease of generation, and effectiveness over a broad range of pH values (CHANG *et al.*, 2000). The efficacy of CD gas to disinfect biofilms has not been studied in detail. A recent study demonstrated that low levels of gaseous CD (0.3 mg/L) and aqueous CD (7 mg/L) have equivalent inactivation of *L.m* cells in a biofilm matrix compared to conventional sodium hypochlorite treatment (50 mg/L). Additional research is needed to establish the CD levels and treatment times required for complete inactivation of biofilm cells. The potential use of CD to inactivate biofilms from food processing equipment surfaces should be further explored.

The discovery that many bacteria use quorum sensing to form biofilms makes it an attractive target for their control (POYART-SALMERON *et al.*, 1990). It is conceivable that quorum sensing inhibition may represent a natural, widespread, antimicrobial strategy with significant impact on biofilm formation. A good understanding of the cell-cell signaling phenomena of bacteria such as *L.m* can be used to control the biofilm formation process by the identification of products that can act as quorum sensing antagonists (SHARMA and ANAND, 2002; SINDE and CERBALLO, 2000). This property can lead to the development of new and efficient natural products for biofilm control.

The literature demonstrates that there is no unique strategy with absolute biofilm control efficiency. Nevertheless, the importance of adequate cleaning and disinfection procedures, in order to avoid *L.m* becoming established in processing environments and thus posing a product contamination threat, is widely accepted. Furthermore, continuous environmental monitoring schemes for *L.m* are of major importance to identify potential contamination sources and as an early warning system for food business operators, especially in food processing plants with low *L.m* prevalence in their food products.

CONCLUSIONS AND FUTURE DIRECTIONS

Pathogenic microorganisms in biofilms are the major source of food contaminations and clinical infections. Preferably, preventing biofilm formation would be a more logical option than treating it. Nutrient and water limitation, equipment design, and temperature control are important in biofilm control. In addition, the choice of material herein is crucial in terms of biofilm formation. The hygienic properties of the material can be altered by specific modifications to render it intrinsically antibacterial and/or less susceptible to attachment (VAN HOUDT and MICHIELS, 2010). The deposition of antifouling layers on stainless steel can influence their hygienic status, as demonstrated by the 81-96% decrease in *L.m* attachment and biofilm formation on polyethylene glycol-modified stainless steel. The modified surface properties were obtained by plasma-enhanced cross-linking of polyethylene glycol on stainless steel (DONG and ZHANG, 2005).

Biofilm detectors have been developed to monitor surface colonization by bacteria and allow the control of biofilms in the early stages of development (PEREIRA *et al.*, 2008). Many authors have suggested that *L.m* may be inhibited by some bacteriocin-producing LAB (SABIA *et al.*, 2002; TYOPPONEN *et al.*, 2003). Newer strategies devised for the bio-control of *Listeria* in planktonic form or in biofilms could include the adsorption of bioactive compounds, such as bacteriocins, onto food-contact surfaces (GUERRIERI *et al.*, 2009). Several attempts have been made to avoid biofilm formation by incorporating antimicrobial products into surface materials, coating surfaces with antimicrobials (GOTTENBOS *et al.*, 2001), and/or modifying the surface physicochemical properties (RODRIGUEZ *et al.*, 2007).

Biosurfactants are compounds with surface-active properties, which are produced by microorganisms. Their use has been suggested as an alternative to synthetic products. Their major advantages over synthetic detergents are their low toxicity and highly biodegradable nature. Biosurfactants may also show antimicrobial and anti-adhesive activities. Biosurfactants have been mentioned as promising multipurpose ingredients, which simultaneously exhibit emulsifier, anti-adhesive, and antimicrobial activities, and they are consequently suitable for many food applications (NITSCHKE and PASTORE *et al.*, 2002; NITSCHKE and COSTA, 2007). Therefore, these compounds of microbial origin could be used as detergent formulations to clean surfaces that come in contact with food and prevent food contamination (FREIRE *et al.*, 2009). Thus, biosurfactants could be utilized in the development of new strategies to retard *L.m* surface colonization and biofilm formation (NITSCHKE *et al.*, 2009; ARAUJO *et al.*, 2011).

Plant-derived compounds have gained widespread interest in the search to identify alternatives for microbial control (ESSAWI and SROUR, 2000). It has been postulated that surface pretreatment with plant extracts produces an unfavorable film that promotes microorganism detachment (SANDASI *et al.*, 2010).

To date, rational equipment design that minimizes laminar product flow, reduces static product, and facilitates cleaning and CIP processes can result in reduced bacterial attachment to processing equipment. The next step is the choice of materials modified by a set of coatings and surface modifications in order to reduce surface adhesion and biofilm formation (SZE, 1981; MANDRACCI and RICCIARDI, 2007). The increased biofilm resistance to convention-

al treatments enhances the need to develop new control strategies (SHARMA and ANAND, 2002). Research on microbial biofilms is proceeding on many fronts, with particular emphasis on elucidation of the genes specifically expressed by biofilm-associated organisms, evaluation of various control strategies (including medical devices treated with antimicrobial agents and antimicrobial locks) for either preventing or remediating biofilm colonization of medical devices, and development of new methods for assessing the efficacy of these treatments.

The majority of bacteria are able to form biofilms displaying a large diversity in architecture, phenotypes, and matrix components. Novel insights include factors contributing to phenotypic heterogeneity within biofilms, such as the identification and characterization of a range of matrix building blocks. To date, the prevention and control of *L.m* biofilms in food processing environments should be based on integrated efforts. The food industry should develop cleaning plans and disinfection programs and monitor their efficacy. In addition, the process equipment should be designed with high standards of hygiene in mind. Finally, a better understanding of how *L.m* attaches, grows, and detaches is urgently needed, and much effort should be invested in research on novel biofilm prevention and control strategies.

REFERENCES

Allison D.G. and Sutherland I.W. 1987. The role of exopolysaccharides in adhesion of freshwater bacteria. J. Gen. Microbiol. 133:1319-1327.

Araujo L.V., Abreu F., Lins U., de Melo Santa Anna L.M., Nitschke M. and Guimaraes Freire D.M. 2011. Rhamnolipid and surfactin inhibit *Listeria monocytogenes* adhesion. Food Research International 44:481-488.

Ashby M.J., Neale J.E., Knott S.J. and Critchley I.A. 1994. Effect of antibiotics on non-growing planktonic cells and biofilms of *Escherichia coli*. J. Antimicrob. Chemother 33:443-452.

Borucki M.K., Peppin J.D., White D., Loge F. and Call D.R. 2003. Variation in biofilm formation among strains of *Listeria monocytogenes*. Appl. Environ. Microbiol. 69:7336-7342.

Bremer P.J., Monk I. and Butler R. 2002. Inactivation of *Listeria monocytogenes*/*Flavobacterium* spp. biofilms using chlorine: impact of substrate, pH, time and concentration. Lett. Appl. Microbiol. 35:321-325.

Brown M.R.W. and Gilbert P. 1993. Sensitivity of biofilms to antimicrobial agents. J. Appl. Bacteriol. Symposium Supplement. 74:87S-97S.

Butler J.L., Stewart J.C., Vanderzant C., Carpenter Z.L. and Smith G.C. 1979. Attachment of microorganism to pork skin and surfaces of beef and lamb carcasses. J. Food Protect. 42:401-406.

Carpentier B. and Chassing D. 2004. Interactions in biofilms between *Listeria monocytogenes* and resident microorganisms from food industry premises. Int. J. Food Microbiol. 97:111-122.

Chae M.S., Schraft H., Hansen L.T. and Mackereth R. 2006. Effects of physicochemical surface characteristics of *Listeria monocytogenes* strains on attachment to glass. Food Microbiol. 23:250-259.

Chang C.Y., Hsieh Y.H., Shih I.C., Hsu S.S. and Wang K.H. 2000. The formation and control of disinfection by-products using chlorine dioxide. Chemosphere 41:1181-1186.

Chatterjee S.S., Hossain H., Otten S., Kuenne C., Kuchmina K., Machata S., Domann E., Chakraborty T. and Hain T. 2006. Intracellular gene expression profile of *Listeria monocytogenes*. Infect. Immun. 74:1323-1338.

Chung K.T., Dickson J.S. and Crouse J.D. 1989. Attachment and proliferation of bacteria on meat. J. Food Protect. 52:173-177.

Conter M., Paludi D., Zanardi E., Ghidini S., Vergara A. and Ianieri A. 2009b. Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. Int. J. Food Microbiol. 128:497-500.

Conter M., Vergara A., Di Ciccio P., Zanardi E., Ghidini S. and Ianieri A. 2009a. Polymorphism of *actA* gene is not related to *in vitro* virulence of *Listeria monocytogenes*. Int. J. Food Microbiol. 137:100-105.

Costerton J.W., Lewandowski Z., Caldwell D., Korber D. and Scott H. 1995. Microbial biofilms. Annual Rev. Microbiol. 49:711-745.

Costerton J.W., Lewandowski Z., de Beer D., Caldwell D., Korber D. and James G. 1994. Biofilms, the customized microniche. J. Bacteriol. 176:2137-2142.

D'Orio V., Paludi D., Conter M., Vergara A. and Ianieri A. 2007. Biofilm formation by environmental strains of *Listeria monocytogenes*: relationship with hydrophobicity, motility and scanning electron microscopy analysis. Food, New options for the industry, *EFFoST/EHEGD* Conference 2007 – Practical applications of research results. Lisbon 14-16 November 2007.

Das J.R., BhaKoo M., Jones M.V. and Gilbert P. 1998. Changes in the biocide susceptibility of *Staphylococcus epidermidis* and *Escherichia coli* cells associated with rapid attachment to plastic surfaces. J. Appl. Microbiol. 84(5):852-8.

Di Bonaventura G., Piccolomini R., Paludi D., D'Orio V., Vergara A., Conter M. and Ianieri A. 2008. Influence of temperature on biofilm formation by *Listeria monocytogenes* on various food-contact surfaces: relationship with motility and cell surface hydrophobicity. J. Appl. Microbiol. 104:1552-61

Dickson J.S. and Koohmaraie M. 1989. Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. Appl. Environ. Microbiol. 55:832-836.

Djordjevic D., Wiedmann M. and McLandsborough L.A. 2002. Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. Appl. Environ. Microbiol. 68:2950-2958.

Dong Y.H. and Zhang L.H. 2005. Quorum sensing and quorum-quenching enzymes. J. Microbiol. 43:101-9.

Donnelly C.W. 2001. *Listeria monocytogenes*: a continuing challenge. Nutrition Rev. 59:183-194.

Dons L., Eriksson E., Jin Y., Rottenberg M.E., Kristensson K., Larsen C.N., Bresciani J. and Olsen J.E. 2004. Role of flagellin and the two-component CheA/CheY system of *Listeria monocytogenes* in host cell invasion and virulence. Infect. Immun. 72:3237-3244.

Dussurget O. 2008. New insight into determinants of *Listeria monocytogenes* virulence. Int. Rev. Cell. Mol. Biol. 270:1-38.

EFSA (European Food Safety Authority). 2007. "Request for updating the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods and scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods and the related risk for human illness - Scientific Opinion of the Panel on Biological Hazards. EFSA J. 599:1-42".

Eklund M.W., Poysky F.T., Paranjpye R.N., Lashbrook L.C., Peterson M.E. and Pelroy G.A. 1995. Incidence and sources of *Listeria monocytogenes* in cold smoking fishery products and processing plants. J. Food Protect. 58:502-508.

Essawi T. and Srour M. 2000. Screening of some Palestinian medicinal plants for antibacterial activity. J. Ethnopharmacol. 70: 343-349.

Farber J.M. and Peterkin P.I. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. 55:476-511.

Flemming H.C. and Wingende J. 2010. The Biofilm matrix. Nature Rev. Microbiol. 8:623-633

Folsom J.P. and Frank J.F. 2006. Chlorine resistance of *Listeria monocytogenes* biofilms and relationship to subtype, cell density, and planktonic cell chlorine resistance. J. Food Protect. 69:1292-1296.

Folsom J.P., Siragusa G.R. and Frank J.F. 2006. Formation of biofilm at different nutrient levels by various genotypes of *Listeria monocytogenes*. J. Food Protect. 69:826-834.

Frank J.F. and Koffi R.A. 1990. Surface adherent growth of *Listeria monocytogenes* is associated with increased resistance to surfactant sanitizers and heat. J. Food Protect. 53:550-554.

Freire D.M.G., Arajuo L.V., Kronemberger F.A. and Nitschke M. 2009. Biosurfactants as emerging additives in food processing. Innovation in food engineering: new techniques and products. 685-705.

Gibson H.J., Taylor H., Hall K.E. and Holah J.T. 1999. Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. J. Appl. Microbiol. 87:41-48.

Gilbert P., Allison D. and McBain A.J. 2002. Biofilms in vitro and in vivo: do singular mechanisms influx cross-resistance? J. Appl. Microbiol. 92:98S-110S.

Giovannacci I., Ermel G., Salvat G., Vendeuvre J.L. and Bellon-Fontaine M.N. 2000. Physicochemical surface properties of five *Listeria monocytogenes* strains from a pork-processing environment in relation to serotypes, genotypes and growth temperature. J. Appl. Microbiol. 88:992-1000.

Gorski L., Palumbo J.D. and Mandrell R.E. 2003. Attachment of *Listeria monocytogenes* to radish tissue is dependent upon temperature and flagellar motility. Appl. Environ. Microbiol. 69:258-266.

Gottenbos B., van der Mei H.C., Klatter F., Nieuwenhuis P. and Busscher H.J. 2001. *In vitro* and *in vivo* antimicrobial activity of covalently coupled quaternary ammonium silane coatings on silicone rubber. Biomaterials. 23:1417-1423.

Gram, L., Bagge-Ravn, D., Ng, Y.Y., Gymoese, P. and Vogel B.F. 2007. Influence of food soiling matrix on cleaning and disinfection efficiency on surface attached *Listeria monocytogenes*. Food Control 18 (10):1165-1171.

Guerrieri E., de Niederhäusern S., Messi P., Sabia C., Iseppi R., Anacarso I. and Bondi M. 2009. Use of lactic acid bacteria (LAB) biofilms for the control of *Listeria monocytogenes* in a small-scale model. Food Contr. 20:861-865.

Habimana O., Meyrand M., Meylheuc T., Kulakauskas S. and Briandet R. 2009. Genetic Features of Resident Biofilms Determine Attachment of *Listeria monocytogenes*. Appl. Environ. Microbiol. 75:7814-7821.

Hall-Stoodley L. and Stoodley P. 2005. Biofilm formation and dispersal and the transmission of human pathogens. Trends Microbiol. 13:7-10.

Herald P.J. and Zottola E.A. 1988. Attachment of *Listeria monocytogenes* to stainless steel surfaces at various temperatures and pH values. J. Food Protect. 53:1549-1552, 1562.

Hibma A.M., Jassim S.A. and Griffiths M.W. 1997. Infection and removal of L-forms of *Listeria monocytogenes* with bred bacteriophage. Int. J. Food Microbiol. 4:197-207.

Hinton A.R., Trinh K.T., Brooks J.D. and Manderson G.J. 2002. Thermophile survival in milk fouling and on stainless steel during cleaning. Transactions of the Institute of Chemical Engineers, 80 (Part C):299-304.

Holah J.T., Bird J. and Hall K.E. 2004. The microbial ecology of high-risk, chilled food factories; evidence for persistent *Listeria* spp. and *Escherichia coli* strains. J. Appl. Microbiol. 97:68-77.

Holah J.T., Higgs C., Robinson S., Worthington D. and Spenceley H. 1990. A conductance-based surface disinfection test for food hygiene. Lett. Appl. Microbiol. 11:255-259.

Hood S.K. and Zottola E.A. 1997. Growth media and surface conditioning influence the adherence of *Pseudomonas fragi*, *Salmonella typhimurium* and *Listeria monocytogenes* cells to stainless steel. J. Food Protect. 60:1034-103.

Huang C.T., Yu F.P., McFeters G.A. and Stewart P.S. 1995. Nonuniform spatial patterns of respiratory activity within biofilms during disinfection. Appl. Environ. Microbiol. 61:2252-2256.

Hung T.T. and Schneewind O. 2004. Assembly of pili in Gram-positive bacteria. Trends in Microbiol. 12:228-234.

Jefferson K.K. 2004. What drives bacteria to produce a biofilm? FEMS Microbiol. Lett. 236:163-173.

Jeong D.K. and Frank J.F. 1994. Growth of *Listeria monocytogenes* at 21°C in biofilms with microorganisms isolated from meat and dairy environments. Lebensm.-Wissenschaft Techn. 27:415-424.

Jeong D.K. and Frank J.F. 1994. Growth of *Listeria monocytogenes* at 10 degrees C in biofilms with microorganisms isolated from meat and dairy processing environments. J. Food Protect. 57:576-586.

Johansen C., Falholt P. and Gram L. 1997. Enzymatic removal and disinfection of bacterial biofilms. Appl. Environ. Microbiol. 9:3724-3728.

Juven B.J. and Pierson M.D. 1996. Antibacterial effects of hydrogen peroxide and methods for its detection and quantification. J. Food Protect. 59:1233-1241.

Kalmokoff M.L., Austin J.W., Wan X-D., Sanders G., Banerjee S. and Farber J.M. 2001. Adsorption, attachment and biofilm formation among isolates of *Listeria monocytogenes* using model conditions. J. Appl. Microbiol. 91:725-734.

Karatan E. and Watnick P. 2009. Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol. Mol. Biol. Rev. 73: 310-47.

Klaeboe H., Rosef O., Fortes E. and Wiedmann M. 2006. Ribotype diversity of *Listeria monocytogenes* isolates from two salmon processing plants in Norway. Int. J. Environ. Health Res.16:375-383.

Krysinski E.P., Brown L.J. and Marchisello T.J. 1992. Effect of cleaners and sanitizers on *Listeria monocytogenes* attached to product contact surfaces. J. Food Protect. 55:246-251.

Kudva I.T., Jelacic S., Tarr P.I., Youderian P. and Hovde C.J. 1999. Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. Appl. Environ. Microbiol. 65:3767.

Kumar C.G. and Anand S.K. 1998. Significance of microbial biofilms in food industry: a review. Int. J. Food Microbiol. 42:9-27.

Landini P. 2009. Cross-talk mechanisms in biofilm formation and responses to environmental and physiological stress in *Escherichia coli*. Research in Microbiol. 160: 259-26

Liu D., Lawrence M.L., Ainsworth A.J. and Austin F.W. 2007. A multiplex PCR for species- and virulence-specific determination of *Listeria monocytogenes*. J. Microbiol. Met. 71:133-140.

Liu Y. and Tay J.H. 2001. Metabolic response of biofilm to shear stress in fixed-film culture. J. Appl. Microbiol. 90:337-342.

López V., Villatoro D., Ortiz S., López P., Navas J., Dávila J.C. and Martínez-Suárez J.V. 2008. Molecular tracking of *Listeria monocytogenes* in an Iberian pig abattoir and processing plant. Meat Sci. 78:130-134.

Lu T.K. and Collins J.J. 2007. Dispersing biofilms with engineered enzymatic bacteriophage, Proc. Nat. Academy Sci. USA 104:11197-11202.

Mahdavi M., Jalali M. and Kermanshahi R.K. 2007. The effect of nisin on biofilm forming foodborne bacteria using microtiter plate method. Res. Pharm. Sci. 2:113-118.

Mandracci P. and Ricciardi C. 2007. Silicon-carbon-oxynitrides grown by plasma-enhanced chemical vapor deposition technique. Thin Solid Films: 515

Mashall K. 1994. Microbial adhesion in biotechnological processes. Curr. Opin. Biotec. 5:296301.

Miettinen M.K., Palmu L., Björkroth K.J., and Korkeala H. 2001. Prevalence of *Listeria monocytogenes* in broilers at the abattoir, processing plant, and retail level. J. Food Prot. 64:994-999.

Ming X.T., Weber G.H., Ayres J.W. and Sandine W.E. 1997. Bacteriocins applied to food packaging materials to inhibit Listeria monocytogenes on meats. J. Food Sci. 62:413-415.

Moens S. and Vanderleyden J. 1996. Functions of bacterial flagella. Crit. Rev. Microbiol. 22:67-100.

Moltz A.G. and Martin S.E. 2005. Formation of biofilms by *Listeria monocytogenes* under various growth conditions. J. Food Protect. 68:92-97.

Morris C.E. and Monier J.M. 2003. The ecological significance of biofilm formation by plant-associated bacteria. Ann. Rev. Phytopat. 41:429-453.

Musk D.J., Banko D.A. and Hergenrother P.J. 2005. Iron salts perturb biofilm formation and disrupt existing biofilms of *Pseudomonas aeruginosa*. Chem. Biol. 12:786-796.

Nikolaev Y.A. and Plakunov V.K. 2007. Biofilm "City of Microbes" or an Analogue of Multicellular Organisms? Microbiol. 76 (2):125-138.

Nitschke M. and Costa S.G.V.A.O. 2007. Review: Biosurfactants in food industry. Trends in Food Science and Technology. 18: 252-259

Nitschke M. and Pastore G.M. 2002. Biossurfactantes: propriedades e aplicações. Química Nova. 25, 772.

Nitschke M., Araújo L.V., Costa S.G.V.A.O., Pires R.C., Zeraik A.E., Fernandes A.C.L.B., Freire D.M.G. and Costiero J. 2009. Surfactin reduces the adhesion of food-borne pathogenic bacteria to solid surfaces. Lett. Appl. Microbiol. 49 (2): 241-247

Oh D.H. and Marshall D.L. 1996. Monolaurin and acetic acid inactivation of Listeria *monocytogenes* attached to stainless steel. J. Food Prot. 59:249-252.

Oliveira K., Oliveira T., Teixeira P., Azeredo J. and Oliveira R. 2007. Adhesion of *Salmonella* Enteritidis to stainless steel surfaces. Braz. J. Microbiol. 38 (2):318-323.

Oliveira M.M., Brugnera D.F., Alves E. and Piccoli R.H. (2010). Biofilm formation by *Listeria monocytogenes* on stainless steel surface and biotransfer potential. Braz. J. Microbiol. 41:7-106.

Palmer J., Flint S. and Brooks J. 2007. Bacterial cell attachment, the beginning of a biofilm. J. Ind. Microbiol. Biotec. 34:577-588.

Pan Y., Breidt F.Jr. and Gorski L. 2010. Synergistic effects of sodium chloride, glucose, and temperature on biofilm formation by *Listeria monocytogenes* serotype 1/2a strains and 4b strains. Appl. Environ. Microbiol. 76 (5):1433-1441.

Pan Y., Breidt F.Jr. and Kathariou S. 2006. Resistance of *Listeria monocytogenes* biofilms to sanitizing agents in a simulated food processing environment. Appl. Environ. Microbiol. 72: 7711-7717.

Pereira A., Mendes J. and Melo L.F. 2008. Using nanovibrations to monitor biofouling, Biotec. Bioeng. 15:1407-1415.

Piette J.P.G. and Idziak E.S. 1991. Role of flagella in adhesion of Pseudomonas fluorescens to tendon slices. Appl. Environ. Microbiol. 57:1635-1639.

Poyart-Salmeron C., Carlier C., Trieu-Cuot P., Courtieu A.L. and Courvalin P. 1990. Transferable plasmid-mediated antibiotic resistance in *Listeria monocytogenes*. Lancet 335:1422-1426.

Rasmussen T.B., Skindersoe M.E., Bjarnsholt T., Phipps R.K., Christensen K.B. and Jensen P.O. 2005. Identity and effects of quorum-sensing inhibitors produced by Penicillium species. Microbiol. 151:1325-1340.

Rieu A., Lemaitre J.P., Guzzo J. and Piveteau P. 2008. Interactions in dual species biofilms between *Listeria monocytogenes* EGD-e and several strains of *Staphylococcus aureus*. Int. J. Food Microbiol. 126:76-82.

Rodas-Suarez O.R., Flores-Pedroche J.F., Betancourt-Rule J.M. Quinones-Ramırez E.I. and Vazquez-Salinas C. 2006. Occurrence and antibiotic sensitivity of *Listeria monocytogenes* strains isolated from oysters, fish, and estuarine water. Appl. Environ. Microbiol. 72:7410-7412.

Rodriguez A., Autio W.R. and McLandsborough L.A. 2007. Effects of inoculation level, material hydration, and stainless steel surface roughness on the transfer of *Listeria monocytogenes* from inoculated bologna to stainless steel and high-density polyethylene. J. Food Protect. 70:1423-1428.

Sabia C., Manicardi G., Messi P., de Niederhäusern S. and Bondi M. 2002. Enterocin 416 K1, an antilisterial bacteriocin produced by *Enterococcus casseliflavus* IM 416K1 isolated from Italian sausages. Int. J. Food Microbiol. 75:163-170.

Safdar A. and Armstrong D. 2003. Antimicrobial activities against 84 *Listeria monocytogenes* isolates from patients with systemic listeriosis at a comprehensive cancer center (1955-1997). J. Clin. Microbiol. 41:483-485.

Sandasi M., Leonard C.M. and Viljoen A.M. 2010. The *in vitro* antibiofilm activity of selected culinary herbs and medicinal plants against *Listeria monocytogenes*. Lett. Appl. Microbiol. 50: 30-35.

SCENHIR 2009. "Assessment of the Antibiotic Resistance Effects of Biocides (on-line) Available at http://ec.europa.eu/health/ph_risk/committees/documents/sc_o_001.pdf".

Schlech W.F., Lavigne P.M., Bortolussi R.A., Allen A.C., Haldane E.V., Wort A.J., Hightower A.W., Johnson S.E., King S.H., Nicholls E.S. and Broome C.V. 1983. Epidemic listeriosis. Evidence for transmission by food. New Engl. J. Med. 308:203-206.

Senczek D., Stephan R. and Untermann F. 2000. Pulsed-field gel electrophoresis (PFGE) typing of *Listeria* strains isolated from a meat processing plant over a 2-year period. Int. J. Food Microbiol. 62:155-159.

Sharma M. and Anand S.K. 2002. Biofilms evaluation as an essential component of HACCP for food/dairy processing industry. A case. Food Contr. 13:469-477.

Simões M. and Vieira M.J. 2009. Persister cells in Pseudomonas fluorescens biofilms treated with a biocide, Proceedings of the international conference processes in biofilms: Fund. to App. 58-62 Davis, CA, Usa.

Simões M., Simoes L.C. and Vieira M.J. 2010. A review of current and emergent biofilm control strategies. LWT - Food Sci. Tec. 43:573-583.

Sinde E. and Cerballo J. 2000. Attachment of Salmonella spp and Listeria monocytogenes to stainless steel, rubber and polytetrafluorethylene: the influence of free energy and the effect of commercial sanitizers. Food Microbiol. 17:439-447.

Smith L., Fratamico P.M. and Novak J.S. 2004. Quorum sensing: a primer for food microbiologists. J. Food Protect. 67:1053-1070.

Somers E.B. and Wong A.C.L. 2004. Efficacy of two cleaning and sanitizing combinations on Listeria monocytogenes biofilms formed at low temperature on a variety of materials in the presence of ready-to-eat meat residue. J. Food. Protect. 67:2218-2229.

Sorum H. and L'Abee-Lund T.M. 2002. Antibiotic resistance in food-related bacteria. A result of interfering with the global web of bacterial genetics, Int. J. Food Microbiol. 78:43-56.

Splendiani A., Livingston A.G. and Nicolella C. 2006. Control membrane-attached biofilms using surfactants. Biotec. Bioeng. 94:15-23.

Stewart P.S. and Costerton J.W. 2001. Antibiotic resistance of bacteria in biofilms. Lancet. 358:135-138.

Stoodley P., Sauer K., Davies D.G. and Costerton J.W. 2002. Biofilms as complex differentiated communities. Ann. Rev. Microbiol. 56:187-209.

Sutherland I.W., Hughes K.A., Skillman L.C. and Tait K. 2004. The interaction of phage and biofilms. FEMS Microbiol. Lett. 232:1-6.

Sze S.M. 1981. "Physics of Semiconductor devices" Wiley, New York.

Takeuchi K., Matute C.M., Hassan A.N. and Frank J.F. 2000. Comparison of the attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Pseudomonas fluorescens* to lettuce leaves. J. Food Protect. 63:1433-1437.

Teixeira P.C., Leite G.M., Domingues R.J., Silva J., Gibbs P.A. and Ferreira J.P. 2007. Antimicrobial effects of a microemulsion and a nanoemulsion on enteric and other pathogens and biofilms. Int. J. Food Microbiol. 118:15-19.

Thévenot D., Dernburg A. and Vernozy-Rozand C. 2006. An updated review of Listeria monocytogenes in pork meat industry and its products. J. Appl. Microbiol. 101:7-17.

Tompkin R.B. 2002. Control of *Listeria monocytogenes* in the food-processing environment. J. Food Protect. 65:709-725.

Tyopponen S., Markkula A., Petaja E., Suihko M.L. and Mattila-Sandholm T. 2003. Survival of *Listeria monocytogenes* in North European type dry sausages fermented by bioprotective meat starter cultures. Food Control. 14:181-185.

Van der Veen S. and Abee T. 2010. Importance of SigB for *Listeria monocytogenes* Static and Continuous-Flow Biofilm Formation and Disinfectant Resistance. Appl. Environ. Microbiol. 76(23):7854-7860.

Van Houdt R. and Michiels C.W. 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. J. Appl. Microbiol. 109 (4):1117-1131.

Zhang Y., Yeha E., Hallb G., Cripeb J., Bhagwatc A.A. and Menget J. 2007. Characterization of *Listeria monocytogenes* isolated from retail foods. Int. J. Food Microbiol. 113:47-53.

Paper received July 27, 2011 Accepted December 20, 2011