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Full Length Research Paper

Adhesive ability and biofilm metabolic activity of *Listeria monocytogenes* strains before and after cold stress

Rihab BEN SLAMA[#]*, Karima BEKIR[#], Hanen MILADI, Amira NOUMI and Amina BAKHROUF

Laboratoire d'Analyses, Traitement et Valorisation des Polluants de l'Environnement et des Produits, Faculté de Pharmacie, Université de Monastir, rue Avicenne 5000 Monastir, Tunisie.

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***Listeria monocytogenes* is an important pathogen responsible for major outbreaks associated with food products. Adhesion to surfaces leads to significant modifications in cell physiology. In this work, the ability of *L. monocytogenes* to produce biofilm and its ability to adhere to abiotic surfaces under cold stress were evaluated. Metabolic activity of biofilm formed by *L. monocytogenes* before and after cold stress was measured *in vitro* using the colorimetric method based on the reduction of the tetrazolium salt 2,3-bis(2methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT). The ability to adhere to abiotic surfaces was determined by the ability of the cells to metabolically reduce bromure de 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) to a formazan dye. Our results show that *L. monocytogenes* strains were able to adhere to abiotic materials with different degrees. In fact, cold stressed strains (-20°C) were more adhesive to polyethylene, glass, polyvinyl chloride and stainless style surfaces than non-stressed cells. Our observations show that the hydrophily varied with cold stress period. At freezing temperature, *L. monocytogenes* was strongly hydrophobic. Genetic studies of adhesive genes of *L. monocytogenes* will be required to fully understand the importance of this observation.**

Key words: *Listeria monocytogenes*, slime production, cold stress, abiotic-surfaces, biofilm formation.

INTRODUCTION

Listeria monocytogenes is an important pathogen responsible for major outbreaks associated with food products. This organism is frequently isolated from food-processing and natural environments (Chae et al., 2006). Growth at refrigerated temperatures and survivability in adverse environments, including those conditions that occur in minimally processed foods, where cells persist due to their ability to attach to different surfaces, have made *L. monocytogenes* a challenge to control. The psychotropic nature of *L. monocytogenes* allows replication in refrigerated, ready-to-eat food products that have been contaminated during processing and packaging. Biofilms allow microorganisms to persist in the environment and resist desiccation, ultraviolet (UV) light and treatment with antimicrobial and sanitizing agents. Biofilms formation by *L. monocytogenes* in food processing environments has thus become a concern for food manufacturers. The capacity of *L. monocytogenes* cells to adhere and colonize inert food contact surfaces such as polypropylenes, rubbers, stainless steel and glass, is now well established.

Biofilm formation by *L. monocytogenes* may enhance bacterial persistence in food processing environments and consequently increase the chances of contributing to post-processing contamination (Beresford et al., 2001). However, the adhesion of *Listeria* depends on the strains

*Corresponding author. E-mail: rbs_23@yahoo.fr. Tel: +21673461000. Fax: +21673461830.

#These authors contributed equally to this paper

Abbreviations: XTT, 2,3-Bis(2methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide; **MTT,** bromure de 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium.

(Harvey et al., 2007), the physiological state of the bacteria (Briandet et al., 1999) and the type of substrate (Sinde and Carballo, 2000). Accordingly, the assessment of the evolution of micro-organisms that naturally contaminate food must take into account the variability of factors influencing the microbial behavior that is, biological factors, physico-chemical and microbial food characteristics and storage conditions (Pouillot et al., 2007). Strains of *L. monocytogenes* are recurrently found on refrigerated products, notably in seafood, for example, ready-to-eat cold or hot smoked salmon, 'gravad' salmon, shrimp, mussels, fermented fish and fish salads, and the link between seafood consumption and listeriosis cases was frequently assessed (Rorvik et al., 2000). That is why fresh-salmon was chosen in this study. The objectives of this study, therefore, was to investigate the ability of *L. monocytogenes* to adhere to abiotic surfaces and to evaluate differences in biofilm formation, slime production and then adhesion ability for *L. monocytogenes* before and after cold stress using staining methods.

MATERIALS AND METHODS

Bacterial strains

Bacteria used in this study were an American type culture collection *L. monocytogenes* strain ATCC 19115 and three other *L. monocytogenes* strains (S1, S2 and S3) isolated from frozen meat according to the standardised French method Nf V 08-055 (AfNor, 1999), for the detection of *L. monocytogenes* in food. Artificially contamination of the fresh salmon, freezing storage conditions and isolation of stressed bacteria were followed as previously described by Miladi et al. (2008).

Cell surface hydrophobicity

Cell surface hydrophobicity was determined according to Sanin et al. (2003) with modifications. Suspended cells were washed three times in phosphate-buffered saline (PBS) (after centrifugation at 6,100 × G for 10 min). Washed cells were resuspended in PBS and the initial optical density was measured at 600 nm. The OD_{600} was then adjusted to 0.3 (OD1) and 3 ml aliquots were transferred to 10 × 100 mm round bottom glass tubes. After that, 0.3 ml of n-hexadecane was added to the suspension and the mixture was mixed vigorously for 2 min. After standing for 15 min, the water phase was removed and its OD_{600} was measured (OD2). The cell surface hydrophobicity was calculated as:

Hydrophobicity (%) = (1-OD2/OD1) × 100

Phenotypic characterization of slime-producing bacteria

Qualitative detection of slime production was studied by culturing the strains on Congo red agar (CRA) plates as described by Freeman et al. (1989). *L. monocytogenes* strains were inoculated into the surface of CRA plates, prepared by mixing 0.8 g Congo red with 36 g sucrose (Sigma) in 1 L of brain heart infusion agar, and were incubated for 24 h at 30°C under aerobic conditions and followed overnight at room temperature (Chaieb et al., 2007). Slime producing bacteria appeared as black colonies, whereas non-slime producers remained non pigmented (Subashkumar et al., 2006).

Biofilm formation assays by *Listeria monocytogenes* strains

Biofilm formation in glass test tubes

Biofilm formation assay was determined according to Wolfe et al. (2004) with modifications. Each *L. monocytogenes* strain was cultured in SWT medium containing (per liter): 5 g of BactoTryptone (Difco), 3 g of yeast extract (Difco), 3 ml of glycerol, and 1 L of distilled water, at 28°C with shaking and then transferred to glass test tubes. The cells were incubated without shaking for 10 h at 28°C, and then stained with 1% crystal violet solution to visualize cells attached to the test tube (Wolfe et al., 2004). After incubation for 15 min, the tubes were rinsed with sterile distilled water. Biofilms formed at the air liquid interface were stained purple. All the strains were tested in triplicate.

Biofilm assessment on polyvinyl chloride (PVC), polyethylene (PE) and stainless style surfaces

For thus, cells were grown for 18 h at 37°C in trypticase soy broth supplemented with 0.6% (w/v) yeast extract (TSB-YE) containing 50 mM of glucose. Batches of medium were inoculated with overnight cell cultures and incubated at 37°C in an orbital shaker operating at 150 rpm. Cells were harvested after 24 h (stationary growth phase), washed once with PBS (pH 7.2), and standardized to a density DO_{600}= 0.3. A volume of 80 µl of the standardized *L. monocytogenes* cells suspensions was applied for all the tested strips (1cm2) placed in a 12-well tissue culture plate. The cells were allowed to adhere for 90 min at 37°C (adhesion phase). Non-adherent cells were removed from the strips by being gently washed with 5 ml PBS. Strips were then submerged in 4 ml of TSB-YE containing 50 mM of glucose. Strips to which no cells were added served as negative controls. Control and experimental strips were incubated at 37°C for 72 h (biofilm growth phase). The percentage of viable cells in biofilms was estimated with the bromure de 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) colorimetric assay based on the ability of cells to metabolically reduce MTT to a water soluble formazan dye (Mosmann, 1983). Strips with biofilms were transferred to 12-well tissue culture plates containing 3 ml PBS/well. Fifty microliters of MTT (5 mg/ml in PBS) were added to each well. Plates were incubated for additional 5 h at 37°C. The supernatant was then removed and the formazan product was determined spectrophotometrically at 492 nm. Experiments were performed in triplicate.

Biofilm formation on polystyrene

Biofilms were produced on commercially available pre-sterilized polystyrene flat-bottom 96 well microtiter plates (Iwaki, Tokyo, Japan) for 24 h on TSB-YE. We have adopted two protocols to evaluate biofilm formation: biofilm metabolic activity assessment using the 2,3-bis(2methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay according to the staining method described previously (Pettit et al. 2005; Sandasi et al. 2010) and the Semi-quantitative adherence assay using Cristal Violet colorimetric as described by Chaieb et al. (2007).

Semi-quantitative adherence assay

Biofilm production by *L. monocytogenes* strains was determined using a semi-quantitative adherence assay on 96-well tissue culture

plates, as described previously (Chaieb et al., 2007). Strains were grown in TSB-YE (Pronadisa, Spain), Following overnight incubation at 30°C, the optical density at 600 nm (OD) of the bacteria was measured. An overnight culture, grown in TSB-YE at 30°C, was diluted to 1:100 in TSB-YE supplement with 2% (w/v) glucose. A total of 200 µl of cell suspensions was transferred in a U bottomed 96-well microtiter plate (Nunc, Roskilde, Denmark). Each strain was tested in triplicate. Wells with sterile TSB-YE alone were served as controls. The plates were incubated aerobically at 30°C for 24 h. The cultures were removed and the microtiter wells were washed twice with phosphate-buffered saline (7 mM Na_2HPO_4, 3 mM NaH_2PO and 130 mM NaCl at pH 7.4) to remove non-adherent cells and dried in an inverted position. Adherent bacteria were fixed with 95% ethanol and stained with 100 µl of 1% crystal violet (Merck, France) for 5 min. The excess stain was rinsed and poured off and the wells were washed three times with 300 µl of sterile distilled water. The water was then cleared and the microplates were air-dried. The optical density of each well was measured at 570 nm using an automated Multiskan reader (GIO. DE VITA E C, Rome, Italy).

Quantification of viable cells in the biofilm by XTT assay

The metabolic activity of cells in biofilm was assessed using the XTT reduction assay according to methods described previously (Pettit et al., 2005; Sandasi et al., 2010) which measures the reduction of a tetrazolium salt by metabolically active cells to a colored water soluble formazan derivative that can be easily quantified colorimetrically. 96-well polystyrene tissue culture plates containing TSB broth with 2% glucose (w/v) were prepared and inoculated in the same way as described for crystal violet assay. XTT (Sigma-Aldrich, Switzerland) solution (1 mg/ml) was prepared in PBS; filter sterilized and stored at -80°C. Menadione (Sigma-Aldrich, Switzerland) solution (1 mM) was prepared in acetone and sterilized immediately before each assay. Following incubation, the biofilms were first washed five times with PBS, and then 100 µl PBS and 12 µl XTT-menadione solutions (12.5:1 v/v) were added to each of the prewashed wells and the control wells. The plate was then incubated for 3 h in the dark at 37°C. Following incubation, 100 µl of the solution was transferred to fresh wells and the color change in the solution was measured with a multiskan reader at 492 nm. The absorbance values for the controls were then subtracted from the values of the tested wells to eliminate spurious results due to background interference. Each assay was repeated three times.

Statistical analyses

Each analysis was performed using the S.P.S.S. 13.0 statistics package for windows. Differences in the degree of biofilm formation (semi-quantitative adherence assay on 96-well tissue culture plates). P-values of < 0.05 were considered as significant. Other analysis was realized between the origin of strains, slime production and glass test tube adherence.

RESULTS

Adhesion to solvent

The results of measuring the adherence of *Listeria* cells to n-hexadecane are summarized in Table 1. This hydrocarbon was able to bind *L. monocytogenes* cells with different degrees function of the physiological state (stressed/non-stressed). Jones et al. (1996) defined only three groups of hydrophobicity to characterize the bacteria according to the effect of this variable on the interaction between bacterial cells and surfaces. Those authors considered cells with hydrophobicity percentage greater than 70% as highly hydrophobic; from 30 to 70% as weakly hydrophobic and those with hydrophobicity lower than 30% as highly hydrophilic. According to this proposal, the reference strain ATCC 19115 showed significant change of cell surface hydrophobicity after cold stress, moving from the highly hydrophilic group (H = 1%) to the weakly hydrophobic (H= 41.6 %) after two year of cold stress. The other bacterial strains maintained their highly hydrophilic cell surface after months of cold stress (H < 30 %) (Table1). In fact, the optimal hydrophobicity percents were observed for two years stressed cells. Nevertheless adherence to hexadecane was lower than 30% for all the strains, thus almost cells were considered as hydrophilic even after cold stress, except for the stressed ATCC strain. Our results show that two years stressed *L. monocytogenes* are the most hydrophobic specie using n-hexadecane as liquid hydrocarbon.

Slime production

Phenotypic slime production was assessed by culturing the investigated strains on CRA plates. Among 12 strains tested in this study, only the two years stressed S3 strain was a slime-producer developing black colonies (Figure 1b) whereas the remaining strains are considered as non-producers since they showed red colonies on CRA plates (Figure 1a) even after freezing incubation.

Biofilm formation in glass test tubes

The results of adherence assay to test glace tube assessed by Cristal Violet stain showed that *L. monocytogenes* strains were more adherent after cold stress than the non-adherent strains (Table 1). After six months of cold stress, only two strains (S2 and S3) were able to form biofilm on glass surface and the two others (ATCC 19115 and S4) were slightly adherent (Figure 2b). After two years of cold stress, strains were fairly adherent (Figure 2a).

Quantitative biofilm formation by *L. monocytogenes* strains on polystyrene

All *L. monocytogenes* strains were screened for their adherence to polystyrene 96 well microtiter plates at different degrees. Biofilm formation was interpreted as highly positive ($OD_{570} \geq 1$), low-grade positive ($0.1 \leq OD_{570} < 1$), or negative ($OD_{570} < 0.1$) (Ben Abdallah et al., 2009). The results showed that *Listeria* was able to form biofilm on polystyrene ($OD_{570} > 1$) and was then

Table 1. Cell surface hydrophobicity, adhesive properties and biofilm formation of *L. monocytogenes* on polystyrene microtiter plates, stainless steel (SS), polyethylene (PE), polyvinyl chloride (PVC) biomaterial, and glass surfaces.

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Stress period (month)	Strain	Hydrophobicity (%) ± SD	CRA	Glass	Polystyrene		SS	PVC	PE
					mean OD_{492} ± SD	mean OD_{570} ± SD	(mean OD_{578} ± SD)	(mean OD_{578} ± SD)	(mean OD_{578} ± SD)
0	ATCC 19115	1.03 ± 0.02	Red	S	0.46 ± 0.15	0.549 ± 0.03	0.0725 ± 0.02	0.31 ± 0.109	0.014 ± 0.057
	S1	1.05 ± 0.01	Red	S	0.63 ± 0.16	0.593 ± 0.28	0.001 ± 0.05	0.48 ± 0.078	0.133 ± 0.041
	S2	3.2 ± 0.02	Red	S	0.53 ± 0.09	0.629 ± 0.26	0.041 ± 0.01	0.38 ± 0.018	0.111 ± 0.059
	S3	2.4 ± 0.01	Red	S	0.54 ± 0.04	0.457 ± 0.18	0.019 ± 0.01	0.39 ± 0.055	0.134 ± 0.052
6	ATCC 19115	22.6 ± 0.1	Red	S	0.61 ± 0.07	1.533 ± 0.38	0.173 ± 0.04	0.46 ± 0.016	0.075 ± 0.030
	S1	6.7 ± 0.02	Red	S	0.7 ± 0.07	1.107 ± 0.28	0.017 ± 0.02	0.55 ± 0.039	0.098 ± 0.009
	S2	7.7 ± 0.3	Red	A	0.74 ± 0.21	0.039 ± 0.02	0.168 ± 0.01	0.59 ± 0.11	0.145 ± 0.025
	S3	3.03 ± 0.2	Red	A	0.34 ± 0.01	0.101 ± 0.03	0.18 ± 0.01	0.19 ± 0.106	0.119 ± 0.034
24	ATCC 19115	41.7 ± 0.4	Red	A	0.59 ± 0.07	2.015 ± 0.15	0.175 ± 0.02	0.44 ± 0.049	0.382 ± 0.168
	S1	9.7 ± 0.2	Red	A	0.83 ± 0.1	0.015 ± 0.02	0.317 ± 0.02	0.68 ± 0.026	0.477 ± 0.003
	S2	16.06 ± 0.1	Red	A	0.84 ± 0.12	1.484 ± 0.45	0.184 ± 0.007	0.69 ± 0.04	0.504 ± 0.004
	S3	6.03 ± 0.2	Black	A	0.67 ± 0.16	1.68 ± 0.52	0.305 ± 0.01	0.52 ± 0.025	0.51 ± 0.02

SS, Stainless style; PE, polyethylene; PVC, polyvinyl chloride; SD, standard deviation; A, adherent strain; SA, slightly adherent strain; CRA, Congo red agar assay.

considered as highly positive after cold exposure. Biofilm formation was significantly increased by cold stress from (0.984, 0.347, 0.337 and 0.092), after six months of cold stress to (1.466, 0.456, 0.855 and 1.22) after two years of cold stress for the ATCC 19115, S1, S2 and S3 strain respectively. The optical density values of XTT reduction estimated at 492 nm were reported in (Table 1). Stressed cells showed the highest potency to adhere to polystyrene plates when compared to non-stressed cells. Also, these bacteria were strong slime producer when tested with cristal violet stainon glass tubes. Biofilm formation assessed by XTT correlate with Crystal Violet assay.

Quantitative biofilm formation by *L. monocytogenes* strains on PVC, PE and SS surfaces

In this study, the metabolic activity of *L. monocytogenes* biofilms was determined by the ability of the attached cells to metabolically reduce MTT to a formazan dye after six months and after two years of freezing exposure. The highest levels of metabolic activity (MTT reduction) were observed for two years stressed cells which were strongly biofilm-producers as determined by the cristal violet assay (DO570>1) in comparison with the non-stressed strains. We found that metabolic activity of *L. monocytogenens* biofilm formed on stainless steel (SS) surfaces differed significantly between the three situations of cold exposure (Table 1). Thus the metabolic reduction of MTT has increased by 0.1, 0.016, 0.127 and 0.161 after six months of cold exposure then by 0.125, 0.316, 0.143 and 0.286 after two years of cold exposure for the ATCC 19115, S1, S2 and S3 strains, respectively. Similar results were shown for the two other types of surfaces PVC and PE. Nevertheless, the adherence affinity of stressed cells differs between the three materials; we noted that, until six months of cold exposure, the highest metabolic activity of biofilm still given on PVC surfaces by the four strains but two years above cells became more adherent to PE surfaces. Even

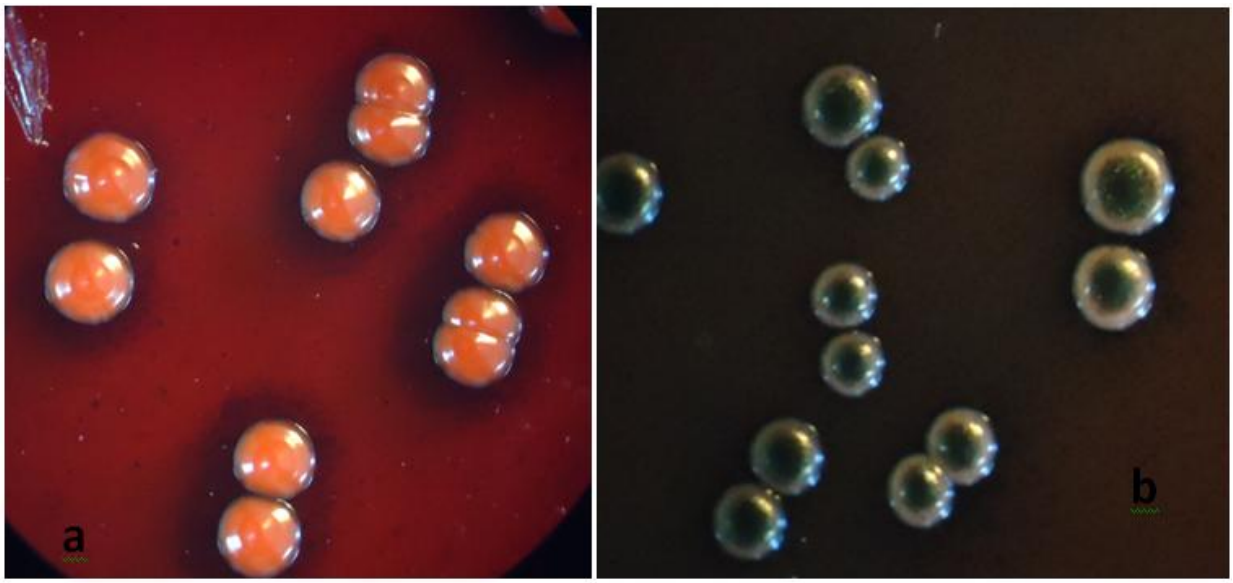

Figure 1. Colorimetric scale for colony analysis of slime production by *L. monocytogenes* using Congo red agar assay. a, Non-producing strain (red); b, slime producing strain (black).

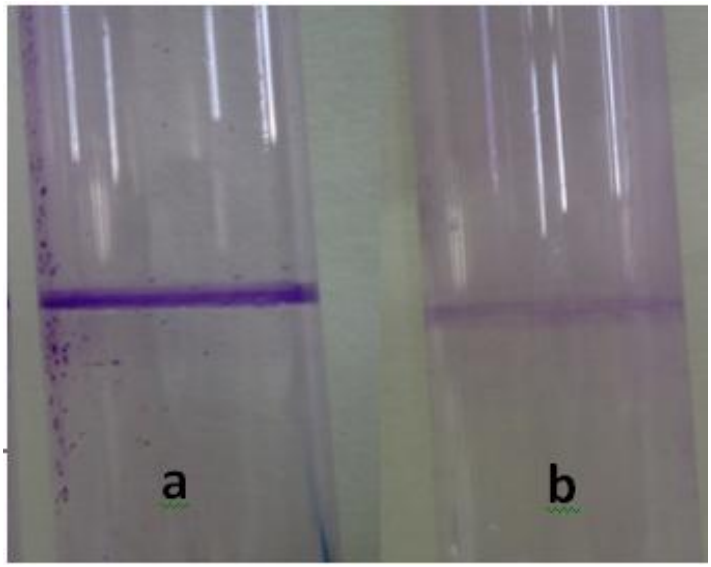

Figure 2. Adherence of *L. monocytogenes* tested strains to test glace tube. a, Adherent strain; b, slightly adherent strains.

stressed the tested strains have shown lowest affinity to adhere to SS surfaces comparing with PVC and PE surfaces.

DISCUSSION

The growth and presence of the food borne pathogen *L. monocytogenes* in processing plants is of increasing concern to the food industry. This study was undertaken to examine the ability of *L. monocytogenes* to adhere to abiotic surfaces commonly used in kitchens and food industry, before and after exposure to cold stress. Differences in biofilm formation were also detected between stressed and non-stressed strains isolated from food samples, with increased biofilm formation ability relative to non-stressed strains. SS, PE, PVC and glass surfaces were selected because of their common use in food-processing plants and because they have different physicochemical characteristics (Blackman, 1996). To

determine whether cold stress would influence biofilms formation, we investigated the ability of the tested strains to metabolically reduce the XTT by biofilms. The XTT assay was previously well described to reproduce quantitative models of biofilm formation and cell attachment (Pompilio et al., 2003; Sen et al., 2003). Biofilms were then indirectly quantified using the MTT assay as described previously by Kouidhi et al. (2010).

The ability of *L. monocytogenes* strains to produce slime was tested by Congo red method. The second part of this work was conducted to determine the ability of *L. monocytogenes* strains to adhere to the test materials. All strains adhered to all materials but to different extents depending on the material and strain. Analysis of the results shows that biofilm formation by the stressed strains was induced with cold stress exposure especially after long period of freezing. The cold incubation of the tested strains had a significant effect on its ability to adhere to test surfaces. A study by Parkar et al. (2001) of thermophilic strains of *Bacillus* species did not have an effect on the degree of bacterial attachment to stainless steel. The authors suggested that attachment of those bacteria is a multi-factorial process, and that cell surface proteins play a significant role in initiation of biofilm formation.

Physicochemical cell surface properties and ability of strains to produce extracellular polysaccharides (EPS) may provide leads towards understanding the mechanism of *L. monocytogenes* biofilm formation. EPS are thought to be an important factor for the process of bacterial attachment to epithelial cells and abiotic surfaces (Adlerbertti et al., 1996; Boulange-Petermann, 1996; Oliveira et al., 1994). Although presence of nucleic acids and proteins has been reported, EPS of biofilms consist largely of polysaccharides (Laspidou and Rittmann, 2002). It was also reported that *L. monocytogenes* strains form high biofilm levels which had more extracellular polysaccharides present than weak biofilm producers (Borucki et al., 2003). Genetic studies targeting carbohydrate synthesis pathways of *L. monocytogenes* will be required to fully understand the mechanism of this observation. In fact, results show that *L. monocytogenes* was significantly ($P < 0.05$) more adhesive to PVC than to PE and SS surfaces after freezing incubation. According to Vogler's criterion (Vogler, 1998) the materials tested here are classified as hydrophobic with the exception of glass, which are considered hydrophilic (Cox et al., 1989; Mafu et al., 1991). SS was sometimes classified as hydrophilic (Chamberlain and Johal, 1988b; Leclercq-Perlat and Lalande, 1994; Mafu et al., 1991).

Several techniques are normally used to determine cell surface characteristics, such as the hydrophobicity of bacteria, their cell surface charge, contact angle measurements and X-ray photoelectron spectroscopy (Bruinsma et al., 2001; Giovannacci et al., 2000). Little is known about cell surface properties of *L. monocytogenes* attachment to solid surfaces. Statistical analysis revealed a significant difference between the metabolic activity obtained after cold stress incubation between incubated cells and control cells. Accordingly, previous studies have mentioned that cold incubation have the potential to increase biofilm formation in *Escherichia coli* (Christine et al., 2008). These findings suggest that regulation of biofilm formation under cold stress conditions plays a role in the pathogenesis of *Escherichia coli.* These results agree with a recent study which reported that the growth temperature and the phase of growth may influence the cell wall composition and thereby modify the surface electrical properties, hydrophobicity and electron donor or electron acceptor character of the bacteria (Giovannacci et al., 2000; Hibma et al., 1996). Thus, we founded that slime production of *L. monocytogenes* isolates is highly correlated with other properties responsible for its firm adherence such as hydrophobicity.

We found that cells with highest biofilm production (as determined by coefficient of variation (CV) and XTT assays) were cells with highest values of hydrophobicity. Our observations showed that the hydrophily varied with cold stress period. Other researchers have also failed to find a correlation between the hydrophobicity of microbial strains and attachment to a surface. For example, 12 strains of *Streptococcus thermophilus* formed biofilms in dairy plants, but the attachment of those strains to stainless steel did not correlate with cellular hydrophobicity (Flint et al., 1997). Fletcher (1996) reported a general trend indicating that hydrophobic cells attach more readily than hydrophilic cells to biotic or abiotic surfaces, presumably due to their reduced stability in the bulk aqueous medium. At freezing temperature, *L. monocytogenes* was strongly hydrophobic which suggests some modifications in cell wall composition. It is well known that some bacteria maintain an optimal degree of membrane fluidity by modifying the cell wall lipid composition with temperature (Hebraud and Potier, 1999) and cell growth (Beck et al., 1988; Jana et al., 2000; Little et al., 1986). Membrane modifications probably occur in *L. monocytogenes* ATCC 19115 because the hydrophily varies with temperature.

Several studies have shown that adhesion of bacteria partly depends upon the nature of the inert surfaces and partly upon the bacterial surface properties (Carballo et al., 1992; Chamberlain and Johal, 1988a; Hood and Zottola, 1995). Hydrophobicity and surface charge are the most important surface properties in the adhesion process as demonstrated in numerous studies (Kalmokoff et al., 2001; Monica et al., 2003; Oliveira et al., 2007). The mechanisms governing the adhesion of *L. monocytogenes* to inert surfaces are not completely understood. In this study, we show that no significant bacterial could be formed at 37°C (low metabolic activity of biofilm) on PE, glass, PVC or on SS surfaces and the initial adherent population increased during freezing incubation, thereby increasing the hydrophobicity of cells

at low temperature which facilitate colonization of the tested surfaces. The combination of these two antagonist parameters, a hydrophobic cell envelope and a hydrophilic surface, leads to a very significant increase of the colonization power of the strain. The increase in cell attachment due to the cold stress suggests that this phenomenon may be in accordance with the development of *Listeria* in food preservation and other control processes (Gandhi and Chikindas, 2007). Our data are consistent with data on persistent strains forming biofilms but do not support a consistent relationship between enhanced biofilm formation and disease incidence.

Conclusion

This study represents the first attempt to study the adhesion ability of *L. monocytogenes* strains to abiotic surfaces while subjected to the cold stress: the most common stress applied in food industry. We have demonstrated that *L. monocytogenes* strains were able to adhere to biomaterials with different degree. The initial adherent population increased during freezing incubation, thereby increasing the hydrophobicity of cells at low temperature which facilitate colonization of the tested surfaces. To summarize, adhesive properties of *L. monocytogenes* strains allow these bacteria to persist in food processing environments and to colonize food products. Under cold stress *Listeria* became more able to attach to abiotic surfaces.

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