Listeria monocytogenes LO28: Surface Physicochemical Properties and Ability To Form Biofilms at Different Temperatures and Growth Phases

Patrick Chavant,¹ Brigitte Martinie,² Thierry Meylheuc,³ Marie-Noëlle Bellon-Fontaine,³ and Michel Hebraud^{1*}

Station de Recherches sur la Viande-Microbiologie¹ and Laboratoire de Microbiologie,² Institut National de la Recherche Agronomique, Saint-Genès Champanelle, and Unité de Recherche en Bioadhésion et Hygiène des Matériaux, Institut National de la Recherche Agronomique, Massy,³ France

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The surface physicochemical properties of *Listeria monocytogenes* LO28 under different conditions (temperature and growth phase) were determined by use of microelectrophoresis and microbial adhesion to solvents. The effect of these parameters on adhesion and biofilm formation by *L. monocytogenes* LO28 on hydrophilic (stainless steel) and hydrophobic (polytetrafluoroethylene [PTFE]) surfaces was assessed. The bacterial cells were always negatively charged and possessed hydrophilic surface properties, which were negatively correlated with growth temperature. The colonization of the two surfaces, monitored by scanning electron microscopy, epifluorescence microscopy, and cell enumeration, showed that the strain had a great capacity to colonize both surfaces whatever the incubation temperature. However, biofilm formation was faster on the hydrophilic substratum. After 5 days at 37 or 20°C, the biofilm structure was composed of aggregates with a three-dimensional shape, but significant detachment took place on PTFE at 37°C. At 8°C, only a bacterial monolayer was visible on stainless steel, while no growth was observed on PTFE. The growth phase of bacteria used to inoculate surfaces had a significant effect only in some cases during the first steps of biofilm formation. The surface physicochemical properties of the strain are correlated with adhesion and surface colonization.

Listeria monocytogenes is a psychrotrophic gram-positive pathogen regularly involved in outbreaks of food-borne disease (12, 26) in which milk (24), cheese (12), meat (16, 35), and vegetables represent the incriminated foodstuffs. This microorganism adheres and forms biofilms on numerous surfaces (15, 27), generally in association with other microorganisms such as *Pseudomonas* spp. Biofilms may represent an important source of contamination for materials or foodstuffs coming into contact with them (20, 21, 29). Several reports have shown that sessile bacterial cells are more resistant to environmental changes and cleaning or disinfection treatments (23). Therefore, there is growing interest in this mode of growth in food industries that aim to improve the microbiological safety of food.

Bacterial biofilms are generally described as surface-associated bacterial communities forming microcolonies surrounded by a matrix of exopolymers (9). Interspersed between these microcolonies are water-filled channels which may serve as a primitive circulatory system (22). Biofilm formation can be separated into four sequential steps (6, 39) governed by physicochemical factors such as hydrophobicity (17), van der Waals and Lewis acid-base characteristics (4, 32), and electrical properties (11). These four steps are (i) conditioning of the surface by macromolecules, (ii) initial adherence, (iii) physical irreversible adherence that involves the production of exopolymers that fix the cells, and (iv) growth of the microorganisms,

which form microcolonies, and coaggregation leading to establishment of the biofilm. Such structures are dynamic systems in which cells grow, die, and/or are released (8), allowing contamination of other surfaces.

The aim of this study was to investigate the kinetics of *L. monocytogenes* LO28 development in biofilms on two different surfaces commonly used in food-processing plants. Adhesion capability was assessed by determining the electrical, hydrophobic, and electron donor and acceptor properties of planktonic cells growing at three temperatures. Biofilm development and structural organization were studied under different conditions of growth and inoculation using microscopic techniques.

MATERIALS AND METHODS

Microorganism, media, and culture conditions. *L. monocytogenes* LO28, serotype 1/2c, was kindly donated by P. Cossard (Institut Pasteur, Paris, France). The strain was maintained on brain heart infusion medium (Difco, Detroit, Mich.) and stored at –20°C. The culture medium consisted of the MCDB 202 chemically defined medium (CryoBiosystem, L'Aigle, France) supplemented with 1% yeast nitrogen base without amino acids (Difco) and 3.6 g of glucose per liter (Carlo-Erba, Val de Reuil, France) and adjusted to pH 7.3.

For each experiment, the strain was grown overnight on brain heart infusion slopes at 37°C. These slopes were used to inoculate a liquid preculture in an Erlenmeyer flask containing 20 ml of supplemented MCDB 202 medium. The preculture was grown to stationary phase in an orbital shaking water bath (150 rpm) at the same temperature as used in the subsequent experiment (8, 20, or 37°C). Erlenmeyer flasks containing 100 ml of supplemented MCDB 202 were inoculated with the preculture to obtain a final optical density at 600 nm (OD₆₀₀) of 0.1 (approximately 10⁷ CFU ml^{–1}). The strain was grown until the mid-log or stationary phase, i.e., for 6 or 16 h at 37°C, 15 or 40 h at 20°C, and 5 or 10 days at 8°C, respectively.

* Corresponding author. Mailing address: SRV-Microbiologie, INRA de Theix, 63122 Saint-Genès Champanelle, France. Phone: 33 4 73 62 46 70. Fax: 33 4 73 62 42 68. E-mail: hebraud@clermont.inra.fr.

Physicochemical experiments. Cells were harvested by centrifugation for 10 min at $7,000 \times g$ at 4°C and then washed twice with and resuspended in the relevant suspending liquid (1.5×10^{-1} or 1.5×10^{-3} M NaCl).

The microbial-adhesion-to-solvents partitioning method, previously described by Bellon-Fontaine et al. (2), is based on comparing microbial cell affinities for monopolar and nonpolar solvents which exhibit similar van der Waals surface tension components. On this basis, the following pairs of solvents were selected: (i) chloroform, an acidic solvent (electron acceptor), and hexadecane, a nonpolar *n*-alkane, and (ii) ethyl acetate, a basic solvent (strong electron donor), and decane, a nonpolar *n*-alkane. Experimentally, a suspension containing approximately 10^8 CFU in 2.4 ml of 1.5×10^{-1} M NaCl (OD_{400} of between 0.6 and 0.7 [A_0]) was vortexed for 60 s with 0.4 ml of a solvent. The mixture was held for 15 min to ensure complete separation of the two phases before a sample (1 ml) was carefully removed from the aqueous phase and the OD_{400} was measured (4). The percentage of cells in each solvent was subsequently calculated by the following equation: percent affinity = $[1 - (A/A_0)] \times 100$. Each experiment was performed in triplicate with three independently grown cultures.

For the electrophoretic mobility measurements, cells were resuspended in 1.5×10^{-3} M NaCl at a final concentration of 10^7 CFU ml^{-1} . The pH of each suspension was adjusted within the range of 2 to 7 by adding nitric acid (0.01 N) or potassium hydroxide (0.01 N). Electrophoretic mobility measurements were taken in a 50-V electric field using a Laser Zetameter (CAD Instrumentation, Limours, France). For each measurement, results were based on the automated video analysis of about 200 bacterial cells. Each experiment was performed in duplicate on two independent cultures.

Biofilm formation. (i) Surfaces and cleaning treatment. The two surfaces used for biofilm experiments were AISI 304 stainless steel (mean roughness = 0.064) and polytetrafluoroethylene (PTFE) (Teflon) (mean roughness = 0.239) (Goodfellow, Cambridge, United Kingdom). Each surface was cut into rectangular chips (3 by 1 cm). Before each experiment, the chips were soaked for 10 min with a 2% solution of the commercial surfactant TFD4 (Franklab S.A., Saint Quentin en Yvelines, France) and rinsed five times for 5 min each with hot tap water and five times for 5 min each with demineralized water. Finally, the surfaces were autoclaved for 15 min at 120°C .

(ii) Adhesion of *L. monocytogenes*. Cells (in the stationary or exponential phase of growth) were harvested by centrifugation ($7000 \times g$, 10 min) and resuspended in their own supernatant to give an OD_{600} of between 0.6 and 0.7. Seven milliliters of the bacterial suspension was poured into a petri dish (55-mm diameter) containing a chip of surface material and stored at 37, 20, or 8°C . The medium was replaced after 2 h and then every 24 h for studies at 37 or 20°C or after 1 and 5 days for studies at 8°C . Cell adhesion and biofilm formation were evaluated at each temperature by cell enumeration and determination of the percentage of contaminated surface after 2 h, 6 h, and 1, 2, 5, and 7 days of contact with planktonic cells.

For cell enumeration, each chip was placed in a petri dish (90-mm diameter) and washed twice for 1 min each with 35 ml of sterile tryptone salt (TS) (Bactotryptone, 0.1%; NaCl, 0.85%) on a orbital shaking table (50 rpm) to remove nonadherent cells. Sessile cells were then detached from the inert surfaces in 5 ml of sterile TS by use of a sonication bath (Deltasonic, Meaux, France) for 3 min at 50 kHz. After serial dilution in TS, the number of CFU was counted on tryptic soy agar (Difco), and the cells were incubated for 24 h at 37°C . Each experiment was performed in duplicate.

To determine the percentage of surface contaminated, the nonadherent cells were removed as described above. Sessile cells were fixed on the support with a solution of 3% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4) and rinsed in the same buffer. Samples were stained for 3 min with a solution of 0.05% acridine orange and then washed twice for 1 min with demineralized water. The chips were then dried in air for 1 h and observed with an Axioplan 2E microscope (magnification, $\times 157.5$) (Carl Zeiss, Jena, Germany) coupled with a camera under UV light filtered through a blue filter. The images were analyzed with Visiolab 1000 software (Biocom, Les Ulis, France) as grey-scale interpretations on the screen. The area covered by the biofilm was converted into a percentage of the total area. For each experiment, two chips were analyzed, and 15 fields were observed per chip.

SEM. Nonadherent cells were removed from the surfaces, and sessile cells were fixed as described above. After postfixation for 1 h with osmic acid vapors, cells were dehydrated using a graded ethanol series (70, 95, and 100% three times for 10 min each) and subjected to an acetone dehydration series of 30, 50, 70, and 100% acetone for 10 min each. Chips were coated with gold in an Emscope SC500 and observed with a Philips SEM 505 scanning electron microscope (SEM).

Statistical analysis. Growth curves and percentages of contaminated surface were analyzed with STATISTICA version 5.0 software (Statsoft, Tulsa, Okla.).

TABLE 1. Affinity of *L. monocytogenes* LO28 to solvents with respect to temperature and growth phase

Temp ($^\circ\text{C}$)	Growth phase	% Affinity (mean $\pm$ SD) for:			
		Chloroform	Hexadecane	Ethyl acetate	Decane
37	Exponential	77 ± 5	65 ± 9	32 ± 3	60 ± 9
	Stationary	85 ± 4	80 ± 2	51 ± 6	78 ± 4
20	Exponential	77 ± 2	65 ± 3	29 ± 4	63 ± 5
	Stationary	59 ± 6	53 ± 10	20 ± 2	41 ± 11
8	Exponential	52 ± 5	29 ± 7	17 ± 1	22 ± 8
	Stationary	30 ± 7	18 ± 12	21 ± 6	13 ± 8

The effects of growth stage, nature of the surface, and temperature were studied by analysis of variance. The mathematical model used in the analysis of variance was as follows: $X_{i,j,k,r} = m + T_i + G_j + S_k + TG_{ij} + GS_{jk} + TS_{ik} + \Sigma_{i,j,k,r}$ where X is the growth or contaminated-surface characteristics; m is a constant term; T is the mean effect of temperature ($i = 3$); G is the mean effect of growth stages ($j = 2$); S is the mean effect of the surface ($k = 2$); TG , GS , and TS are mean effects of first-order interactions; Σ is the residual variations; and r is the number of repetitions ($r = 3$).

Modeling of *L. monocytogenes* LO28 biofilm formation for the two types of surfaces and the different temperatures was carried out with the use of isoreponse surfaces (31). This analysis consists of regression by least-squares analysis using polynomials of the fourth degree and was done using STATISTICA software.

RESULTS

Influence of temperature and growth phase on *L. monocytogenes* surface physicochemical properties. The data in Table 1 show the microbial-adhesion-to-solvents results obtained for *L. monocytogenes* growing in supplemented MCDB 202 medium at different temperatures and growth phases. Under all conditions, the highest affinity of *L. monocytogenes* was observed with chloroform, the electron acceptor solvent. Moreover, the cell affinity with chloroform was always higher than that with hexadecane, the nonpolar solvent. These observations indicated an electron-donating nature of *L. monocytogenes* LO28 (basic surface properties). In the same way, the weak affinity of the strain for the electron donor solvent (ethyl acetate) in comparison to the associated nonpolar solvent (decane) revealed a weak electron-accepting nature of the strain. In stationary phase as well as in mid-log phase, the affinity of the strain for the nonpolar solvents (hexadecane and decane) decreased with incubation temperature, demonstrating that the hydrophilic properties of the cell surface of the strain were negatively correlated with incubation temperature.

The data in Fig. 1 represent the electrophoretic mobility of bacterial cells at between pH 2.0 and 7.0 based on incubation temperature and growth phase. The standard deviation of the mean electrophoretic mobility value was $0.3 \mu\text{m/s/V/cm}$. Under all conditions of growth, cells were always negatively charged, and an isoelectric point could be determined at any pH that was investigated.

Kinetics of biofilm formation. (i) Cell growth. The data in Fig. 2 show the kinetics of growth of *L. monocytogenes* LO28 at 8, 20, and 37°C on stainless steel and PTFE surfaces inoculated with cells in either the stationary or mid-log phase of growth. The experimental conditions used, and particularly the supplemented MCDB medium, resulted in a minimum of 10^7 CFU/ cm^2 after 2 h of contact with the substrata. At this time, the initial adherent population was greater on stainless steel at

FIG. 1. Electrophoretic mobility (EM) of *L. monocytogenes* LO28 at 37°C (A), 20°C (B), and 8°C (C) in stationary (■) and mid-log (□) phase. Error bars indicate standard deviations.

37°C (Δ log units = 0.2 to 0.6), while it was greater on PTFE at 20°C (Δ log units = 0.4 to 0.5) and was equivalent on the two surfaces at 8°C. Under all experimental conditions except at 8°C on PTFE, the number of attached cells increased with time

(Δ log units = 0.4 to 1.4). The maximum number of adhering cells was reached after 1, 2, or 5 to 7 days for the two surfaces at 37, 20, and 8°C, respectively. At 37 and 8°C, there was better growth of cells on stainless steel than on PTFE. For the first

FIG. 2. Biofilm growth of *L. monocytogenes* LO28 at 37°C (A), 20°C (B), and 8°C (C) on stainless steel (squares) and PTFE (circles). Surfaces were inoculated with bacteria in either the stationary (filled symbols) or mid-log (open symbols) phase of growth.

FIG. 3. Isoresponse surface of sessile cell enumerations (log CFU per square centimeter) as a function of time on stainless steel (A and C) and PTFE (B and D) after inoculation with cells in the mid-log (A and B) or stationary (C and D) phase of growth.

48 h, the opposite was observed at 20°C, and after 48 h, the population on PTFE decreased slightly whereas that on stainless steel increased slightly. At low temperature, the colonization of both surfaces was very slow, and a reduction in the adherent population was observed on PTFE.

Biofilm formation, with respect to temperature and time after the adhesion step, is shown in Fig. 3 as isoresponse surfaces in which each curve corresponds to the same population level. The patterns for each surface were similar regardless of the growth phase of the inoculum. There were no significant differences either in the efficiency of adhesion or in the kinetics of surface colonization when the inoculum was with mid-log or stationary-phase cells. In other respects, the isoresponse surfaces clearly indicate differences between hydrophilic and hydrophobic substrata. The density of adherent cells on stainless steel was greater than 10^8 CFU/cm² for a large range of temperatures (from 15 to 37°C) and time (at and after 48 h) and was never below 10^7 CFU/cm². On the other hand, the adherent population on PTFE never reached the level of that on stainless steel, and a density higher than 10^8 CFU/cm² was observed only between 18 and 30°C and between 24 h and 4 days. After 4 days, a slight reduction in the adherent population was observed in this range of temperature.

(ii) Percentage of surface contaminated. The percentages of surface colonized, as a function of the different parameters

studied, are represented in Fig. 4. The results showed that, regardless of growth temperature and phase of growth, the stainless steel chips were completely overlaid by the bacterial biofilm by the end of 5 days. This occurred only at 20°C on PTFE. The colonization of stainless steel chips was rapid. At least 80% of the chip surface was covered by the biofilm after 2 h at 37 and 20°C. For both surfaces, the colonization reached 100% after 24 h at 37°C but decreased after 2 days because of detachment. Detachment was also observed on stainless steel at 20°C after 24 h of colonization. At 37 and 20°C, the hydrophilic surface was again completely colonized, whereas the contamination of PTFE at 37°C decreased to 35% after 7 days. At low temperature, the results were in agreement with those obtained by enumeration and showed that *L. monocytogenes* LO28 could not colonize the hydrophobic surface. After an initial adhesion covering 18 or 43% of the surface with stationary- or exponential-phase cells, respectively, the colonization decreased to about 5% under the two conditions at 24 h after inoculation. Slight differences due to the phase of growth of cells used to inoculate the two surfaces were observed. Generally, these differences were observed only for the first 2 days and showed a faster colonization when surfaces were contaminated by cells in the exponential phase of growth. On stainless steel at 37 and 20°C, this faster colonization was followed by detachment preceding a new colonization phase.

FIG. 4. Percentages of surface colonized by *L. monocytogenes* LO28 at 37°C (A), 20°C (B), and 8°C (C) after 2 and 6 h and 1, 2, 5, and 7 days on stainless steel inoculated with cells in the stationary (■) or mid-log (□) phase of growth and on PTFE inoculated with cells in the stationary (▒) or mid-log (▨) phase of growth.

The statistical representation of the percent colonization of the two surfaces according to temperature and time (Fig. 5) emphasizes the results obtained. The isoresponse surfaces clearly show higher and faster colonization of stainless steel than of PTFE at all temperatures. The initial colonization of the hydrophilic surface covered 50% at low temperature and at least 80% at 20°C and above. These values were always lower for the hydrophobic surface. Patterns of colonization on PTFE were similar despite the physiological state of the bacteria. On stainless steel, there were slight differences, with a complete covering of the surface at a broader range of temperature with the inoculum of stationary-phase cells.

SEM. Figures 6, 7, and 8 represent the kinetics of biofilm formation at 37, 20, and 8°C, respectively, observed by SEM on stainless steel and PTFE. The micrographs are all of surfaces inoculated with stationary-phase cells, but similar data were obtained with inoculation of exponential-phase cells (data not shown).

At 37°C, the two surfaces were rapidly colonized by the formation of bacterial mats. Three-dimensional structures appeared in a few hours and constituted aggregates that evolved to dense bacterial mats at the end of 2 days, in which spaces or channels could be distinguished with high magnification. These spatial structures gained increasing importance on stainless

steel, while on PTFE, detachment was clearly visible in the electron micrographs. At 20°C, colonization was slower than at 37°C, and formation of aggregates was visible only at the end of 2 days for stainless steel and 7 days for PTFE. The three-dimensional structure of the biofilm was identical to that observed at 37°C, but no detachment of aggregates occurred on PTFE during the 7 days of the experiment. At 8°C, *L. monocytogenes* slowly formed a bacterial mat on stainless steel, but this mat was not very dense, and only small and dispersed aggregates were visible. The bacteria were arranged mainly as a monolayer. In contrast, fewer and fewer cells were present on PTFE with increasing time in comparison with the initial adherent population. Finally, the average length of cells measured for each temperature on both surfaces (data not shown) revealed that at 8°C the cells were 1.5 and 2 times longer than at 20 and 37°C, respectively.

DISCUSSION

The aim of this study was to determine the surface physico-chemical properties of *L. monocytogenes* LO28 and to investigate its ability to adhere and to form biofilm on abiotic surfaces at different temperatures and in the log and stationary phases.

FIG. 5. Isoresponse surface of the percentages of contaminated surface as a function of time on stainless steel (A and C) and PTFE (B and D) after inoculation with cells in the mid-log (A and B) or stationary (C and D) phase of growth.

Stainless steel and PTFE were selected because of their common use in food-processing plants and because they have different physicochemical characteristics (3). 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 (13, 36).

The affinity of bacterial cells as determined by the comparison of the two pairs of solvents was higher for the electron acceptor solvent and weaker for the electron donor solvent under all conditions of the study, indicating a strong electron donor nature and a weak electron acceptor nature of the bacteria. Lewis acid-base interactions indicated that the cells had basic surface properties that correlated with their hydrophilic characteristics. These results are consistent with those of Mafu et al. (27), who used a hydrophobic-interaction chromatography technique to illustrate the same hydrophilic characteristic for the *L. monocytogenes* strain Scott A. Our observations showed that the hydrophily varied with the temperature and growth phases. As previously described by Smoot and Pierson (36), hydrophily increased as the growth temperature decreased, except at 37 and 20°C for mid-log phase cells, where the cells shared the same properties. Hydrophily was greater

for mid-log-phase cells than for stationary-phase cells at 37°C, but this trend was the opposite at 20 and 8°C. At low temperature, *L. monocytogenes* was strongly hydrophilic at either growth phase, 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 (14) and cell growth (1, 19, 25). Membrane modifications probably occur in *L. monocytogenes* LO28 because the hydrophily varies with temperature and growth phase, but these modifications do not influence the global charge of bacterial cells, which were always electronegatively charged under all of the conditions studied. The results of the electrophoretic mobility tests were surprising, because generally most bacterial cells have an isoelectric point of between pH 2.0 and 3.5 (30). Nevertheless, this cell surface property seems to be characteristic of *L. monocytogenes*, because several authors have reported the same result for strains of *L. monocytogenes* from different origins (4, 11, 13, 28). The electronegative global charge over the pH range studied may indicate the presence of compounds with a very low pKa on the cell surfaces (4).

Our experiments demonstrated that *L. monocytogenes* LO28 adhered and formed biofilm with a three-dimensional shape on

FIG. 6. SEM observations of *L. monocytogenes* biofilm formation at 37°C on stainless steel (left panels) and PTFE (right panels) at 6 h (A), 2 days (B), and 7 days (C) after inoculation with stationary-phase cells. Bars, 10 µm.

surfaces commonly used in food-processing plants. Whatever the growth phase of the cells, the strain completely or almost completely colonized stainless steel and PTFE surfaces, except at low temperature for PTFE. Several authors (3, 27) reported the capacity of different strains of *L. monocytogenes* to colonize such surfaces; however, with the chemically defined medium MCDB 202 and the growth conditions used in this study, the cell density in biofilms reached up to 3×10^8 CFU/cm², which had not previously been obtained.

An analysis of variance (see Materials and Methods) shows that the nature of the surface and the temperature were the main factors which statistically affected adhesion and colonization ($P < 0.01$). Thus, better adhesion and colonization were observed on the stainless steel, confirming the hydrophilic character of *L. monocytogenes* LO28 and the importance of this property in these processes (38). Moreover, no bacterial mat could be formed at 8°C on PTFE, and the initial adherent population decreased during the first days of the experiment, thereby increasing the hydrophilicity of cells at low tempera-

ture, which made colonization of the hydrophobic surface very difficult or even impossible. The combination of these two antagonist parameters, a hydrophilic cell envelope and a hydrophobic surface, leads to a very significant decrease of the colonization power of the strain. It is noteworthy that the presence of similar initial adherent populations does not imply the same kinetics of colonization of the two surfaces, which suggests that adhesion and colonization phases could require different molecular adaptations.

Significant detachment occurred at 37°C on PTFE, but only after the surface was completely colonized. It would seem that the rapid colonization of the surface resulted in the formation of cellular aggregates based on a relatively low number of adherent cells. Consequently, the aggregates became too voluminous and detached themselves because of the weak interactions of the few adherent cells with the hydrophobic PTFE. Interestingly, a phase of detachment took place after 1 day at 20°C on stainless steel, but this was transitory and the surface was almost completely colonized again 24 h later. At 20°C, *L.*

FIG. 7. SEM observations of *L. monocytogenes* biofilm formation at 20°C on stainless steel (left panels) and PTFE (right panels) at 6 h (A), 2 days (B), and 7 days (C) after inoculation with stationary-phase cells. Bars, 10 μ m.

monocytogenes colonized PTFE more slowly but without detachment, and at the end of 1 week, a three-dimensional structure with voluminous aggregates was visible. Biofilms could be stable at 20°C on PTFE due to the presence of flagella. Several authors have described the positive influence of flagellation and motility on the different stages of biofilm formation (33, 34). Flagellation could facilitate the contact with the substratum by overcoming any electrostatic repulsion forces (15) and in this way could help to form a more important layer of cells directly in contact with the surface. Such a phenomenon may explain the significant difference in biofilm stability observed on PTFE between 37 and 20°C. This study confirms that biofilms are dynamic structural entities in which detachment, growth, and movement of microcolonies take place (37). This dynamic may be at the origin of dissemination of microorganisms and contamination of surfaces, such as, for example, in food industries.

The observations of *L. monocytogenes* biofilms by SEM

showed a significant spatial shape composed of aggregates surrounded by voids. These spaces could be the consequence of the treatments undergone by the samples before SEM observations or could correspond to water channels, as described by several authors (7, 10, 22). These water channels allow the distribution and circulation of nutrients and oxygen inside the biofilm. The greater cell length observed at low temperature, already described as well for planktonic cells (18), than for sessile cells growing under conditions of stress (5) gave evidence of an impaired physiology. The growth medium and the methodology used in this work show that it is possible to obtain a significant population of sessile cells with a three-dimensional shape. From a practical point of view, our results demonstrate that the use of PTFE (hydrophobic) surfaces in cold rooms may minimize the development of *L. monocytogenes* biofilms in food plants. In other respects, the size of the population obtained ($\geq 10^8$ CFU/cm²) indicates the need for further investigations about the biofilm physiology and mecha-

FIG. 8. SEM observations of *L. monocytogenes* biofilm formation at 8°C on stainless steel (left panels) and PTFE (right panels) at 6 h (A), 2 days (B), and 7 days (C) after inoculation with stationary-phase cells. Bars, 10 μ m.

nisms of resistance to environmental stresses or to cleaning and disinfection treatments. Such studies are in progress in our laboratory, in particular by a proteomic approach.

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